

CARDIOVASCULAR GENOMIC MEDICINE

Role of Genetic Analysis in the Management of Patients With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a recognized cause of sudden cardiac death, which may be prevented by timely detection and intervention. Clinical diagnosis of ARVC is fraught with difficulties in both index cases and relatives owing to the nonspecific nature of associated features, diverse phenotypic manifestations, and a lack of conspicuous abnormalities in the early, "concealed" phase. During the past 7 years, researchers have isolated causative mutations in several components of the desmosome, shedding light on the molecular mechanisms underlying the disease and offering the promise of genetic testing as a diagnostic tool. Sequence analysis is likely to be the mainstay of genotyping in ARVC because of marked allelic heterogeneity, frequent "private" mutations, and digenicity in a minority, highlighting the importance of comprehensive genetic screening. The main technical obstacle to implementation of genotyping in clinical practice will be the prohibitive costs of performing sequence analysis of a genomic region exceeding 40 kb. Nevertheless, the success rate of genotyping in ARVC is of the order of 40%, and key clinical applications include confirmatory testing of index cases to facilitate interpretation of borderline investigations and cascade screening of families. The latter is particularly attractive in ARVC, because age-related penetrance otherwise demands lifelong clinical reassessment of extended families. A role for genetic analysis in prognostication is more tenuous at present, but increasing identification of individuals with early and familial disease underscores the need for a definitive risk stratification algorithm in this population. (*J Am Coll Cardiol* 2007;50:1813-21) © 2007 by the American College of Cardiology Foundation

Perhaps more so than any other inherited cardiovascular disorder, arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) has undergone a paradigm shift in the 30 years since its name was first coined (1). The salient structural finding of fibrofatty replacement of the right ventricular myocardium was originally thought to occur as a developmental anomaly, leading to the use of the term dysplasia. Key clinical and pathological studies subsequently led to reclassification of the disease as a cardiomyopathy with a familial preponderance (2-4). Involvement of the left ventricle is now commonly recognized (5-7). The most significant advance of the current decade, however, has been identification of causative mutations in components of the desmosome (Table 1), the specialized intercellular junctions that anchor intermediate filaments to the cytoplasmic mem-

brane in adjoining cells, thereby forming a supportive network (Fig. 1) (8-30). Besides offering the promise of deoxyribonucleic acid-based diagnosis, the gene identification studies spurred the desmosomal model of ARVD/C (8), which has provided the first glimpse into the molecular underpinning of the disease.

Desmosomes are most prevalent in tissues exposed to frictional and shear stress, such as the myocardium and epithelium, where they play a key role in imparting mechanical strength, both via cell-cell adhesion and through transmission of force between the junctional complex and the intermediate filaments in the cytoskeleton (Fig. 1) (8). The underlying premise of the model is that defects in components of desmosomes may compromise their function, predisposing to myocyte detachment and death. Significant myocyte loss may be accompanied by an inflammatory response. The regenerative capacity of the myocardium is limited, necessitating repair by fibrofatty substitution. Rather than being a gradual and continuous process, extension of the disease to previously unaffected regions of the myocardium may occur during intermittent "hot phases," periodic exacerbations of an otherwise-quiescent disease, sometimes

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Abbreviations and Acronyms

ARVD/C = arrhythmogenic right ventricular dysplasia/cardiomyopathy

ICD = implantable cardioverter-defibrillator

PKP = plakophilin

TGF = transforming growth factor

accompanied by a transient increase in arrhythmic activity. It is probable that the majority of “hot phases” are clinically silent, whereas others are accompanied by mild symptomatic flare-ups; rarely, however, the outcome may be an unheralded arrhythmic event in an individual with previously stable disease.

Of the 2 additional genes linked with ARVD/C, the cardiac ryanodine receptor gene RyR2 causes a distinct clinical entity, ARVD2, characterized by juvenile sudden cardiac death and effort-induced polymorphic ventricular tachycardia (31). The typical electrocardiographic features of ARVD/C do not occur, and structural abnormalities are limited to mild regional wall motion abnormalities of the right ventricle, with preservation of global systolic function. As such, the phenotype of ARVD2 bears closer resemblance to familial catecholaminergic polymorphic ventricular tachycardia, which is also associated with mutations in RyR2 (8,14).

