DATABASES

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

The ARVD/C Genetic Variants Database: 2014 Update

Elisabetta Lazzarini,[1,2] Jan D. H. Jongbloed,[2] Kalliopi Pilichou,[1] Gaetano Thiene,[1] Cristina Basso,[1] Hennie Bikker,[3] Bart Charbon,[2] Morris Swertz,[2] J. Peter van Tintelen,[2,3,4†] and Paul A. van der Zwaag[2*†]

[1]*Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Padua, Italy;* [2]*Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;* [3]*Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands;* [4]*Durrer Center for Cardiogenetic Research, Utrecht, The Netherlands*

Communicated by Alastair Brown

Received 29 October 2014; accepted revised manuscript 5 February 2015.
Published online 10 February 2015 in Wiley Online Library (www.wiley.com/humanmutation). DOI: 10.1002/humu.22765

ABSTRACT: Arrhythmogenic cardiomyopathy (ACM) is an inherited cardiac disease characterized by myocardial atrophy, fibro-fatty replacement, and a high risk of ventricular arrhythmias that lead to sudden death. In 2009, genetic data from 57 publications were collected in the arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) Genetic Variants Database (freeware available at http://www.arvcdatabase.info), which comprised 481 variants in eight ACM-associated genes. In recent years, deep genetic sequencing has increased our knowledge of the genetics of ACM, revealing a large spectrum of nucleotide variations for which pathogenicity needs to be assessed. As of April 20, 2014, we have updated the ARVD/C database into the ARVD/C database to contain more than 1,400 variants in 12 ACM-related genes (*PKP2, DSP, DSC2, DSG2, JUP, TGFB3, TMEM43, LMNA, DES, TTN, PLN, CTNNA3*) as reported in more than 160 references. Of these, only 411 nucleotide variants have been reported as pathogenic, whereas the significance of the other approximately 1,000 variants is still unknown. This comprehensive collection of ACM genetic data represents a valuable source of information on the spectrum of ACM-associated genes and aims to facilitate the interpretation of genetic data and genetic counseling.
Hum Mutat 36:403–410, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: cardiomyopathy; database; genetics

Introduction

Arrhythmogenic cardiomyopathy (ACM; MIM #107970) is an inherited cardiac disease, clinically characterized by electrical abnormalities and high frequency of re-entrant arrhythmias, mainly originating from the right ventricle, which may lead to palpitations, syncope, and even sudden death at a young age [Thiene et al., 1998]. The right-sided form is predominant and referred to as arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). The clinical manifestations of the disease mostly occur between the second and forth decade of life, and it is one of the major causes of sudden death in the young and athletes [Corrado et al., 2003 , 2008]. The clinical diagnosis is based on the fulfillment of the task force criteria proposed in 1994 [McKenna et al., 1994], and revised in 2010 [Marcus et al., 2010]. The main pathological feature of the disease is a progressive loss of the right ventricular myocardium and its replacement by fibrous or fibro-fatty tissue in a process that originates from the epicardium and spreads to the endocardium. The biventricular and left dominant forms of ACM, characterized by the same pathologic process, are now being reported more frequently [Sen-Chowdhry et al., 2008]. Therefore, the wider definition of "ACM," which encompasses the right sided, the biventricular, and the left sided subtypes, is replacing the classical description of "arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)." Moreover, 30 years after the first descriptions of the disease, the original designation of "dysplasia" is no longer suitable for describing a cardiomyopathy whose features and pathophysiology are genetically determined [Basso et al., 2010].

Familial occurrence of ACM is common. In approximately 30%–50% of cases, it is transmitted through an autosomal-dominant pattern of inheritance, with variable expressivity and age-dependent penetrance [Basso et al., 2009]. However, growing evidence suggests that the inheritance pattern is more complex. Recessive forms have been described originally in association with cutaneous disorders (Naxos disease and Carvajal syndrome) [McKoy et al., 2000; Norgett et al., 2006]. Further, recessive inheritance as homozygosity or compound or digenic/oligogenic heterozygosity occur also in nonsyndromic ACM forms. Therefore, the low penetrance of ACM may be explained as a result of recessive or recessive—"like" inheritance patterns [Xu et al., 2010; Rigato et al., 2013; Bhonsale et al., 2015]. One example of recessively transmitted nonsyndromic ACM where rare homozygous missense variants lead to a clinical phenotype is the *DSG2* p.T335A variant, which is associated with an ACM phenotype in homozygous individuals, or often appears together with other rare desmosomal variants in heterozygous probands [Rasmussen et al., 2014].

Presently, causative mutations in ACM have primarily been described in genes encoding the main components of the cardiac desmosome: desmoplakin (*DSP*), desmoglein-2 (*DSG2*), plakophilin-2 (*PKP2*), desmocollin-2 (*DSC2*), and plakoglobin (*JUP*) [Rampazzo et al., 2002; Gerull et al., 2004; Pilichou et al., 2006; Syrris et al., 2006; Asimaki et al., 2007]. Desmosomes are multiprotein complexes that link intermediate filaments of adjacent cardiomyocytes providing structural and functional integrity.

†These are shared last authors.

*Correspondence to: Paul A. van der Zwaag, Department of Genetics, UMCG, PO Box 30001, Groningen 9700 RB, The Netherlands. E-mail: p.a.van.der.zwaag@umcg.nl

Contract grant sponsors: Netherlands Heart Foundation (grant 2007B132), the Registry of Cardio-Cerebro-Vascular Pathology, Veneto Region, Venice, Italy, and Research Grant TRANSAC, Padua, Italy and the Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development, and the Royal Netherlands Academy of Sciences.

