Arrhythmogenic Right Ventricular Cardiomyopathy: Growing Evidence for Complex Inheritance

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Genetics of Arrhythmogenic Right Ventricular Cardiomyopathy

The identification of the genetic underpinnings of the rare Mendelian cardiac diseases associated with sudden cardiac death (SCD) in the young has led to an improvement in the management of patients with some of these disorders. For example, genetic testing in the long-QT syndrome (LQTS) provides an important means of confirming the clinical diagnosis and allows for cascade familial screening in affected families. Moreover, in this disorder, numerous genotype–phenotype relationships have been uncovered, such as the relation between the affected gene and arrhythmogenic triggers, and genotype-specific response to pharmacotherapy. In the LQTS, genetic testing, which identifies a (putative) mutation in \( \approx \)70% of probands, has joined traditional risk factors, such as sex and the extent of QTc-interval prolongation, as an independent prognostic risk factor. This contrasts sharply with other disorders associated with SCD in the young, 1 of which is arrhythmogenic right ventricular cardiomyopathy (ARVC), where the role of genotype as a prognostic factor is unclear.

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ARVC is a progressive disease of the myocardium, which leads to cardiomyopathic changes involving pathological fatty infiltration and cardiomyocyte loss and SCD from ventricular arrhythmias at a young age. Although it has classically been considered to affect the right ventricle (hence the name), the increasing awareness of the disease has led to the recognition of forms that present left ventricular or biventricular involvement. Genetic studies have identified a number of genes for the disorder; most of these encode components of the cardiac desmosome (DSG2, DSP, JUP, and PKP2), although other genes have also been implicated (eg, TMEM43 and PLN). About 50% to 70% of probands harbor a causal or possibly causal mutation in a desmosomal gene. However, although gene identification has provided important molecular insight for initiation of mechanistic studies to increase our understanding of the pathogenesis of the disease, the use thus far of these gene discoveries in patient management has been rather disappointing. At the base of this problem are the difficulties encountered in cosegregation analysis in affected families where low disease penetrance, and variable disease expression and severity among carriers of a putatively causal ARVC gene variant, is commonly encountered. In essence, not all mutation carriers of the putative familial mutation develop cardiomyopathic changes, and not all develop life-threatening arrhythmia.

The variability in clinical disease presentation among carriers of a putatively causal ARVC gene variant may stem from different causes. The cardiomyopathic changes associated with ARVC may be challenging to diagnose, and thus the difficulty in accurately assessing the extent of cardiomyopathic changes in patients could contribute to at least some of the apparent variability in clinical signs of the disease. Notwithstanding, other factors, both environmental and genetic, most likely contribute. As for nongenetic factors, age and intense physical activity are, for example, long-suspected modulators of disease expression. With respect to additional genetic factors, variable disease severity may stem from the presence of multiple mutations in either the same gene (compound heterozygosity) or different genes (digenic heterozygosity). The role of such inheritance in the modulation of disease severity has been demonstrated clearly in LQTS where compound or digenic mutations in the known LQTS-associated genes are found in 8% to 10% of patients, along with longer QTc intervals, a higher incidence of arrhythmia, and more severe symptoms in individuals carrying 2 mutations. In ARVC, multiple mutations in \( \geq 1 \) ARVC genes are found in 6% to 15% of probands. In this issue of Circulation Cardiovascular Genetics, Rigato et al extend on previous observations that compound or digenic inheritance of desmosomal gene mutations may be associated with a more severe phenotype. By studying 134 mutation carriers from 44 consecutive families during an observation period of 22 to 52 years, they demonstrate that carriership of multiple mutations in desmosomal genes and male sex are independent predictors of life-time arrhythmic events. Although there are certain limitations to the study, by demonstrating that a higher mutational load is associated with an increased risk of arrhythmic events, this study provides the first evidence for a possible role for genetic testing in arrhythmic risk stratification, which could possibly allow for optimization of therapy for SCD prevention on the basis of genetic findings.