The second extradesmosomal gene implicated in ARVD/C is transforming growth factor (TGF)- β 3, which stimulates production of components of the extracellular matrix. Mutations in the untranslated regions of TGF- β 3, predicted to result in overexpression, have been isolated in one large family and an unrelated proband with ARVD1 linkage (32,33). That 2 ARVD1 kindreds lacked mutations in TGF- β 3 is a caveat in gauging its overall contribution to the genetic profile of ARVD/C (8,33). Nevertheless, in vitro studies suggest that TGF- β may modulate expression of desmosomal genes, lending further support to the hypothesis that impaired desmosomal function is the “final common pathway” in ARVD/C (14,32).

Although genetic studies have provided important insights into the pathogenesis of ARVD/C, the anticipated role of genotyping in the clinical arena has not yet been realized. This article aims to outline both the current status

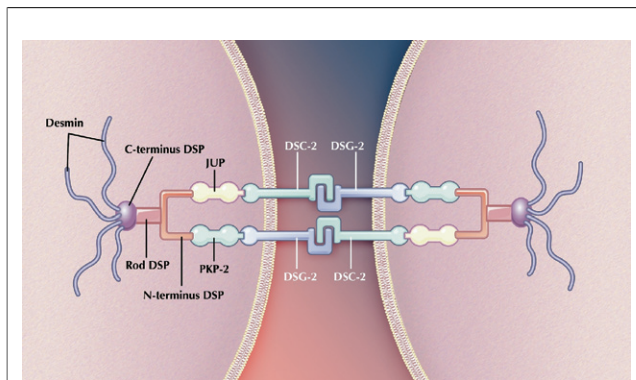


Figure 1 Schematic of Desmosomal Structure

The desmosomal cadherins, desmoglein (DSG)-2 and desmocollin (DSC)-2 comprise the transmembrane component of the desmosomal complex. Their extracellular domains interface directly with their counterparts on neighboring cells. The intracellular portions of the desmosomal cadherins interact with proteins of the armadillo family: junctional plakoglobin (JUP) and plakophilin (PKP)-2, which in turn bind to the N-terminal domain of desmoplakin (DSP). At its C-terminal, DSP anchors desmin intermediate filaments to the cell surface. Modified from Sen-Chowdhry et al. (8).

of molecular genetic analysis as a clinical tool in ARVD/C and the prospects for expansion of its scope in the future.

Clinical Applicability of Genetic Testing

Despite the enthusiasm with which the discovery of new genes is generally hailed, assimilation of genetic analysis into clinical practice tends to be a slow and arduous process (34). Hypertrophic cardiomyopathy is the most common inherited cardiovascular disease, with an estimated prevalence of 1 in 500 in the population. Contemporary estimates suggest that around 60% of index cases with hypertrophic cardiomyopathy harbor mutations in one of the known disease-causing genes, and that more than 80% of these individuals could be successfully genotyped by screening 2 genes in particular: β -myosin heavy chain and myosin binding protein C (34). Nevertheless, for a variety of reasons pertaining to accessibility, allocation of limited resources, and per-

Table 1 Summary of Desmosomal Genes Implicated in ARVD/C

Gene (Symbol), Locus	Exons (n), Transcript Size (kb)	Mode of Inheritance	Number of Reported Mutations	Type of Reported Mutations	Associated Phenotype	References
Plakoglobin (JUP) 17q21	14, 2.4	AR	1	Deletion	Naxos disease	(1)
Desmoplakin (DSP) 6p24	24, 8.9	AD AR	>10 3	Various Missense Nonsense Deletion	ARVC ARVC, skin disorder, woolly hair ARVC, skin disorder, woolly hair Carvajal syndrome	(2-6) (7) (8) (9)
Plakophilin (PKP)-2 12p11	14, 2.6	AD AR	>50 1	Various Cryptic splice site	ARVC ARVC	(5,10-17)
Desmoglein (DSG)-2 18q12	15, 3.4	AD	>20	Various	ARVC	(5,18-20)
Desmocollin (DSC)-2 18q12	17, 3.1	AD	3	Deletion, insertion, splice site	ARVC	(21,22)

AD = autosomal dominant; AR = autosomal recessive; ARVD/C = arrhythmogenic right ventricular dysplasia/cardiomyopathy.

ceived cost-effectiveness, genetic testing is offered to families with hypertrophic cardiomyopathy in only a few specialized centers.