Defective desmosomal components might impair cell adhesion, leading to myocyte detachment, and death followed by fibro-fatty repair. It has also been hypothesized that impaired desmosome assembly results in the release and nuclear translocation of JUP, where it acts as a competitor of β-catenin and suppresses the canonical Wnt signaling. This leads to an enhanced expression of adipogenic and fibrogenic genes and thus to a transcriptional switch from myogenesis to adipogenesis [Garcia-Gras et al., 2006].

Although ACM is widely considered a desmosomal disease [van Tintelen et al., 2007], mutations in extradesmosomal genes have also been associated with ACM or clinically overlapping syndromes, suggesting there is some genetic heterogeneity. These include the ryanodine receptor 2 gene (*RYR2*) mutations in the untranslated regions of the transforming growth factor beta3 gene (*TGFB3*) and the transmembrane protein 43 gene (*TMEM43*) [Tiso et al., 2001; Beffagna et al., 2005; Merner et al., 2008]. More recently, mutations in the genes desmin (*DES*), titin (*TTN*), phospholamban (*PLN*), lamin A/C (*LMNA*), and αT-catenin (*CTNNA3*) have also been discovered [van Tintelen et al., 2009; Taylor et al., 2011; van der Zwaag et al., 2012; Quarta et al., 2012; van Hengel et al., 2013].

In 2008, the ARVD/C Genetic Variants Database was constructed to provide a comprehensive collection of all genetic variants, including common polymorphisms in the ACM-related genes, along with all the available information on their effect, thereby facilitating their clinical interpretation [van der Zwaag et al., 2009]. The database is publicly available via http://www.arvcdatabase.info, and http://grenada.lumc.nl/LOVD2/ARVC/home.php, its copy in the Leiden Open Variation Database [Fokkema et al., 2005], which is a free Web-accessible database collecting DNA variations in specific genes. The ARVD/C database contains data from a broad screening of the scientific literature as well as unpublished variants identified during routine genetic screening in several contributing laboratories. Since its launch, the database has aided scientists in verifying if a specific variant has already been described and published and aided clinicians in providing a proper diagnosis and counseling of mutation carriers and their families. It has been accessed more than 200,000 times from over 50 different countries, highlighting the need for an easy-to-use registry of disease-associated variants. However, such a genetic database needs to be constantly revised and updated as new genes, variants, and papers appear, and the pathogenicity of variants is revised. It has been suggested, for instance, that the missense variant p.R490W in *PKP2* may not be a causative mutation as it is located in exon 6, which is skipped in the cardiac isoform PKP2a [Gandjbakhch et al., 2011]. In another example, the p.A897KfsX4 frameshift variation in *DSC2* was reclassified from pathogenic to a single-nucleotide polymorphism after it was identified in 1.5% of control subjects [De Bortoli et al., 2010].

The ARVD/C database has now been upgraded. The new version contains details on 12 ACM-related genes, an update of all nucleotide variants published in the literature (referencing over 160 studies) and now contains more than 1,400 distinct variants, of which 411 are reported to be pathogenic mutations. It also includes a section on negatively screened candidate genes.

Database Update

The database has been updated with data available from online publications derived from literature searches on Entrez PubMed through April 20, 2014, using the search terms reported on our Website (http://www.arvcdatabase.info/general/searchterms.aspx). New genes, variants, and references have been added, and information related to variants such as classification and clinical reports have been modified to reflect the findings of the most recent studies. The structure of the first version of the database has been maintained, and the information coming from literature was manually annotated. A summary of this update reporting all the newly included studies appears in the "News" section on the database homepage. In order to give a comprehensive overview of the spectrum of nucleotide variants in ACM-related genes, all the variants reported in the literature were annotated independently from the form of cardiomyopathy and linked to the respective case report.

Nomenclature

All variants are described according to the Human Genome Variation Society nomenclature guidelines [den Dunnen and Antonarakis, 2000] and numbered with respect to the genes' reference sequences (*DSC2*:NM_024422.3; *DSG2*:NM_001943.3; *DSP*:NM_004415.2; *JUP*:NM_021991.2; *PKP2*:NM_004572.3; *CTNNA3*:NM_013266.3; *DES*:NM_001927.3; *LMNA*:NM_170707.3; *PLN*:NM_002667.3; *TGFB3*:NM_003239.3; *TMEM43*:NM_024334.2; *TTN*:NM_133378.4). The nomenclature of all variants has been checked using Mutalyzer (http://www.LOVD.nl/mutalyzer) [Wildeman et al., 2008].

Database Information

The revised database provides information on all reported variants for the ARVD/C genes with the correct nomenclature and references to the papers. Every variant is linked to a summary page giving the following information: gene and locus, exon–intron boundaries, coding DNA change based on the reference sequence, protein change, type of alteration (nonsense, missense, splice site, insertion–deletion, synonymous, frameshift, intronic), the putative pathogenic nature of every variant reported and the studies associated with each variant. The classification of variants in our database is documented from information published in the literature and responsibility for study content therefore remains with the respective authors, not with the curators. In silico prediction data were updated for each missense variant based on the following methods: Grantham score, PolyPhen, and SIFT [Grantham, 1974; Ng and Henikoff, 2003; Ramensky et al., 2002; Liu et al., 2011]. Patient information, as reported in the respective publications, has also been included together with the number of patients described per family and data on the variant frequency in control subjects. Variants present in more than one scientific report have been linked to the detailed reports in a specific Web page.

