Arrhythmogenic RV Cardiomyopathy/Dysplasia

Recent Advances

Springer
This book covers all the recent research highlights of arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), a recently discovered heart muscle disease which is a major threat to the life of affected young people. It summarizes nearly 25 years of investigation on the etiology, genetics, pathology, clinical features, diagnosis, and treatment of ARVC/D. In particular, a 5-year research program supported by grants from both the European Community and the National Heart, Lung and Blood Institutes has contributed to the discovery of seven disease-causing genes, thus opening new avenues for the early identification of affected patients and prevention of sudden death.

A Workshop was held in Venice, Italy, October 3, 2005, as part of the Venice Arrhythmia Meeting, where the European and American investigators presented and discussed several major achievements which are now reported in this book. As a result of these coordinated efforts, great advances have been made in the recognition and understanding of the disease, which are summarized in this book.

Molecular genetics has established this cardiomyopathy as a familial disorder caused by mutation of the genes that encode cell junction proteins, resulting in defective cellular adhesion with specific immunohistochemical and ultrastructural alterations. Remodelling at the intercalated disk may trigger a cascade of events including apoptotic cell death, fibrofatty replacement, and electrical instability. The left ventricle may be involved early in the course of some genetic types of this disease. This finding alters the traditional concept of a disease confined to the right ventricle.

Genetic screening can detect symptomatic carriers in the early stage of the disease. Magnetic resonance imaging with gadolinium permits in vivo identification of fibrous tissue. Electroanatomic mapping can reveal areas of fibrofatty replacement of the right ventricular myocardium. The implantable cardioverter defibrillator has been shown to prevent sudden cardiac death. Whether it should be implanted for secondary as well as for primary prevention is still controversial.

Screening prior to participation in competitive sports has been found to be effective for the identification of subjects at risk and is life-saving by disqualifying affected individuals and avoiding competitive type effort. Sudden death of young athletes declined fivefold after implementation of preparticipation screening, mainly due to identification of ARVC/D.

A panel of experts have contributed to writing this monograph, which will be an essential reference for clinicians and scholars in human genetics, pathology, cardiology, and radiology as well as in forensic and sports medicine.

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Andrea Nava is Professor of Cardiology at the University of Padua, Italy. He first recognized the familial occurrence of ARVC/D in the Veneto Region and has made major contributions to the early identification, discovery of defective genes and phenotypic expression of the disease. He is in charge of the family screening program that consists of clinical and genetic investigations.

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Gaetano Thiene is Professor of Cardiovascular Pathology and Director of the Institute of Pathological Anatomy at the University of Padua, Italy. He was trained both in Cardiology and Pathology. He first reported ARVC/D as a major cause of sudden, unexpected death in the young and in athletes and established diagnostic criteria for endomyocardial biopsy. He coordinates the European study and has implemented the European Registry of ARVC/D supported by the European Commission.
The first monograph on arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) was published 10 years ago [1]. Since then, there have been major advancements in the basic knowledge of the disease as well as a better understanding of the diagnosis and treatment. A workshop was held in Venice, Italy on October 3, 2005, where research on various aspects of this disease, both biological and clinical was presented.

This book has assembled contributions in the form of a monograph rather than as the publication of Proceedings. In addition, some topics were added in a similar format to that of the first monograph [1], which followed a meeting on ARVC/D held in Paris in 1996.

In the last 10 years our understanding of this disease has been impressive. This is the logical consequence of a research strategy with clear goals.

At the turn of the millennium, following a series of meetings of experts from both sides of the Atlantic, it became evident that we had to merge the expertise of scientists and clinicians attracted by the mystery of ARVC/D and its devastating physical and social consequences into an “army” for the fight against the disease.

An International Registry was considered mandatory in order to collect study material and concentrate efforts on this rare disease [2].