Genotyping in ARVD/C lags behind the status quo in hypertrophic cardiomyopathy, but is quickly catching up and, as a result of the difficulties inherent in clinical diagnosis (35), may ultimately take on a far more important role therein than in other inherited cardiovascular disorders. Priori and Napolitano (34) have proposed a scoring system (Table 2) to determine the clinical applicability of genotyping in Mendelian diseases, based on both technical and clinical criteria. According to the guidelines, genetic testing is indicated in diseases achieving a score of ≥ 3 , which include hypertrophic cardiomyopathy, long-QT syndrome, dilated cardiomyopathy in association with conduction defects, and familial catecholaminergic polymorphic ventricular tachycardia. At the other end of the spectrum are diseases in which genotyping remains a research activity, with limited scope for clinical application. Dilated cardiomyopathy falls in this latter category, attaining a score of < 1 , largely because of marked genetic heterogeneity and the consequently poor pick-up rate from genotyping. In disorders scoring from 1 to 3, of which ARVD/C and Brugada syndrome are examples, genetic testing has a potential clinical role that remains to be fully defined (34). The main factors influencing this categorization warrant further discussion in the specific context of ARVD/C.

What Proportion of Patients With ARVD/C Can Be Genotyped?

The clinical feasibility of genetic testing is dependent in large part on its success rate in the target population. In a recent UK referral center sample, mutation screening of the

5 desmosomal genes hitherto implicated in ARVD/C (Table 1) in 69 unrelated individuals allowed successful genotyping of 20 (~30%) (7). No mutations were identified in plakoglobin. In a contemporaneous Italian series, mutation screening of the “big 3” ARVD/C genes (i.e., desmoplakin, plakophilin [PKP]-2, and desmoglein-2, in order of frequency) allowed successful genotyping of 32 of 80 unrelated index cases (40%). Inclusion of TGF- $\beta 3$ effected a modest increase in the detection rate to 42.5% (26,36).

A key departure from the United Kingdom-Italian experience in other published cohorts has been the frequency of mutations in PKP-2 (7,36). Gerull et al. (18) first reported 27% prevalence of PKP-2 defects in 120 unrelated index cases with ARVD/C. The proportion was still greater in the U.S. cohort described by Dalal et al. (22), in which 25 of 58 unrelated ARVD/C probands had PKP-2 mutations. van Tintelen et al. (20) reported a similar 43% frequency of PKP-2 mutations in their series of 56 ARVD/C index cases in the Netherlands, which exceeds the overall pick-up rate from genotyping in the United Kingdom and Italian cohorts. The most striking finding in the Dutch study was that 16 of 23 (70%) of probands with demonstrable familial disease had mutations in PKP-2, suggesting that plakophilin-2 is the major determinant of familial ARVD/C in the Netherlands (20).

Dalal et al. (22) failed to identify common haplotypes among individuals with identical PKP-2 mutations in the U.S. study. In the Dutch cohort, however, haplotype analysis of index cases with 4 apparently recurrent PKP-2 mutations revealed allele sharing, which is consistent with founder effects (20). Founder mutations have previously been recognized in the Dutch population in conjunction with benign familial cholestasis, type I diabetes mellitus, L-DOPA-responsive dystonia, familial hypercholesterolemia, protein C deficiency, and hypertrophic cardiomyopathy (the 2373insG mutation in the myosin binding protein C gene) (20,37). As noted by van Tintelen et al. (20), the 70% pick-up rate from mutation screening of a single gene is unique among inherited myocardial diseases, strengthening the case for clinical use of genetic testing in the management of familial ARVD/C in the Dutch population. A similar argument can be made in favor of screening the Naxos population for carriers of the 2157del2 mutation in plakoglobin, for which a founder effect has also been demonstrated (9).