Variants

The first version of the database included eight genes and 481 variants, of which 144 were pathogenic, and information was derived from 57 references [van der Zwaag et al., 2009]. In this revised version of the database, more than 1,400 variants are annotated in all 12 ACM-related genes, of which about 411 are reported as pathogenic. The database now also includes variants present in extradesmosomal genes such as *DES, PLN, CTNNA3, TTN, LMNA*, distributed as follows: 150 indels, 172 intronic, 725 missense, 92 nonsense, 64 splice site, 185 synonymous, 38 in the untranslated regions (Table 1). Out of the 733 newly added sequence changes in the revised database, 198 were updated with new references and 535 were uploaded for the first time.

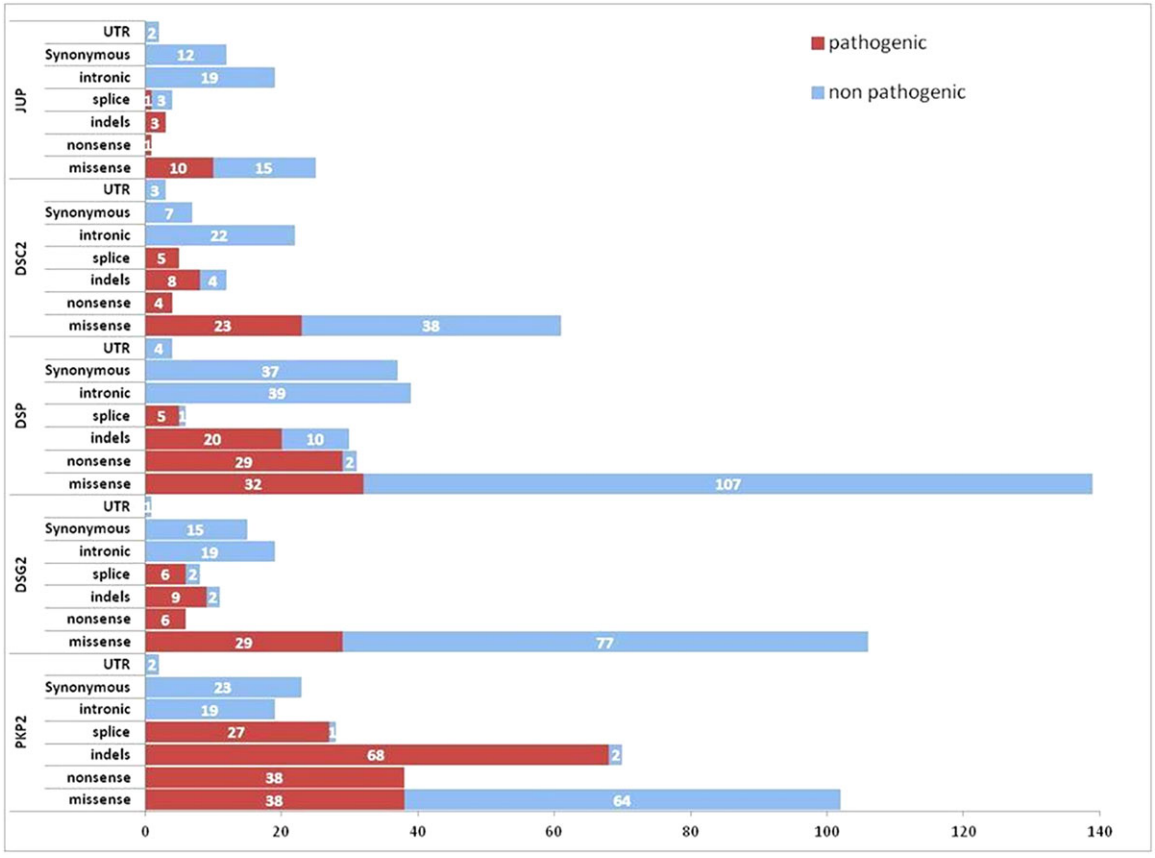

Figure 1. Desmosomal genes variants sorted per type (untranslated region, synonymous, intronic, splice site, insertions/deletions, nonsense, missense) and classification (pathogenic [red/dark], nonpathogenic [blue/light]).

Table 1. Overview of All the Variants in 12 Genes Reported in the Database

Variant	Reported classification			Total
	Pathogenic	Unknown/ unclassified variant	No known pathogenicity	
Insertion/deletion	117	9	24	150
Intronic	-	1	171	172
Missense	159	227	339	725
Nonsense	84	2	6	92
Splice site	49	6	9	64
Synonymous	-	37	148	185
Untranslated region	2	6	30	38
Total	411	288	727	1426

Table 2. Distribution of Variants in Desmosomal Genes

Variant	Reported classification			Total
	Pathogenic	Unknown/ unclassified variant	No known pathogenicity	
Insertion/deletion	108	10	8	126
Intronic	-	1	117	118
Missense	132	213	88	433
Nonsense	78	2	-	80
Splice site	44	5	2	51
Synonymous	-	36	58	94
Untranslated region	-	4	8	12
Total	362	271	281	914

Desmosomal Variants

Of the 914 variants described in desmosomal genes, 362 are reported as pathogenic mutations (approximately 88% of the total pathogenic variants reported). The most frequently mutated gene is *PKP2*, which is responsible for 41.6% of all pathogenic variants, followed by *DSP* (21.2 %), *DSG2* (12.2%), *DSC2* (9.7%), and *JUP* (3.6%). A detailed overview of mutation types in the five desmosomal genes is given in Table 2 and Figure 1.

Extradesmosomal Variants

There are 512 variants in extradesmosomal genes currently reported in the database, of which 49 are currently considered pathogenic variants (approximately 12%). Of these pathogenic variants, two mutations are located in *TGFB3* (0.4% of all extradesmosomal variants), three in *TMEM43* (0.6%), 16 in *LMNA* (3%), four in *PLN* (0.8%), 12 in *DES* (2.3%), 10 in *TTN* (2%), and two in *CTNNA3* (0.4%).