It was then decided to apply for grants to the European Commission (EC) and to the National Institute of Health (NIH). Two teams were created, one in Europe coordinated by Gaetano Thiene [3] and one in North America coordinated by Frank Marcus [4]. Utilizing a similar database and having some Core Laboratories in common, the two projects were initiated. The structure was somewhat different: the European Registry enrolled patients who were both previously diagnosed as well as those with the recent onset of symptoms, whereas the North American Registry enrolled only newly diagnosed patients. Guidelines for diagnostic criteria and protocols were implemented. Genetic investigation was an integral part of both studies. Fortunately, the two projects were approved and funded for 5 years, thus allowing the start of a major interdisciplinary study of ARVC/D. The results exceeded our best expectations, resulting in numerous important publications in well-recognized cardiovascular journals. In this monograph the advances in our knowledge will be summarized in didactic presentations.

A brief overview of the major advances is as follows:

1. The genetic background of this hereditary-familial, monogenic disease has been clarified. Despite genetic heterogeneity with rare variants, it has been demonstrated that both autosomal and recessive forms are due to defects of genes encoding desmosomal proteins of the intercalated disc: plakoglobin [5], desmoplakin, [6] plakophilin [7], desmoglein [8], and desmocollin [9]. This explains why the disease is now called a desmosomal cardiomyopathy [10, 11]. To date, seven disease genes have been identified during the course of the EC and NIH research projects – an unbelievable achievement. Genetic screening is now feasible for the detection of gene carriers and early clinical diagnosis [12].

2. The pathological substrate of the disease has been clarified at the ultrastructural level with evidence of remodeling of intercalated disc (widening of intercellular space with abnormal desmosomes) [13]. These structural abnormalities can potentially trigger a cascade of events following parietal stretch (apoptotic cell death, fibrofatty replacement, electrical instability). There is now evidence that the left ventricle is also involved. In some variants of the disease it has been shown that the left ventricle is primarily affected, thus expanding the previous concept that the disease is confined to the right ventricle [14-16]. The diagnostic role of endomyocardial biopsy has been improved by updating morphometric parameters.

3. Both the advantages and limitations of imaging modalities have been clarified and are beginning to be subjected to quantitative analysis. Magnetic resonance imaging is being expanded in scope not only to study the morphology and dysfunction of the ventricles, but also to identify tissue composition, particularly fibrosis utilizing gadolinium late enhancement.
4. With regard to advances in the invasive diagnostic techniques, electroanatomic mapping is being evaluated to detect areas of decreased electrical activity, which has been found to correspond to diffuse segmental fibrofatty atrophy [17]. This may be important not only for the diagnosis of the disease, but also for the identification of areas that may be the target for catheter ablation. Nevertheless, the precise diagnostic role of electroanatomic mapping needs further clarification. Also the role of ablation for treatment of ventricular arrhythmias needs to be reinvestigated utilizing the technique of electroanatomic mapping.

5. It is indisputable that the implantable cardioverter defibrillator (ICD) has been lifesaving in patients with ARVC/D who have malignant ventricular arrhythmias including hemodynamically unstable ventricular tachycardia [18, 19]. The efficacy of the ICD in this setting is astonishing and recalls the miracle of the resuscitation of Lazarus, friend of Jesus Christ, from the tomb, painted by Giotto in the Scrovegni Chapel in Padua, where Jesus said “veni foras, Lazare” (John’s Gospel chapter 11, line 43-44) (Fig. 1). Whether the ICD should be employed as primary as well as for secondary prevention is still controversial.

6. Primary prevention of sudden death in the young and in athletes from ARVC/D may be possible by lifestyle changes, particularly avoiding participation in vigorous and certainly in competitive sports. Preparticipation screening for those who engage in competitive sports has been shown to be highly effective for identification of the individuals at risk, including those with ARVC/D. In Italy, sudden death of young athletes declined five fold after the implementation of preparticipation screening primarily due to identification of cardiomyopathies [20]. The recognition of ARVC/D as a disease entity, as well as the utilization of strict diagnostic criteria [21], accounts for this important achievement.