In the absence of founder mutations, as in the original study by Gerull et al. (18) and the subsequent American series (22), an alternative explanation must be sought for the discrepancy in the prevalence of PKP-2 mutations. Differences in subject selection may be at least partly responsible. At most referral centers, individuals with symptomatic arrhythmia of right ventricular origin account for the majority of index cases. In comparison, the United Kingdom cohort included a much greater proportion of cases identified by familial evaluation after the death of the proband from pathologically proven ARVD/C (7). It has also been suggested that some of the numerous missense changes may

Table 2 Priori-Napolitano Criteria to Define Applicability of Genetic Testing in Clinical Practice

Criteria	Points
Technical aspects	
Percentage of genotyped patients	
≥ 50	3
30 to 49	2
10 to 29	1
Unknown or ≤ 10	0
Size of the genomic region to screen, kb	
≤ 1	1
> 1 to 3	0
> 3 to 8	-0.5
> 8 to 13	-1
> 13	-1.5
Clinical aspects	
A, Presymptomatic diagnosis is clinically relevant	0.5
B, Identification of silent carriers is clinically relevant	0.5
C, Results influence risk stratification	0.5
D, Results influence therapy/lifestyle	0.5
E, Reproductive counseling is clinically justified	0.5

Reprinted, with permission, from Priori and Napolitano (34).

be pathogenic only when accompanied by additional genetic polymorphisms (7). Assuming, however, that all published mutations are pathogenic in isolation, the pooled results from four recent genotype–phenotype correlation studies (Table 3) yield a pick-up rate of ~40% from mutation screening of the known ARVD/C genes, with the exception of RyR2.

Although the inclusion of polymorphisms may have resulted in overestimation of the success rate of genotyping, the 40% figure may equally represent an underestimate, for a number of reasons. No data were available regarding the prevalence of mutations in desmoplakin or desmoglein-2 in the Dutch and U.S. cohorts screened for PKP-2 disease (20,22,36). Additional single-gene studies from the United Kingdom, Italy, and U.S. were omitted from the pooled data in Table 3 because the overlap with other published cohorts from the same centers was not known (12,19,27–30). Furthermore, existing studies reflect early experience with genotyping in ARVD/C and incorporate only a small proportion of the patient population at each major referral center. Finally, gene identification studies in ARVD/C are ongoing, with additional components of the desmosome-intermediate filament complex and associated proteins the primary candidates. Comprehensive screening of all known and candidate genes is likely to place the detection rate firmly in the 40% to 50% range, sufficient to justify clinical application in the near to immediate future.

What Technical Considerations Influence the Cost-Effectiveness of Genetic Testing in ARVD/C?

In the Priori-Napolitano scoring system, the size of the coding regions of the genes implicated in a particular disease serves as an indirect estimate of the cost of genotyping (34). Although this measure is useful as a preliminary guide, the technical factors impacting on the cost-effectiveness of genetic testing in ARVD/C merit more detailed exploration. Genetic analysis may be considered a 2-step process, entailing the initial search for a causative mutation(s) in an affected individual, and subsequent confirmatory or predictive testing for the presence of a known mutation in relatives.

Marked allelic diversity appears to be the rule for the main ARVD/C genes. Furthermore, although the number

of mutations so far reported in desmocollin-2 and plakoglobin are too small to allow comment, current experience indicates a preponderance of private mutations in the “big 3” genes in ARVD/C, a scenario comparable with that of the beta-myosin heavy chain gene in hypertrophic cardiomyopathy (8–30,38). Frequent private mutations are contingent upon a relatively high spontaneous mutation rate, although this alone is not sufficient to account for their apparent failure to perpetuate in the gene pool and become “common.” One possibility is that most of these mutations occur de novo, with increased penetrance and high lethality resulting in genetic death. Clinical experience, however, suggests otherwise; ARVD/C is familial rather than sporadic in the majority of cases (7) and sudden death a relatively rare occurrence in a typical kindred. The alternative and more plausible explanation is that these mutations are far more prevalent in the general population than presently appreciated, and that milder phenotypic manifestations may be under-recognized.