Transforming Growth Factor β3

There are 24 variants currently described in the transforming growth factor β3 (*TGFB3*) gene. So far, only two variants in the 5′ and 3′ UTRs of the gene encoding transforming growth factor β3 have been described as pathogenic [Beffagna et al., 2005]; however, the real pathogenicity of these variants in AC is still debated.

Transmembrane Protein 43

There are 37 recently identified variants included in the database, currently reporting 80 variants in *TMEM43*. Thus far, three pathogenic mutations have been identified in *TMEM43*: two missense (p.P111L; p.S358L) variants and one splice-site (c.705+7G>A) variant. The most common variant reported is the founder mutation p.S358L, located in a highly conserved transmembrane domain of the protein and first identified in families in Newfoundland [Merner et al., 2008].

Lamin A/C

Mutations in the *LMNA* gene, encoding for a constituent of the nuclear lamina, are related to heterogeneous clinical phenotypes, including dilated cardiomyopathy (DCM). In 2012, four *LMNA* pathogenic variants were linked to clinically severe forms of ACM and to one case of sudden death [Quarta et al., 2012]; therefore, this gene was added to the database as associated with ACM. At present, 49 *LMNA* variants have been described in ACM and DCM patients. Of these 49 variants, 16 have been considered pathogenic and only four have been associated with an ACM phenotype.

Phospholamban

Mutations in the *PLN* gene, a regulator of the SERCA pump, are associated both with DCM and ACM. To date, 15 variants have been described, four of them pathogenic: two missense, one nonsense, one in-frame deletion. The in-frame deletion p.R14del is a founder mutation, which was first identified in Dutch patients with ACM and/or DCM and has been reported by seven studies so far [Van der Zwaag et al., 2012].

Desmin

Although *DES* mutations are related to heterogeneous phenotypes of skeletal myopathies and cardiomyopathies, the first comprehensive study on *DES* mutations in patients with ACM dates from 2009 [van Tintelen et al., 2009]. Of 29 *DES* variants described in ACM and *DES*-related patients, only 12 are pathogenic: 10 (84%) of them are missense mutations, one (8%) is an in-frame deletion, and one (8%) is a splice-site mutation. Just four of the 11 known pathogenic *DES* variants have been described in patients with ACM.

Titin

Eight missense mutations in *TTN*, a gene encoding the myofilament *TTN*, were described for the first time in ACM probands in 2011 [Taylor et al., 2011]. Presently, more than 300 variants, eight of them carried by ACM patients, are included in the database. Although the pathogenicity of TTN missense variants is questionable, "radical" mutations' pathogenicity has been well established for DCM [Herman et al., 2012], of which clinical manifestation may resemble ACM.

α T-Catenin

Mutations in *CTNNA3*, a component of area composita, have recently been associated with ACM after the identification of two pathogenic missense mutations in two unrelated patients with ACM [van Hengel et al., 2013]. These novel pathogenic mutations have been included in the database together with common polymorphisms and nonpathogenic variants identified by studies using candidate gene approaches and finding negative results.

Ryanodine Receptor-2

The gene *RYR2* encodes the cardiac ryanodine receptor, involved in calcium homeostasis and excitation-contraction coupling in cardiomyocytes. Mutations in *RYR2* were first identified in a dominant form of ACM associated with catecholaminergic polymorphic ventricular tachycardia [Tiso et al., 2001]. Whether mutations in *RYR2* are causative for ACM is controversial, as confirmed by the original authors (personal communication). Therefore, *RYR2* mutations were not included in the current version of the database.

Variants Reported in ACM Patients

Only 372 of the 411 variants reported as pathogenic mutations in the 12 ACM genes have been described in patients with ACM. Out of the 362 pathogenic variants located in desmosomal genes and the 49 pathogenic variants in extradesmosomal genes, 348 and 24, respectively, have been observed in patients with ACM. Of these, 93.5% of the 372 variants occur specifically in desmosomal genes, whereas only a minority (6.5%) are present in extradesmosomal genes (Fig. 2). These data confirm *PKP2* as the most often mutated gene and emphasize the significance of mutations in extradesmosomal genes in the clinical setting. Often, a specific mutation that has been reported only once is eventually found to be shared by more patients. This highlights the need for an international network/registry that collects patient details in order to perform a correlation between the spectrum of variants and the prevalence in ACM.

Genotype–Phenotype Correlation

The study of the genetic basis of ACM over the last 10 years has advanced our understanding of its pathogenesis and allowed genotype–phenotype correlation for an increasing number of patients.

Mutations in desmosomal genes account for approximately half of the ACM cases including single and multiple mutations carriers, showing one or more than one mutation in the same gene (compound heterozygotes) or else in different genes (digenic heterozygotes). These desmosomal mutations are not rare as they have been identified in 1% [Syrris et al., 2006] to 14% of cases [Den Haan et al., 2009]. The presence of multiple mutations has already been reported to lead to an earlier onset or to a more severe phenotype of the disease [Bauce et al., 2010; Xu et al., 2010]. Recently, Rigato et al. (2013) observed higher disease penetrance in carriers of multiple mutations associated with a more acute phenotypic expression. They showed that this genetic status is the most significant risk factor for malignant arrhythmic events and sudden death, and they demonstrated that the risk is higher in males. In the same