Defining Pathogenicity

Importantly, the work of Rigato et al hinges on the correct classification of genetic variants as pathogenic mutations, which is not a simple task. Considering that cosegregation analysis within families is of limited use in this regard (for the
reasons discussed above) and considering the unavailability of in vitro assays that could shed light on the causality of gene variants, estimation of the deleteriousness of gene variants identified in patients currently relies heavily on the absence of the variants in samples of the general population. Thus, like previous genetic studies conducted in ARVC, by necessity, Rigato et al\(^a\) attributed involvement of the identified protein-altering desmosomal gene variants in the disease largely on the basis of their absence in a modestly sized sample of ethnically matched control individuals. Using a similar definition for calling a genetic variant a mutation, a screen by Kapplinger et al\(^\text{\text{a}}\) for genetic variants in desmosomal genes conducted in a sample of 427 ostensibly healthy controls of various ethnicities identified novel protein-altering genetic variants in 16\% of the individuals tested. On the basis of population prevalence of ARVC (1/1000–1/5000),\(^2\) we found that not all of the variants identified in this latter study can be pathogenic. When the analysis of Kapplinger et al\(^\text{\text{a}}\) was limited to variants that lead to nonmissense mutations (in-frame and frameshift insertions and deletions, splice junction, and nonsense mutations), this percentage was reduced to 0.5. Considering this and the fact that nonmissense variants are found frequently in patients with ARVC (16 of 48 in the current study are nonmissence),\(^10\) newly identified nonmissense variants in desmosomal genes are likely to represent bona fide ARVC-associated mutations. In contrast, as novel/rare missense variants are prevalent in controls,\(^12\) the discovery of a missense mutation in patients with ARVC presents an enormous interpretive challenge. The interpretive challenge of genetic variants identified in patients has become even more evident with the nearly comprehensive maps of genetic variation in thousands of human exomes and genomes that have now become available through large-scale sequencing projects, such as the 1000 Genomes Project\(^13\) and the National Heart, Lung, and Blood Institute Exome Sequencing Project.\(^14\) A survey we conducted in the Exome Sequencing Project\(^14\) demonstrates that >8\% of the subjects carry a rare (minor allele frequency, <1\%) protein-changing variant in one of the ARVC-associated genes (Table; 523 variants in 6503 individuals; not all individuals have been completely screened for all genes). Again, in this control group, most of the novel/rare variants are missense variants, whereas the percentage of nonmissense variants is low (19/523). In the Exome Sequencing Project, because the variants are not listed per individual, it is not possible to identify the number of subjects with compound/digenic heterozygous mutations. The Exome Sequencing Project data also demonstrated that certain genetic variants, previously deemed pathogenic on the basis of their absence in a few hundred control individuals, were actually present in the general population when one considered a larger sample.\(^15\) However, considering the complex (multigenic) inheritance of ARVC, the presence of a particular variant at low frequency in the general population does not rule out the possibility that the particular variant contributes to the disease when it is co-inherited with other pathogenic variants.

Complicating the issue even further, the absolute mutational load (ie, the total number of novel or rare variants in a patient) does not per se imply an increased disease burden because different variants might have different direction of effects (protective versus deleterious) or 1 variant may mitigate the effect of the other. For instance, several studies have for the years demonstrated how specific gene variants in SCN5A (encoding the major sodium channel isoform in heart and implicated in a variety of primary electric disorders, including LQTS and conduction disease) mitigate the effect of another variant.