Recurrent or “common” ARVD/C mutations are few but noteworthy, particularly in PKP-2, where R79X, 2146-1G>C, S140F, and S50fsX110 are key examples (18,19). In the absence of founder effects, however, the proportion of common mutations in the ARVD/C population is too limited to advocate screening for specific defects as a first-line approach. Neither do there appear to be any mutational hotspots in the “big 3” genes, with defects occurring in the N-terminus, rod, and C-terminus of desmoplakin, every exon of PKP-2, and spread through the functional domains of desmoglein-2 (8–30). The upshot is that in attempting to genotype ARVD/C patients, no shortcut can obviate the need for systematic sequencing of the desmosomal genes, although it may be prudent to begin the search with the “big 3” genes, particularly PKP-2.

The burden on both resources and effort does not end here, however. An additional corollary of frequent private mutations is a substantial likelihood of isolating a novel mutation, with no information available regarding its pathogenicity. In general, it may be safe to assume a causative role for deletions, frameshift, and nonsense mutations, but the same does not apply to single amino acid substitutions. Once a novel missense change has been identified, and its absence from ethnically matched control subjects verified, it

Table 3 Pooled Results From Major Genetic Studies to Estimate Overall Detection Rate From Genotyping in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

Study Location	Number Screened	PKP-2	DSP	DSG-2	DSC-2	TGF-β3	Total
London, United Kingdom	69	6	7	5	2		20
the Netherlands	56	24					24
Padua, Italy	80	11	13	8		2	34
U.S.	58	25					25
Composite	263	66	20	13	2	2	103
Overall detection rate							39.2%

DSC = desmocollin; DSG = desmoglein; DSP = desmoplakin; PKP = plakophilin; TGF = transforming growth factor.

becomes necessary to screen other family members and establish causality through correlation of clinical and genetic findings. Finally, emerging data suggest that an important minority of ARVD/C patients are homozygous, double-heterozygous, or compound-heterozygous (7,25,27). Digenic inheritance in ARVD/C is consistent with experience in both long QT-syndrome and hypertrophic cardiomyopathy (38) and underscores the importance of screening every coding region of all known disease-causing genes, even after isolation of a putative pathogenic mutation.

Confirmatory and predictive testing in relatives with and without clinical features of the disease is more straightforward. Allelic heterogeneity precludes any role for genetic probes, however, and although polymerase chain reaction-restriction fragment length polymorphism analysis is relatively inexpensive, utility is limited to mutations that fall within restriction sites. Consequently, direct bidirectional sequencing of the exon containing the defect remains the preferred approach for known-mutation detection in ARVD/C, affording the highest diagnostic accuracy of any available technique.

Sequence analysis is therefore the mainstay of both gene identification studies and predictive testing in ARVD/C. Despite its reliability, genetic sequencing is an effort and cost-intensive process, particularly when the genes are large, as is the case in ARVD/C. Comprehensive screening of all 5 desmosomal genes, including flanking intronic sequences for each exon, requires coverage of a genomic region of approximately 40 kb, posing the single greatest obstacle to cost-effective genetic testing for ARVD/C in a clinical setting. Nevertheless, it is likely that the clinical benefits of genetic analysis will ultimately outweigh the technical drawbacks.

Is Presymptomatic Diagnosis Clinically Relevant?

Presymptomatic diagnosis is the aspect of ARVD/C in which genotyping is most likely to find a niche. That sudden cardiac death is the first clinical manifestation of the disease in more than 50% of index cases may be the most compelling argument in favor of adopting a proactive approach to familial evaluation (39,40). One of the foremost clinical challenges in ARVD/C is timely diagnosis of the so-called “concealed” phase, during which a dearth of symptoms may belie significant arrhythmic risk (1,35). Recent studies suggest that asymptomatic status does not imply subclinical disease (7,41), and presymptomatic diagnosis may therefore be possible in a significant proportion of family members through standard, noninvasive clinical assessment. Genetic analysis, however, is invaluable in cascade screening of families, the benefits of which are 2-fold: first, in affording a lifetime of reassurance to gene-negative relatives, who will constitute approximately 50% of those tested and, second, in allowing clinical resources to be targeted to proven gene-carriers. A subset of genetically affected individuals will have an unremarkable evaluation but remain at risk of “hot

phases,” underscoring the importance of aggressively investigating new onset symptoms (8,42). The availability of molecular diagnosis intensifies the need for a definitive solution to the ensuing challenge, that of reliable risk stratification in early ARVD/C.