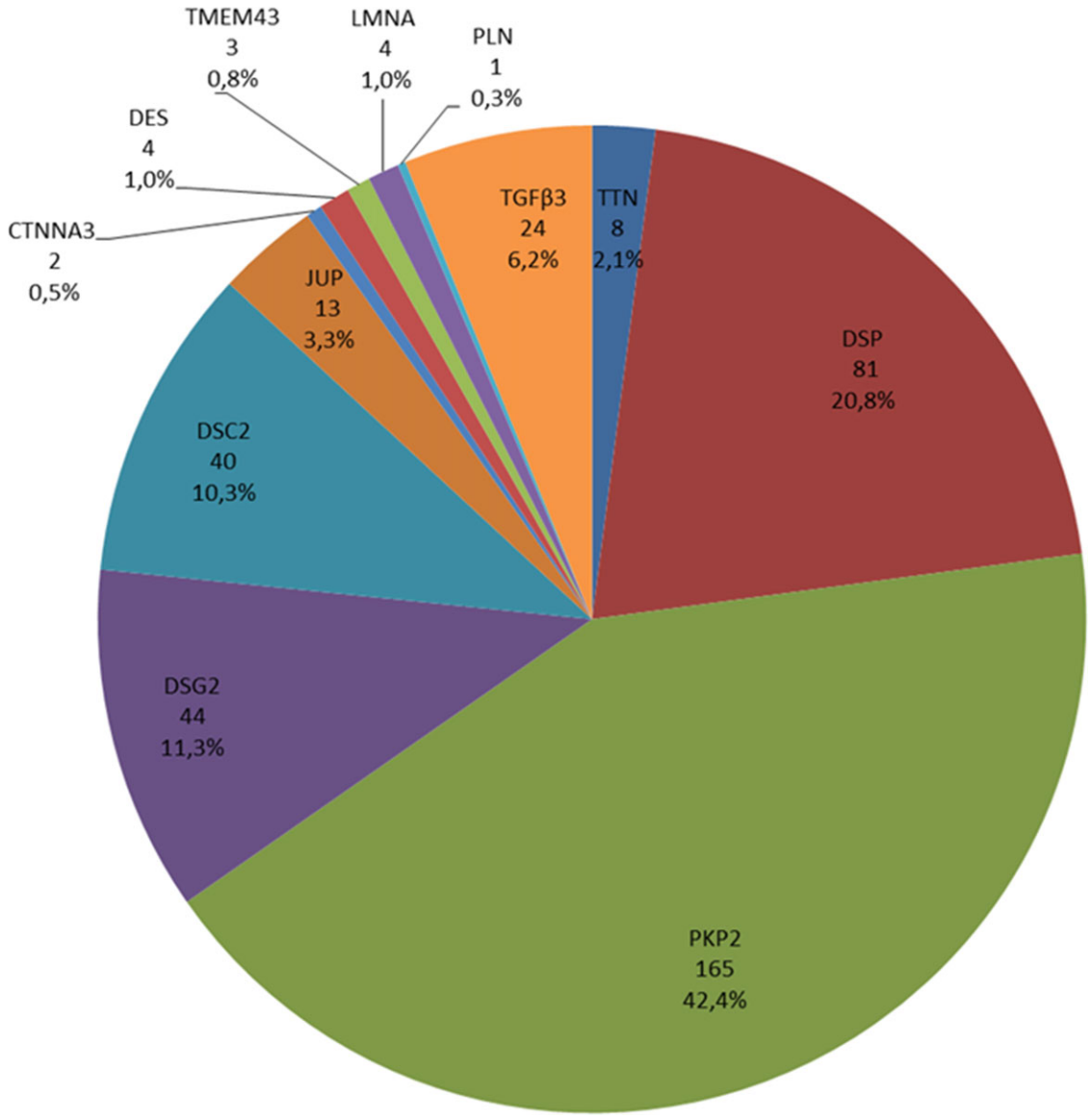

Figure 2. The 372 pathogenic variants (26% of the 1,426 total variants) distributed across 12 ACM-related genes that have been described in patients with ACM.

way, a higher incidence of syncopal events was observed in multiple mutations carriers, especially digenic heterozygous patients, in the Chinese population [Bao et al., 2013]. The authors suggested a gene dose effect on the phenotype presentation by showing a higher number of ventricular tachycardia and syncopal events in multiple mutation carriers compared with single mutation carriers and noncarriers.

The need to correctly evaluate the pathogenic nature of nucleotide variants has been stressed by different studies. [Kapplinger et al., 2011] aimed to determine the background of genetic variations in the five desmosomal ACM-related genes in patients in comparison with healthy controls, and 58% of patients and 16% of controls were found to carry genetic variations that could be classified as "mutations." Specifically, the authors called each variant exclusively observed in ACM patients but absent in a large, ethnically matched, control cohort a "mutation." Radical mutations (insertions–deletions, splice-site mutations, nonsense mutations) believed, with high probability, to be ACM-associated, were found in 0.5% of controls versus 43% of ACM cases. In contrast, the frequency of rare missense mutations was similar in both ACM and control cohorts. Notably, rare missense mutations occurred in residues of *PKP2* and *DSG2* in ACM patients more often than in controls, and these mutations were highly conserved among species and/or critical regions for desmosome formation of DSP and DSG2 (N-terminal domain). The authors concluded that the type and localization of specific mutations might have an equivalent role in explaining variable expressivity of ACM in families, as previously demonstrated for compound and digenic heterozygosity [Kapplinger et al., 2011].