7. Recent developments from in vitro and in vivo analyses of mutated proteins in transgenic mice are providing mechanistic explanations, with targeted therapies on the horizon for affected patients [22-25]. These studies suggest that sudden cardiac death in patients with ARVC/D may be prevented by different approaches (Fig. 2):

**Fig. 1** • The resuscitation of Lazarus, painted by Giotto in the Scrovegni Chapel in Padua (C. 1304), is compared to the rescue from cardiac arrest by ICD; ecg tracing, courtesy of Dr. Moss

**Fig. 2** • Diagram illustrating the various levels of interventions for sudden death prevention in ARVC/D
1. Avoiding the trigger, such as strenuous exercise in patients who are identified as having the disease by clinical or genetic screening;
2. Preventing life-threatening arrhythmias using drug therapy or ablation;
3. ICD implantation, an extremely effective therapy to treat life-threatening ventricular arrhythmias that can result in cardiac arrest.

The selection of appropriate therapy for the individual patient awaits further investigation.

All the above-mentioned therapeutic and preventive measures are palliative, not curative. The definitive cure of the disease is still elusive. Cardiac transplantation is employed to treat end-stage cardiac failure or for refractory electrical instability, but this therapy is not without problems, particularly the need to prevent acute rejection as well as allograft vasculopathy. Prevention of myocyte apoptotic death, inflammation, and fibrofatty replacement, the basic mechanisms of myocardial injury and repair, will require understanding the pathogenetic mechanisms of ARVC/D. At present, replacement of the defective genes (gene therapy) is theoretically possible only by disease identification at the early embryonic stage, with preimplantation genetic diagnosis, an issue that raises major ethical questions [26].

Thus, although the genetic basis of ARVC/D has been largely clarified, there is much work to be done to better understand the cell biology of the disease, to know how to slow disease progression, and ultimately to prevent disease transmission.

Finally, some historical notes. It was in 1961 that Professor Sergio Dalla Volta from the University of Padua reported cases with “auricularization of the right ventricular pressure” with an amazing fibrofatty, nonischemic pathology of the right ventricle [27]. One of those patients survived until 1995, and underwent transplantation due to end-stage cardiac failure. Interestingly, the heart specimen showed severe right ventricular enlargement with a nearly normal left ventricle (Fig. 3).

Attention was focused on the disease following the clinical description of ARVC/D by Marcus et al. in 1982 [28]. In 1988 Nava et al. elucidated the pattern of transmission in family members [29], and Thiene et al. discovered the disease as a previously unrecognized and important cause of sudden death in the young [30]. However, the first description of the disease can be traced to the book De Motu Cordis et Aneurismatibus by Giovanni Maria Lancisi, published in 1736. (Dr. Arnold Katz, personal communication) (Fig. 4). In the 5th chapter of this book, paragraph 47, Lancisi reported a family with disease recurrence in four generations. Signs and symptoms were palpitations, heart failure, dilatation and aneurysms of the right ventricle, and sudden death, all features consistent with the current diagnostic criteria of the disease. Thus, we know that the disease is not new, only newly investigated. Nevertheless, tremendous strides have been made in recognition and understanding of the disease which are summarized in this monograph.
References

Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a progressive cardiomyopathy with different clinical-pathological patterns: (a) “silent” cardiomyopathic abnormalities localized in the right ventricle in asymptomatic victims of sudden death; (b) “overt” disease characterized by segmental or global right ventricular structural changes, often associated with histological evidence of left ventricular involvement and underlying symptomatic ventricular arrhythmias; and (c) “end-stage” biventricular cardiomyopathy mimicking dilated cardiomyopathy, leading to progressive heart failure and eventually requiring heart transplantation [1]. A scoring system to establish the diagnosis of ARVC/D has been developed on the basis of the presence of major and minor criteria encompassing structural, histological, electrocardiographic, arrhythmic, and genetic features of the disease [2].

The clinical manifestations of the disease mostly occur between the second and fourth decade of life; they include electrocardiographic depolarization/repolarization changes, arrhythmias of right ventricular origin, and structural and functional abnormalities of the right ventricle. In ARVC/D, the myocardi um of right ventricular free wall is partially or almost entirely replaced by fibro-fatty tissue [3-5], and involves the epicardium, midmyocardium, and usually spares the subendocardium. The anterior right ventricular outflow tract, the apex, and the inferior-posterior wall are primarily involved [6]. Ventricular tachycardias are thought to be due to re-entrant mechanism, due to slow conduction within the myocardiocytes embedded in fibrous tissue and fat.