Is Identification of Silent Carriers Clinically Relevant?

The clinical manifestations of ARVD/C may develop at any age, although childhood cases are rare and onset during the pubertal growth spurt is common. Where the age-distribution of ARVD/C differs most noticeably from hypertrophic cardiomyopathy is in the prevalence of late onset disease. Recognition of delayed morphological conversion in hypertrophic cardiomyopathy has prompted revision of the guidelines for familial evaluation; whereas relatives attaining physical maturity were previously presumed to be genetically unaffected, reassessment every 5 years throughout adulthood is now advocated (43). Nevertheless, late onset hypertrophic cardiomyopathy is found primarily in conjunction with mutations in myosin binding protein C and is otherwise the exception rather than the rule (44). Conversely, age-related penetrance may be a defining characteristic of ARVD/C (41), which may be related at a molecular level to progressive exposure to mechanical stress. Long-term endurance athletes appear to have structurally severe forms of the disease (7), a finding supported by animal studies in which endurance training accelerated the development of right ventricular dysfunction and arrhythmia in heterozygous plakoglobin-deficient mice (45). The disease may progress more slowly in individuals leading a sedentary life, with myocardial injury accumulating as part of the aging process.

Silent carriers, in the truest sense of the term, are genetically affected individuals who remain free from clinical manifestations of the disease throughout their lifetimes. Because symptoms are a poor guide to disease severity (7), determining the prevalence of silent carriers in ARVD/C will require lifelong clinical follow-up of gene-positive individuals, with re-evaluation at suitable intervals. Although this approach is to be encouraged in ARVD/C because of age-related penetrance (41) and the “hot-phase” phenomenon, long-term outcomes in genotyped individuals with autosomal-dominant ARVD/C must be awaited.

From a pragmatic standpoint, silent carriers are individuals who, at any point in time, lack evidence of clinically penetrant disease but retain the capacity to transmit the disease to their children. As a corollary of variable penetrance (8,41), silent carriers as such are frequent in ARVD/C and may, in small families, give the false impression of sporadic disease. Referral centers specializing in familial disease have responded to this problem by routinely offering assessment to second- and sometimes third-degree relatives which, when continued long-term to avoid missing occult disease, poses a significant burden on clinical resources (35). No less concerning is the psychological impact on relatives, who must reconcile themselves to

lifelong screening without any prospect of definitive reassurance, on the off-chance that they may be gene-carriers. In ARVD/C, the alternative is no more palatable because it entails presuming that a normal clinical work-up indicates gene-negative status in adult relatives, and accepting that a minority of them or their children may present catastrophically with an arrhythmic event. Cascade screening based on genotyping comes to the fore here, by obviating the need to test the children of gene-negative individuals, and identifying silent carriers whose children can in turn be offered genetic analysis.

Is Confirmatory Testing Clinically Relevant?

Confirmatory testing is defined herein as the use of genotyping to corroborate clinical suspicion of disease in an index case. As such, it should be distinguished from genetic screening to verify clinical status in relatives with borderline investigations. The neurodegenerative disorder Huntington's disease is a classic example of a condition in which confirmatory testing is of clinical relevance. When faced with a patient exhibiting cognitive, motor, or psychiatric symptoms suggestive of Huntington's disease, neurologists may request genetic analysis to determine the presence of the causative expanded trinucleotide repeat on the short arm of chromosome 4. The number of CAG repeats provides a definitive answer; <35 excludes the disease, whereas 36 to 40 indicates incomplete and >40 fully penetrant disease (46). In contrast to Huntington's, most inherited cardiovascular disorders are genetically heterogeneous and hence less amenable to confirmatory testing. Marfan's disease, caused by mutations in the gene encoding the matrix protein fibrillin 1, represents a possible exception; however, more than 90% of affected individuals have private mutations, and the prohibitive cost of sequencing all 65 exons has limited the utility of genotyping as a clinical tool (47). Equally, in Marfan's as in most other inherited cardiovascular diseases, the phenotype in index cases is often clear-cut and the diagnosis readily established on clinical grounds alone.