The progression of LV involvement ACM in a clinically overlapping DCM pattern needs to be addressed by studying large ACM cohorts. A prevalent LV involvement has been linked with mutations in *DSG2* [Fressart et al., 2010], *PLN* [Groeneweg et al., 2013], and *DSP* [Bauce et al., 2005; Norman et al., 2005; Rigato et al., 2013]. Defects in the C-terminal of DSP have been associated with early and predominant involvement of the left ventricle and a high occurrence of sudden death [Sen-Chowdhry et al., 2005]. However,

all these studies were carried out in small cohorts. A comprehensive genotype–phenotype study remains too challenging due to the clinical and genetic heterogeneity of ACM and the difficult interpretation of the true pathogenicity of variants. Given that ACM is a rare disease, current clinical genetic correlation studies are too inefficient to be able to determine the probability of missense mutations being pathogenic biomarkers due to the low number of enrolled patients, lack of information regarding cosegregation in their families, and the absence of functional studies. The available data are further biased by the study of symptomatic patients, who are predominantly the individuals catching the attention of clinicians. Likewise, genetic screening is generally performed for main ACM-related genes and, thus far, disease penetrance evaluations are based on single gene studies.

Conclusions and Future Prospects

In the last 10 years, molecular genetic research has led to the identification of a broad mutation spectrum of more than 1,400 variants in 12 ACM-related genes. Although 372 pathogenic mutations have been described in ACM patients, the molecular cause remains unidentified in approximately 50% of ACM patients; other unidentified genes or modifiers could be involved in the pathogenesis of the disease. Next-generation sequencing approaches have detected new causal variants in other cardiomyopathies [Girolami et al., 2014; Schaefer et al., 2014] so their application in ACM also looks promising.

The revised ACM Genetic Variants Database now represents the most comprehensive source of information concerning ACM-related variants, providing an overview of all known genetic information on ACM. These data highlight the complex genetic nature of ACM and the importance of identifying the pathogenic nature of genetic variants and the degree of their contribution to the development of the phenotype. In the clinical setting, this database can be used in conjunction with broad population-based variant databases such as the 1000 Genomes Project (http://www.1000genomes.org/), the Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), and the Exome Aggregation Consortium (http://exac.broadinstitute.org/); these database provide information regarding minor allele frequencies, whereas our database provides literature and in silico predictions information. In combination, these data can be used to correctly pinpoint the pathogenicity of each genetic variant. Database users are invited to submit new variants and patient data and to discuss the classification of recorded variants.

High clinical variability and age-dependent penetrance make the interpretation of a variant's pathogenicity difficult, even though its value is well-recognized for cascade family screening, as well as for the correct classification of "borderline" ACM clinical phenotype. The identification of truly disease-causing variants is extremely helpful to confirm whether a patient suspected of having ACM genuinely does have the disease. However, unless clear pathogenicity is established by cosegregation data and, for example, functional data, variants should be interpreted very cautiously and not be used to "diagnose" ACM. Nonetheless, the major role is in family-cascade screening, where it is used to identify asymptomatic family members carrying the gene defect in order to be given continuous clinical support and to exclude nonmutation-carrying family members from regular cardiological investigation. Indeed, professional recommendations and the role of genetic testing have been described in details by Ackerman et al. (2011).

Finally, the impact of common genetic polymorphisms to the phenotypic variability still needs to be investigated. Recent studies advocate a protective role for these "innocuous" variants [Splawski et al., 2002; Burke et al., 2005] or, on the contrary, an additional risk factor when their effect is added to other nucleotide variants [Bezzina et al., 2013].

Limitations

The database update was focused on providing a comprehensive list of all known variants and information described in all ACM-related genes together with phenotypic information. However, genetic variants in ACM genes have been reported in patients affected by ACM and other inherited cardiomyopathies, thus the distribution of the variants annotated in the database should not be considered as intended only for the ACM population.

Moreover, variant classification is often debatable given the lack of functional studies and/or family cosegregation with phenotype. As a consequence, new data from more recent studies frequently changes the tags of these variants from "disease causing" to "rare polymorphisms."

Acknowledgment

The authors thank Jackie Senior and Kate Mc Intyre for editing the manuscript.

References

Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, Camm AJ, Ellinor PT, Gollob M, Hamilton R, Hershberger RE, Judge DP, et al. 2011. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Europace 13:1077–1109.

Asimaki A, Syrris P, Wichter T, Matthias P, Saffitz JE, McKenna WJ. 2007. A novel dominant mutation in plakoglobin causes arrhythmogenic right ventricular cardiomyopathy. Am J Hum Genet 81:964–973.

Bao J, Wang J, Yao Y, Wang Y, Fan X, Sun K, He DS, Marcus FI, Zhang S, Hui R, Song L. 2013. Correlation of ventricular arrhythmias with genotype in arrhythmogenic right ventricular cardiomyopathy. Circ Cardiovasc Genet 6:552–556.

Basso C, Corrado D, Marcus FI, Nava A, Thiene G. 2009. Arrhythmogenic right ventricular cardiomyopathy. Lancet 373:1289–1300.

Basso C, Corrado D, Thiene G. 2010. Arrhythmogenic right ventricular cardiomyopathy: what's in a name? From a congenital defect (dysplasia) to a genetically determined cardiomyopathy (dystrophy). Am J Cardiol 106:275–277.

Bauce B, Basso C, Rampazzo A, Beffagna G, Daliento L, Frigo G, Malacrida S, Settimo L, Danieli G, Thiene G, Nava A. 2005. Clinical profile of four families with arrhythmogenic right ventricular cardiomyopathy caused by dominant desmoplakin mutations. Eur Heart J 26:1666–1675.

Bauce B, Nava A, Beffagna G, Basso C, De Bortoli M, Rigato I, Mazzotti E, Steriotis A, Marra MP, Towbin JA, Thiene G, Danieli GA, Rampazzo A. 2010. Multiple mutations in desmosomal proteins encoding genes in arrhythmogenic right ventricular cardiomyopathy/dysplasia. Heart Rhythm 7:22–29.