Familial occurrence of ARVC/D is rather common. Evidence has been found for autosomal dominant inheritance with variable penetrance in about 50% of cases [7].

ARVC/D has been reported in different human populations [8-10], although it is not known if the disease is equally prevalent in different geographical areas. Ten years ago, we estimated that prevalence rate of ARVC/D in the Veneto region (northeast Italy) is about 6:10000 [11]. This figure is probably low, because many cases escape diagnosis. In Italy, 12.5%-25% of sudden deaths in athletes under the age of 35 are due to undiagnosed ARVC/D [12].

Two international registries have been established; one in North America and one in Europe, to determine the clinical, pathological, and genetic features of ARVC/D, to validate diagnostic criteria, and to define strategies for disease management and sudden death prevention [13-15].

Since identification of the first ARVC/D locus in 1994 by Rampazzo et al. [11], ten loci have been detected [11, 16-24], but only five disease genes have been identified [22-26] (Table 1.1).

Disease Genes

The first identified ARVC/D gene in a dominant form was ryanodine receptor-2, involved in ARVD2 [25]. In ARVD2, there is fibro-fatty substitution of the myocardial tissue, though much less pronounced than in the typical ARVC/D. The distinctive feature of this form is the presence of polymorphic, effort-induced arrhythmias. RYR2 is one of the largest human genes (105 exons), encoding a 565Kda protein located in the membrane of smooth sarcoplasmic reticulum. The homo-tetrameric structure known as cardiac ryanodine receptor plays a pivotal role in intracellular calcium homeostasis and excitation-contraction coupling in cardiomyocytes [27, 28]. All RYR2 mutations detected in ARVD2 patients were missense resulting in substitutions involving amino acids highly conserved through evolution in critical domains of the protein [25, 29].

Mutations in the human RYR2 gene have also been associated with catecholaminergic polymorphic ventricular tachycardia (CPVT; OMIM 604772) [30, 31] and familial polymorphic ventricular tachycardia (FPVT); OMIM 604772) [32, 33]. Putative pathogenic mutations in RYR2 have been reported in
20 out 240 patients referred for long-QT syndrome genetic testing [34].

All RYR2 mutations described to date cluster in three specific domains: the N-terminal amino-acid residues 176-433, the centrally located residues 2246-2504, and the C-terminal residues 3778-4959. Detection of RYR2 mutations in both ARVD2 and CPVT patients raises the question of the possible existence of a single genetic defect, different phenotypes of which might be simply due to variable expression and incomplete penetrance. Both ARVD2 and CPVT-RyR2 missense mutations would alter the ability of the calcium channel to remain closed. Intense adrenergic stimulation due to emotional or physical stress can lead to calcium overload, thus triggering severe arrhythmias. The functional role of mutations R176Q, L433P, N2386I, and T2504M, previously detected in ARVD2 patients [25], was recently investigated [35]. RyR2 mutants N2386I and R176Q/T2504M exhibited enhanced sensitivity to caffeine activation and increased Ca2+ release, in agreement with the current hypothesis that defective RyR2 causes Ca2+ leak. In contrast, RyR2 L433P mutation showed reduced response to caffeine activation. This mutation might be interpreted as a “loss-of-function.” Therefore, RyR2 mutations might be either “gain-of-function” or “loss-of-function,” thus suggesting heterogeneity in functional consequences of RyR2 mutations. Even with this additional information, the question of whether ARVD2 and CPVT are different diseases due to different mutations of the RYR2 gene still remains unsettled.