For these reasons at least, confirmatory testing is not included as a distinct criterion in the Priori-Napolitano scoring system (34). The myriad caveats notwithstanding, however, confirmatory testing has the potential to play a key role in ARVD/C because of the unique challenges that clinical diagnosis may pose (35). In everyday clinical practice, arrhythmia of right ventricular origin remains the primary reason to suspect ARVD/C and idiopathic right ventricular outflow tract tachycardia the main differential diagnosis (35). Isolated right heart failure is a far less common presentation. With growing awareness of ARVD/C among clinicians, typical electrocardiographic abnormalities such as right precordial T-wave inversion may also arouse suspicion, whether in the context of arrhythmic symptoms or as an incidental finding. It will be apparent, however, that the clinical features of disease are nonspecific; even right ven-

tricular aneurysms and epsilon waves, both highly characteristic of ARVD/C, may also occur in sarcoidosis (48).

The 1994 Task Force diagnostic guidelines were designed to facilitate clinical diagnosis by excluding phenocopies (1). Developed through expert consensus in the pregenetics era, when experience with ARVD/C was limited to symptomatic index cases and sudden death victims, the criteria fulfill their aim of standardizing diagnosis, with high specificity attained at the cost of reduced sensitivity (35,39). Modifications have been proposed to enhance their sensitivity in the context of familial disease (39). Early diagnosis in index cases, however, remains an unsolved problem. Much attention has recently focused on imaging techniques, such as cardiovascular magnetic resonance and 3-dimensional and/or contrast-enhanced echocardiography. Both almost certainly have an important role to play in early detection, but high diagnostic accuracy is difficult to achieve when interpreting subtle wall motion abnormalities in the thin-walled, trabeculated, pyramidal right ventricle (49).

Furthermore, emerging data suggest that ventricular arrhythmia in ARVD/C may occur in the absence of histological substrate. Kaplan et al. (50) describe a 5-year-old girl with Naxos disease who demonstrated frequent ventricular extrasystoles (>14,000 in 24 h) and depolarization abnormalities characteristic of ARVD/C, including localized QRS prolongation and epsilon waves. After her death from leukemia 2 years later, extensive macroscopic and histological assessment of her heart failed to identify fibrofatty replacement or other features of ARVD/C, leukemic infiltrates, or degenerative features suggestive of chemotherapy-related injury. However, immunofluorescent studies and electron microscopy revealed reduced localization of mutant plakoglobin to cell-cell junctions, diminished expression of the gap junction protein connexin-43, and a decreased number and size of gap junctions (50). Impaired mechanical coupling from desmosomal dysfunction is postulated to compromise electrical coupling through gap junction remodeling (50).

More recently, Kirchhof et al. (45) have reported spontaneous ventricular tachycardia of right ventricular origin in heterozygous plakoglobin-deficient mice without evidence of either abnormal histology or altered connexin-43 expression. The mechanism underlying the arrhythmia remains unexplained, although mechano-electrical feedback is one possibility. The clinical implications are more readily apparent: patients with ventricular arrhythmia may have underlying ARVD/C without there being any other clinical pointer to the diagnosis. Follow-up of these patients is therefore imperative, even if treatment modalities succeed in suppressing both symptoms and arrhythmia (35). In centers with a particular interest in early ARVD/C, familial assessment may be a first-line strategy to facilitate diagnosis in the index case. This approach involves consolidating isolated phenotypic features in family members to attain a unifying diagnosis and is sometimes successful, but will clearly disappoint in the case of a de novo mutation in the proband.