### Table. Rare Variants (MAF<1\%) in ARVC Genes in the Exome Variant Server Database

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Clinical Relevance</th>
<th>DSC2</th>
<th>DSG2</th>
<th>DSP</th>
<th>JUP</th>
<th>PKP2</th>
<th>PLN</th>
<th>TMEM43</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coding insertion/deletion</td>
<td>Unknown</td>
<td>...</td>
<td>...</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>6</td>
</tr>
<tr>
<td>Frameshift</td>
<td>Unknown</td>
<td>4</td>
<td>2</td>
<td>...</td>
<td>...</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>7</td>
</tr>
<tr>
<td>Missense</td>
<td>Pathogenic</td>
<td>66</td>
<td>78</td>
<td>182</td>
<td>58</td>
<td>69</td>
<td>1</td>
<td>40</td>
<td>494</td>
</tr>
<tr>
<td>Probable nonpathogenic</td>
<td>...</td>
<td>2</td>
<td>...</td>
<td>...</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Probable pathogenic</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Missense near-splice</td>
<td>Unknown</td>
<td>64</td>
<td>72</td>
<td>180</td>
<td>58</td>
<td>68</td>
<td>1</td>
<td>39</td>
<td>481</td>
</tr>
<tr>
<td>Splice</td>
<td>Pathogenic</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
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<td>7</td>
</tr>
<tr>
<td>Unknown</td>
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<td>...</td>
<td>1</td>
<td>2</td>
<td>...</td>
<td>...</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop gained</td>
<td>Unknown</td>
<td>1</td>
<td>...</td>
<td>2</td>
<td>...</td>
<td>1</td>
<td>...</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>83</td>
<td>188</td>
<td>62</td>
<td>74</td>
<td>1</td>
<td>43</td>
<td>523</td>
<td></td>
</tr>
</tbody>
</table>

Variants are presented per gene and categorized on mutation type and according to functional annotation in dbSNP. In total, 523 variants are found in a total of 13 006 alleles (in 8\% of 6503 individuals). Some of these variants are called (probable-) pathogenic in the dbSNP database. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; dbSNP, Single-Nucleotide Polymorphism Database; and MAF, minor allele frequency.
co-inherited in cis (on same allele) or in trans (on opposite allele) in the same gene.16–18

Of note, some of the current variant databases, such as the latest versions of Single-Nucleotide Polymorphism Database (eg, version 132 and later), contain (with and without intent) variants published as being pathogenic (Table).19 Simply filtering out any variant present in these databases as harmless is therefore a risky strategy because these reputedly pathogenic variants will be filtered out as well. Clearly, the difficulties encountered in the interpretation of DNA variants highlights the need for the cardiac community to find a means of standardizing genetic testing platforms and depth of clinical phenotyping. This is required to support the exchange of genotype and phenotype data across centers, which is necessary to further our understanding of the contribution of such variants to these rare diseases as recently done for the CFTR gene in cystic fibrosis.20 Although indispensible, such an effort in variant annotation in extended patient sets will likely take years of careful studies until it reaches a mature stage where it may be used in routine clinical care.

Other Considerations and Perspectives for the Future

What else is to be learned from the increasing awareness of the genetic complexity of ARVC? Importantly, the study of Rigato et al10 stresses the necessity to screen the entire panel of ARVC-related desmosomal genes even after a single gene mutation has been identified. As these investigators point out in their discussion, these findings have important implications for genetic counseling. The mode of inheritance is different depending on the manner of interaction of the identified variants, but as 1 mutation may already be pathogenic, a carrier of a double hit has obviously much higher chances of transmitting an affected allele to his/her offspring than a patient carrying only 1 affected allele. The emerging genetic complexity clearly necessitates that clinical application of genetic testing should be performed at referral centers with expertise in inherited cardiovascular disorders. Furthermore, ARVC remains a multifactorial disease in which genetics is 1 of the facets to be considered alongside clinical evaluation.

The sample size of the study of Rigato et al10 is limited (22 of the total number of 134 mutation carriers had the composite end point arrhythmia/SCD), prohibiting a more fine-grained subdivision according to known or suspected confounding factors, such as affected gene, mutation type and location, medication, age of diagnosis, sports activity, and whether the patient was the proband or was identified through cascade family screening. Nonetheless, the findings of the current study clearly warrant a larger prospective study. Setting up a prospective registry of patients with ARVC ensuring unbiased inclusion of (multiple-)mutation positive and negative patients for long-term follow-up is an essential next step to determine prognostic risk factors for patient stratification to which the study of Rigato et al10 has given a first insight.

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None.

References


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