The first disease gene linked to autosomal dominant ARVC/D showing typical right ventricular phenotype was Desmoplakin (DSP) [22]. In 2002, genome scan in a family with ARVC/D indicated a linkage with a region of chromosome 6 short arm including DSP gene. DNA sequencing of all DSP exons in the affected persons of this family revealed a missense mutation in exon 7 (C1176G; AGC→AGG) (Fig. 1.1). The involved amino acid (Ser299Arg) is at the center of a coiled, charged region, separating the two short helices of DSP subdomain Z. The amino acid substitution suppresses a putative phosphorylation site, which, on the other hand, is fully conserved in related proteins belonging to the same family. This mutation is thought to disrupt a protein kinase C phosphorylation site which is involved in plakoglobin binding and in clustering of desmosomal cadherin-plakoglobin complexes. Desmoplakin, together with plakoglobin, anchors to desmosomal cadherins, forming an ordered array of nontransmembrane proteins, which bind to keratin intermediate filaments (Fig. 1.2) [36]. The primary structure of desmoplakin contains three functional domains: the N-terminal, which binds to the desmosome via connection with plakoglobin and plakophilin; the rod segment, which is predicted to form a dimeric coil; and the C-terminal domain, which binds intermediate filaments [37]. Alternative splicing of the protein produces two isoforms, desmoplakin I and desmoplakin II. The cDNAs encoding these two highly related proteins differ in a 1.8 Kbase sequence that is missing in DSPII, most likely due to differential splicing of a longer transcript [38].
Mutations in the desmoplakin gene have been shown to be responsible for some cases of an autosomal dominant skin disorder (striate palmoplantar keratoderma) without cardiac involvement [39-41]; a dominant form of ARVC/D without skin disease [22]; an autosomal recessive condition characterized by dilated cardiomyopathy, woolly hair, and keratoderma (so-called Carvajal syndrome) [42], an autosomal recessive condition characterized by ARVC/D, woolly hair, and keratoderma [43] and a left-sided ARVC/D named arrhythmogenic left ventricular cardiomyopathy (ALVC) [44].

Mutations in DSP gene were detected in different families: they include twelve missense, two nonsense, and two splice-site mutations. In our experience, DSP mutations may account for a considerable number of ARVC/D cases.

In 2004, Gerull et al. [23] selected plakophilin-2 (PKP2) as candidate gene because a homozygous deletion caused a lethal cardiac defect in mice [45]. PKP2 gene encodes plakophilin-2, an essential protein of the cardiac desmosome. By sequencing all 14 exons of the PKP2 gene, including flanking intronic splice sequences, the authors identified 25 different heterozygous mutations (twelve insertion-deletion, six nonsense, four missense, and three splice site mutations) in 32 of 120 unrelated ARVC/D probands [23]. Plakophilin-2 is an armadillo-related protein, located in the outer dense plaque of desmosomes. It links desmosomal cadherins to desmoplakin and the intermediate filament system (Fig. 1.2). Plakophilins are also present in the nucleus, where they may play a role in transcriptional regulation [46]. Gerull et al. [23] speculated that lack of plakophilin-2 or incorporation of mutant plakophilin-2 in the cardiac desmosomes might impair cell-cell contacts and, as a consequence,
might disrupt association between adjacent cardiomyocytes.

The frequency of PKP2 mutations among ARVC/D cases ranged from 11% to 43% in different studies [47-49]. These differences might be attributed to different geographical origin of cases or simply to selection bias.

Recently, we decided to shift from linkage studies in ARVC/D families to a candidate gene approach. Thus, we screened different genes encoding desmosomal proteins. When analyzing DSG2 gene (Desmoglein-2, the only isoform expressed in cardiac myocytes), we detected nine heterozygous mutations in eight of 50 unrelated individuals with ARVC/D which proved negative for mutations of DSP, PKP2, and TGFβ3 genes [24]. Among these, five were missense mutations, two were insertion-deletions, one was a nonsense and one was a splice site mutation; one patient had two different DSG2 mutations (compound heterozygote). Endomyocardial biopsy, obtained from five patients, showed intercalated disc paleness, decreased desmosome number, and intercellular gap widening [24]. Mutations in DSG2 gene were also detected in an independent study [50]. It is interesting to note that, in this study, there was one patient with compound-heterozygous mutations in DSG2 (Fig. 1.3).