Thus, despite its modest pick-up rate of 40% to 50% and the size of the genomic region requiring sequence analysis, screening of the desmosomal genes may eventually become part of the clinical work-up for an individual with suspected ARVD/C, although a negative result will not exclude the disease. Identification of a desmosomal mutation may also be of value in the exclusion of phenocopies of ARVD/C. At the time of framing of the Task Force criteria, left ventricular involvement was considered an endstage complication of the disease, occurring after the onset of global right ventricular dysfunction, and leading ultimately to biventricular pump failure (1). Both pathological and clinical studies since then have highlighted left ventricular involvement as a common feature of ARVD/C (5,6), which may occur in the setting of preserved right ventricular function (7). Three distinct patterns of disease expression have recently been identified: the well-known “classic” phenotype, in which the predilection for the right ventricle persists throughout the disease course; “biventricular,” with parallel involvement of both ventricles; and the increasingly recognized “left-dominant” variant. Arrhythmia of left ventricular origin and lateral ECG abnormalities are observed in the “biventricular” and “left-dominant” forms, which may be misdiagnosed as dilated cardiomyopathy. The main distinction is that dilated cardiomyopathy typically presents with heart failure, whereas symptoms of arrhythmia predominate among patients with arrhythmogenic cardiomyopathy (7,8). In late presenters, clarification of the diagnosis may depend on identifying features such as prominent wall motion abnormalities, aneurysms, and myocardial fat, but the isolation of a desmosomal mutation is particularly persuasive. Early data suggest that the extent of left ventricular involvement may be more marked in individuals with desmoplakin disease or chain-termination mutations, but the prognostic impact thereof is not currently known (7).

Does Genotyping Influence Lifestyle, Risk Stratification, or Therapy?

On the basis of currently available data, any attempt to gauge the potential influence of genotyping on risk stratification and therapy is likely to be premature. In accordance with the desmosomal model, it may be prudent for proven ARVD/C gene carriers to avoid highly strenuous activity, particularly endurance training (7,8,45). Without knowledge of the penetrance of a mutation, however, some are apt to consider such blanket recommendations unnecessarily restrictive. Similarly, the dual influences of incomplete penetrance and variable expressivity confound the adverse prognostic impact of carrying an ARVD/C mutation to the point where neither prenatal diagnosis nor proscriptive reproductive counseling are clinically justified, particularly as effective therapies are available (8).

In contrast to hypertrophic cardiomyopathy, a family history of premature sudden death does not appear to be a key indicator of adverse prognosis in ARVD/C (51), sug-

gesting that the ultimate role of genotyping in risk stratification will be limited. Variations in disease expression between families carrying the same mutation, and among members of the same family, suggest that modifier genes and environmental influences contribute significantly to the overall phenotype in ARVD/C (7,24,41). Nevertheless, mutations at the ARVD5 locus, for which the gene responsible remains unidentified, appear to be particularly malignant, and DNA haplotyping has been advocated to facilitate early diagnosis and prophylactic placement of an implantable cardioverter-defibrillator (ICD) (52). Dalal *et al.* (22) conducted a systematic comparison of ARVD/C patients with ($n = 25$) and without ($n = 33$) mutations in PKP-2. Patients harboring PKP-2 mutations had earlier onset of both symptoms and ventricular arrhythmia compared to those who did not, although the incidence of appropriate ICD interventions was similar in the 2 subgroups. Analysis of ICD firing rates allowed identification of a number of risk factors in patients without PKP-2 mutations, including inducibility at electrophysiological study, the presence of previous spontaneous ventricular tachycardia, and diffuse right ventricular disease. Curiously, these predictors did not influence the frequency of ICD interventions among PKP-2-positive patients (22). Numbers, however, remain small and validation in large-scale genotype-phenotype correlation studies is awaited.

Conclusions

Comprehensive mutation screening of all known and candidate genes is liable to yield a success rate of at least 40% in ARVD/C. The major technical obstacle to its implementation in clinical practice will be the prohibitive costs of performing sequence analysis of a genomic region exceeding 40 kb. The frequency of private mutations in ARVD/C families, coupled with digenicity in a minority, nonetheless suggests that sequence analysis will remain the mainstay of both gene identification studies and predictive testing. In the clinical arena, genotyping will be of most value in enabling cascade screening of relatives, particularly attractive in ARVD/C because variable and age-related penetrance otherwise necessitate lifelong clinical reassessment of extended families. Both the subtlety of clinical abnormalities in early ARVD/C and the diverse phenotypic manifestations of the disease, which include left ventricular arrhythmia and ECG changes, suggest a potential role for genotyping in confirmatory testing of index cases. At present, data from genotype-phenotype studies are too limited to permit speculation on the possible impact of genetic analysis on risk stratification or therapy.

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