Beffagna G, Occhi G, Nava A, Vitiello L, Ditadi A, Basso C, Bauce B, Carraro G, Thiene G, Towbin JA, Danieli GA, Rampazzo A. 2005. Regulatory mutations in transforming growth factor-beta3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. Cardiovasc Res 65:366–373.

Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud JB, Simonet F, Verkerk AO, Schwartz PJ, Crotti L, Dagradi F, Guicheney P, Fressart V, et al. 2013. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. Nat Genet 45:1044–1049.

Bhonsale A, Groeneweg JA, James CA, Dooijes D, Tichnell C, Jongbloed JD, Murray B, Te Riele AS, vanden Berg MP, Bikker H, Atsma DE, deGroot NM, et al. 2015. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. Eur Heart J.[Epub ahead of print]. DOI: http://dx.doi.org/10.1093/eurheartj/ehu523

Burke A, Creighton W, Mont E, Li L, Hogan S, Kutys R, Fowler D, Virmani R. 2005. Role of SCN5A Y1102 polymorphism in sudden cardiac death in blacks. Circulation 112:798–802.

Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. 2003. Does sports activity enhance the risk of sudden death in adolescents and young adults? J Am Coll Cardiol 42:1959–1963.

Corrado D, Basso C, Schiavon M, Pelliccia A, Thiene G. 2008. Pre-participation screening of young competitive athletes for prevention of sudden cardiac death. J Am Coll Cardiol 52:1981–1989.

De Bortoli M, Beffagna G, Bauce B, Lorenzon A, Smaniotto G, Rigato I, Calore M, Li Mura IE, Basso C, Thiene G, Lanfranchi G, Danieli GA, et al. 2010. The p.A897KfsX4 frameshift variation in desmocollin-2 is not a causative mutation in arrhythmogenic right ventricular cardiomyopathy. Eur J Hum Genet 18:776–782.

Den Dunnen JT, Antonarakis SE. 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat 15:7–12.

Den Haan AD, Tan BY, Zikusoka MN, Lladó LI, Jain R, Daly A, Tichnell C, James C, Amat-Alarcon N, Abraham T, Russell SD, Bluemke DA, et al. 2009. Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circ Cardiovasc Genet 2:428–435.

Fokkema IF, den Dunnen JT, Taschner PE. 2005. LOVD: easy creation of a locus-specific sequence variation database using an "LSDB-in-a-box" approach. Hum Mutat 26:63–68.

Fressart V, Duthoit G, Donal E, Probst V, Deharo JC, Chevalier P, Klug D, Dubourg O, Delacretaz E, Cosnay P, Scanu P, Extramiana F, et al. 2010. Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/cardiomyopathy: spectrum of mutations and clinical impact in practice. Europace 12:861–868.

Gandjbakhch E, Charron P, Fressart V, Lorin de la Grandmaison G, Simon F, Gary F, Vite A, Hainque B, Hidden-Lucet F, Komajda M, Villard E. 2011. Plakophilin 2A is the dominant isoform in human heart tissue: consequences for the genetic screening of arrhythmogenic right ventricular cardiomyopathy. Heart 97:844–849.

Garcia-Gras E, Lombardi R, Giocondo MJ, Willerson JT, Schneider MD, Khoury DS, Marian AJ. 2006. Suppression of canonical Wnt/β-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J Clin Invest 116:2012–2021.

Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, MacRae CA, Peters S, Grossmann KS, et al. 2004. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. Nat Genet 36:1162–1164.

Girolami F, Iascone M, Tomberli B, Bardi S, Benelli M, Marseglia G, Pescucci C, Pezzoli L, Sana ME, Basso C, Marziliano N, Merlini PA, et al. 2014. Novel alpha-actinin 2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: a massively parallel sequencing study. Circ Cardiovasc Genet 7:741–750.

Grantham R. 1974. Amino acid difference formula to help explain protein evolution. Science 185:862–864.

Groeneweg JA, van der Zwaag PA, Jongbloed JD, Cox MG, Vreeker A, de Boer RA, van der Heijden JF, van Veen TA, McKenna WJ, van Tintelen JP, Dooijes D, Hauer RN. 2013. Left-dominant arrhythmogenic cardiomyopathy in a large family: associated desmosomal or nondesmosomal genotype? Heart Rhythm 10:548–559.

Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, et al. 2012. Truncations of titin causing dilated cardiomyopathy. N Engl J Med 366:619–628.

Kapplinger JD, Landstrom AP, Salisbury BA, Callis TE, Pollevick GD, Tester DJ, Cox MG, Bhuiyan Z, Bikker H, Wiesfeld AC, Hauer RN, van Tintelen JP, et al. 2011. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. J Am Coll Cardiol 57:2317–2327.

Liu X, Jian X, Boerwinkle E. 2011. dbNSFP: a lightweight database of human nonsynonymous SNPs and their functional predictions. Hum Mutat 32:894–899.

Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MG, Daubert JP, Fontaine G, Gear K, et al. 2010. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. Circulation 121:1533–1541.

McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G, Camerini F. 1994. 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 71:215–218.

McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. 2000. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet 355:2119–2124.

Merner ND, Hodgkinson KA, Haywood AF, Connors S, French VM, Drenckhahn JD, Kupprion C, Ramadanova K, Thierfelder L, McKenna W, Gallagher B, Morris-Larkin L, et al. 2008. 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 82:809–821.

Ng PC, Henikoff S. 2003. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 31:3812–3814.

Norgett EE, Lucke TW, Bowers B, Munro CS, Leigh IM, Kelsell DP. 2006. Early death from cardiomyopathy in a family with autosomal dominant striate palmoplantar keratoderma and woolly hair associated with a novel insertion mutation in desmoplakin. J Invest Dermatol 126:1651–1654.

Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, Syrris P, Sen-Chowdhry S, Rowland E, Crosby A, McKenna WJ. 2005. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. Circulation 112:636–642.

Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, Lorenzon A, Frigo G, Vettori A, Valente M, Towbin J, Thiene G, Danieli GA, et al. 2006. Mutations in desmoglein-gene are associated with arrhythmogenic right ventricular cardiomyopathy. Circulation 113:1171–1179.

Quarta G, Syrris P, Ashworth M, Jenkins S, Zuborne Alapi K, Morgan J, Muir A, Pantazis A, McKenna WJ, Elliott PM. 2012. Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy. Eur Heart J 33:1128–1136.

Ramensky V, Bork P, Sunyaev S. 2002. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30:3894–3900.

Rampazzo A, Nava A, Malacrida S, Beffagna G, Bauce B, Rossi V, Zimbello R, Simionati B, Basso C, Thiene G, Towbin JA, Danieli GA. 2002. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. Am J Hum Genet 71:1200–1206.

Rasmussen TB, Nissen PH, Palmfeldt J, Gehmlich K, Dalager S, Jensen UB, Kim WY, Heickendorff L, Mølgaard H, Jensen HK, Baandrup UT, Bross P, et al. 2014. Truncating plakophilin-2 mutations in arrhythmogenic cardiomyopathy are associated with protein haploinsufficiency in both myocardium and epidermis. Circ Cardiovasc Genet 7:230–240.

Rigato I, Bauce B, Rampazzo A, Zorzi A, Pilichou K, Mazzotti E, Migliore F, Marra MP, Lorenzon A, De Bortoli M, Calore M, Nava A, et al. 2013. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. Circ Cardiovasc Genet 6:533–542.

Schaefer E, Helms P, Marcellin L, Desprez P, Billaud P, Chanavat V, Rousson R, Millat G. 2014. Next-generation sequencing (NGS) as a fast molecular diagnosis tool for left ventricular noncompaction in an infant with compound mutations in the MYBPC3 gene. Eur J Med Genet 57:129–132.

Sen-Chowdhry S, Syrris P, McKenna WJ. 2005. Desmoplakin disease in arrhythmogenic right ventricular cardiomyopathy: early genotype-phenotype studies. Eur Heart J 26:1582–1584.

Sen-Chowdhry S, Syrris P, Prasad SK, Hughes SE, Merrifield R, Ward D, Pennell DJ, McKenna WJ. 2008. Left-dominant arrhythmogenic cardiomyopathy: an under-recognized clinical entity. J Am Coll Cardiol 52:2175–2187.

Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, Cappuccio FP, Sagnella GA, Kass RS, Keating MT. 2002. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. Science 297:1333–1336.

Syrris P, Ward D, Asimaki A, Sen-Chowdhry S, Ebrahim HY, Evans A, Hitomi N, Norman M, Pantazis A, Shaw AL, Elliott PM, McKenna WJ. 2006. Clinical expression of plakophilin-2 mutations in familial arrhythmogenic right ventricular cardiomyopathy. Circulation 113:356–364.

Syrris P, Ward D, Evans A, Asimaki A, Gandjbackhch E, Sen-Chowdhry S, McKenna WJ. 2006. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. Am J Genet 79:978–984.

Taylor M, Graw S, Sinagra G, Barnes C, Slavov D, Brun F, Pinamonti B, Salcedo EE, Sauer W, Pyxaras S, Anderson B, Simon B, et al. 2011. Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. Circulation 124:876–885.

Thiene G, Nava A, Corrado D, Rossi L, Pennelli N. 1998. Right ventricular cardiomyopathy and sudden death in young people. N Engl J Med 318:129–133.

Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, Larderet G, Brahmbhatt B, Brown K, Bauce B, Muriago M, Basso C, et al. 2001. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum Mol Genet 10:189–194.

van der Zwaag PA, Jongbloed JD, van den Berg MP, van der Smagt JJ, Jongbloed R, Bikker H, Hofstra RM, van Tintelen JP. 2009. A genetic variants database for arrhythmogenic right ventricular dysplasia/cardiomyopathy. Hum Mutat 30:1278–1283.

van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, et al. 2012. 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 14:1199–1207.

van Hengel J, Calore M, Bauce B, Dazzo E, Mazzotti E, De Bortoli M, Lorenzon A, Li Mura IE, Beffagna G, Rigato I, Vleeschouwers M, Tyberghein K, et al. 2013. Mutations in the area composita protein αT-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. Eur Heart J 34:201–210.

van Tintelen JP, Hofstra RM, Wiesfeld AC, van den Berg MP, Hauer RN, Jongbloed JD. 2007. Molecular genetics of arrhythmogenic right ventricular cardiomyopathy: emerging horizon? Curr Opin Cardiol 22:185–192.

van Tintelen JP, van Gelder IC, Asimaki A, Suurmeijer AJ, Wiesfeld AC, Jongbloed JD, van den Wijngaard A, Kuks JB, Spaendonck-Zwarts KY, Notermans N, Boven L, van den Heuvel F, et al. 2009. Severe cardiac phenotype with right ventricular predominance in a large cohort of patients with a single missense mutation in the DES gene. Heart Rhythm 6:1574–1583.

Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PE. 2008. Improving sequence variant descriptions in mutation databases and literature using the Mutalyzersequence variation nomenclature checker. Hum Mutat 29:6–13.

Xu T, Yang Z, Vatta M, Rampazzo A, Beffagna G, Pilichou K, Scherer SE, Saffitz J, Kravitz J, Zareba W, Danieli GA, Lorenzon A, et al. 2010. Compound and digenic heterozygosity contributes to arrhythmogenic right ventricular cardiomyopathy. J Am Coll Cardiol 55:587–597.