In 2005, our group identified the gene involved in ARVD1 [26]. The large critical interval for ARVD1 included 40 known genes; five of them (POMT2, KIAA0759, KIAA1036, C14orf4, and TAIL1) were unsuccessfully screened for pathogenic ARVC/D mutations [51, 52]. Among genes mapped to the ARVD1 critical region and expressed in myocardium, transforming growth factor-beta3 (TGFβ3) appeared to be a good candidate, since it encodes a cytokine stimulating fibrosis and modulating cell adhesion. After previous analyses failed to detect any mutation in the coding region of this gene, mutation screening was extended to the promoter and untranslated regions (UTRs). A nucleotide substitution (c.-36G>A) in 5′ UTR of TGFβ3 gene was detected in all affected subjects belonging to a large ARVD1 family. After the investigation was extended to 30 unrelated ARVC/D index patients, an additional mutation (c.1723C>T) was identified in the 3′ UTR of one proband. In vitro expression assays of constructs containing the mutations showed that mutated UTRs were twofold more active than wild type [26].

TGFβ3 is a member of the transforming growth factor superfamily, which includes a diverse range of proteins regulating many different physiological processes. TGFβ1, -β2, and -β3 are the prototype of the TGFβ superfamily. They inhibit proliferation in most types of cells and induce apoptosis of epithelial cells. Conversely, they stimulate mesenchymal cells to proliferate and produce extracellular matrix and they induce a fibrotic response in various tissues in vivo.

Finding TGFβ3 mutations associated with ARVC/D is very interesting, since it is well established that TGFβs stimulate mesenchymal cells to proliferate and to produce extracellular matrix components. Since mutations in UTRs of the TGFβ3 gene, detected in ARVC/D, showed enhanced gene expression in vitro, it is likely that they could promote myocardial fibrosis in vivo. Myocardial fibrosis may disrupt electrical and mechanical behavior of myocardium and extracellular matrix abnormalities may predispose to reentrant ventricular arrhythmias. In agreement with this hypothesis, endomyocardial biopsy in the two probands in which TGFβ3 UTR mutations were detected showed extensive replacement-type fibrosis. Moreover, it has been shown that TGFβs modulate expression of genes encoding desmosomal proteins in different cell types. CDNA microarray analysis, performed on RNA from cardiac fibroblasts incubated in the presence or in the absence of exogenous TGFβs, revealed increased expression of different genes, including plakoglobin [53]. Yoshida et al. [54] reported that TGFβ1 exposure of cultured airway epithelial cells increases the content of desmoplakins I and II. This suggests that regulation of cell–cell junctional complexes may be an important effect of TGFβs. Therefore, overexpression of TGFβ3, caused by UTRs mutations, might affect cell-to-cell junction stability, thus leading to disease expression similar to that observed in ARVC/D due to mutations of genes encoding desmosomal proteins.

**Fig. 1.3** • Pedigree of the proband carrying two DSG2 mutations (988G>A, 1881-2A>G). Hatched symbol represents an individual of unknown disease status. Presence (+) or absence (−) of the DSG2 mutation is indicated.
Desmosomes are important cell-cell adhesion junctions, predominantly found in the epidermis and heart. They couple cytoskeletal elements to plasma membrane at cell-cell or cell-substrate adhesions. Whereas adherens junctions are linked with microfilaments at cell-cell interfaces, desmosomes anchor stress-bearing intermediate filaments at sites of strong intercellular adhesion. The resulting scaffold plays a key role in providing mechanical integrity to tissues such as epidermis and heart, which experience mechanical stress. Desmosomes include proteins from at least three distinct gene families: cadherins, armadillo proteins, and plakins (Fig. 1.2). Desmosomal cadherins include desmogleins and desmocollins; members of both subfamilies are single-pass transmembrane glycoproteins, mediating Ca2+-dependent cell-cell adhesion. Armadillo proteins include plakoglobin and plakophilins (PKP1-3). The plakin family proteins include desmoplakin, plectin, and the cell envelope proteins envoplakin and periplakin. Desmoplakin (involved in ARVD8), plakophilin-2 (involved in ARVD9), desmoglein-2 (involved in ARVD10), and plakoglobin (involved in Naxos syndrome, the autosomal recessive form of ARVC/D) are desmosomal proteins. Based on present evidence we may conclude that different defects in proteins of desmosomal complex lead to ARVC/D. Therefore, additional components of the desmosomal complex may be targets for pathogenic mutations leading to ARVC/D.

Molecular Pathogenesis

The reported involvement of different desmosomal proteins in ARVC/D and the discovery that some RYR2 mutations may produce ARVD2 leads us to propose a comprehensive hypothesis on the molecular pathogenesis of ARVC/D [22]. According to this hypothesis, the predilitation of involvement of the right ventricle in ARVC/D might be due to greater dilatation and thinning of its wall, in comparison with the left ventricular free wall. Possibly, defective proteins in cardiac desmosomes might impair cell-to-cell contacts and, hence, might affect the response of ventricular myocardium to mechanical stretch. This alteration would occur preferably in myocardial areas subjected to high strain, like the right ventricular outflow tract, the apex, and subtricuspid areas.

According to present knowledge, mechanical forces applied to adherens junctions activate stretch-sensitive calcium channels via cadherins’ mechanical intracellular signaling [55]. Data on stretch-activated channels in ventricular cardiomyocytes point to the relevance of these channels in transduction of mechanical forces into a cellular electrochemical signal, via increase of intracellular calcium concentration [56-58].

Volume overload of the right ventricle in a patient with genetically defective intercellular junctions (as in case of mutant plakoglobin, desmoplakin, plakophilin, desmoglein, or TGFβ3) would produce unusual stretching resulting in excessive calcium load. Stretching of cardiomyocytes is known to modulate the elementary calcium release process from ryanodine receptor release channels [59]. Therefore, a genetically impaired response to mechanical stress might adversely affect intracellular calcium concentration and excitation-contraction coupling, thus producing arrhythmias. On the other hand, volume overload of the right ventricle in carriers of RYR2 mutations would cause calcium overload, because of defective Ca++ homeostasis. The existence of a dominant form of ARVC/D due to RyR2 mutations supports the hypothesis of a key pathogenic role of intracellular calcium overload in the molecular pathogenesis of the disease.

Mutation Screening

We performed mutation screening in 90 unrelated probands fulfilling the International ARVC/D Task Force criteria; the screening by DHPLC and subsequent DNA sequencing involved coding sequences of known ARVC/D genes. Plakophilin-2 was involved in 21% of cases, desmoplakin-2 in 20%, desmoglein-2 in 11%, and TGFβ3 in 2% (unpublished results). In 46% of cases no mutation was detected. This is not surprising, since in 50% of reported ARVC/D loci (ARVD3, ARVD4, ARVD5, ARVD6, ARVD7) the involved gene has not been identified.

In our series of patients screened for mutations, eight compound heterozygotes were detected, suggesting that this condition may be more frequent than expected among ARVC/D patients. It is difficult to establish whether all of these cases are compound heterozygotes for pathogenic mutations, since it is almost impossible to discriminate between a rare variant with pathogenic effect and a rare DNA polymorphism.

Present knowledge on the molecular genetics of the dominant forms of ARVC/D may permit detection of asymptomatic carriers in families with ARVC/D. However, it must be noted that genotype-phenotype correlations may be established only for clearly pathogenic mutations (i.e., nonsense, frameshifts, splice-site
mutations with evidence of modified RNA length, etc.) and once all genes reportedly involved in ARVC/D would have been screened.

Mutation screening is time- and effort-consuming. Routine methods (direct sequencing of coding segments or DHPLC followed by DNA sequencing) reach about 98% detection rate due to undetectable mutations in intronic sequences or in regulatory elements, or unexpected large deletions. Moreover, the presence of compound heterozygotes carrying one mutation in a known ARVD gene and one mutation in a gene still unknown might produce misleading results. Therefore, genetic assessment of asymptomatic relatives of ARVC/D patients still poses several technical, clinical, and ethical problems. However, identification of additional genes involved in dominant forms of ARVC/D and collection of data regarding pathogenic mutations in known genes will provide information to establish safe protocols for genetic investigation in families with ARVC/D, genetic counseling, and risk assessment.

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P.S. Following submission of this manuscript, mutations in desmocollin-2 gene, encoding a desmosomal cadherin, have been reported to be associated with ARVC/D [60-62].

References