Assessing the Significance of Pathogenic Mutations and Autopsy Findings in the Light of 2010 Arrhythmogenic Right Ventricular Cardiomyopathy Diagnostic Criteria

A Clinical Challenge

Kalliopi Pilichou, PhD; Gaetano Thiene, MD; Cristina Basso, MD, PhD

A rrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease associated with significant genotype and phenotype heterogeneity. The structural features of ARVC consist of progressive fibrofatty replacement of myocytes and, clinically, the disease has been associated with ventricular arrhythmias at risk of sudden cardiac death. Disruption of the desmosomal complex could lead to ARVC pathognomonic features by at least 3 different mechanisms: (1) collapse or weakening of desmosomes by physical strain accounting for cardiomyocyte death and subsequent replacement by fibrous tissue and adipocytes; (2) reduced expression of desmosomal proteins might impair expression of interacting proteins at the intercalated disk (eg, gap junction or ion channel proteins) and promote ventricular arrhythmogenesis, even in the absence of fibrofatty tissue replacement; or (3) mutations in desmosome-associated proteins (eg, JUP) may result in Wnt signal transduction changes.

In this issue of Circulation Cardiovascular Genetics, Kirchner et al report a family with clinical history of arrhythmias and ARVC Task Force Criteria, associated with a PKP2 missense mutation previously described as a Dutch founder mutation. Although in-frame, frame-shift, nonsense, and splicing-site mutations (so-called radical) constitute the vast majority of genetic alterations identified in mutation-positive ARVC cases, missense mutations may also cause disease. In this article, the investigators demonstrated that the final common pathway, even for missense mutations, might be haploinsufficiency with lack of desmosomal assembly caused by the mutant PKP2, unable to reach the membrane layers.

To validate these findings, the authors evaluated other putative pathogenic PKP2 mutations, and they demonstrated decreased expression of PKP2 mutant proteins in the plasma membrane. Finally, in vitro assays determined the implication of calpain proteases pathway that leads to the degradation of mutant PKP2 proteins. Therefore, the authors advocated haploinsufficiency as the most likely cause for the genesis of dominant ARVC caused by mutations in PKP2, supported by their in silico predictions and in vitro data.

The investigators have also highlighted the need for molecular assays to discern pathogenic mutations from no pathogenic unique variants (“genetic noise”). Indeed, about 20% unique ARVC variants predicted by in silico softwares have a benign/tolerated effect. Moreover, 16% of healthy individuals without

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Five of the 8 different genes associated with ARVC encode desmosomal proteins (desmplakin [DSP], plakophilin-2 [PKP2], desmoglein-2 [DSG2], desmocollin-2 [DSC2], plakoglobin [JUP]) and account for up to 50% of ARVC-unrelated probands. Therefore, ARVC is considered a disease of the desmosome. Desmosomes, crucial for normal morphogenesis during all stages of embryogenesis, are found at the intercalated disk subadjacent to adheres junction ensuring mechanical cohesion of cardiomyocytes in the beating heart. These multiprotein complexes appear as multilayered, bilaterally symmetrical structures featuring a pair of electron-dense plaques that sandwich an extracellular region of ≈35 nm width. This extracellular space is bisected by a central dense line (dense midline) comprising the desmosomal cadherin ectodomains (DSG2, DSC2).

Altogether, >350 mutations have been reported in the 5 putative, causative ARVC desmosomal genes. The prevalence of PKP2 mutations among apparently unrelated individuals with ARVC ranges from 11% to 43%, which makes PKP2 the most common gene mutated in ARVC. PKP2, a member of the armadillo family, links to DSP and to other desmosomal elements via the N-terminal head domain, promoting junction assembly and function. Similar to its adheres junction counterparts (ie, β-catenin), a PKP2 isofrom is also present in the nucleus, contributing to the RNA polymerase III holoenzyme complex. The desmosomal protein PKP2 has been shown to interact also with gap junction and ion channel proteins.

From the Department of Cardiac, Thoracic, and Vascular Sciences, Cardiovascular Pathology, University of Padua, Padova, Italy.

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Correspondence to Cristina Basso, MD, PhD, Cardiovascular Pathology, Department of Cardiac, Thoracic, and Vascular Sciences, University of Padua Medical School, Via A. Gabelli, 61 35121 Padova, Italy. E-mail cristina.basso@unipd.it


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ARVC carry a desmosomal gene mutation. This is of utmost importance because, by contrast to the 1994 criteria, the family history category of the 2010 criteria includes molecular genetic information. In particular, the identification of a pathogenic mutation in a first-degree relative has become a major criterion for the diagnosis of ARVC. Because of the diagnostic implications, caution in the interpretation of genetic-screening results is highly recommended. A pathogenic mutation is challenging to establish; even if a desmosomal gene mutation is identified, determining whether the individual will be affected is difficult because of the variable disease penetrance.

Another crucial aspect is the need for accurate interpretation of autopsy findings, because of the role of postmortem diagnosis of ARVC in the clinical assessment of family members. In the family reported by Kirchner et al, the postmortem reports are vague and not documented. In particular, in patient II-1 (a 76-year-old man who died suddenly after gastric bleeding) the autopsy features are confusing and not in keeping with ARVC. These data, together with the negative clinical findings, do not support a cosegregation of the PKP2 mutation with the disease with 2 possible implications, either that this family member is an healthy carrier of a PKP2 mutation or that the PKP2 mutation is nonpathogenic. We cannot exclude that the other 2 PKP2 mutation carriers, who are clinically affected, do carry a compound mutation in a yet unknown ARVC susceptibility gene. Furthermore, in patient III-4, who died suddenly on effort at the age of 32 years (no genetic data available), the authors report that autopsy investigation suggested ARVC, but no gross and microscopic features are available. Overall, no histopathologic data and illustrations are provided to support a clear-cut diagnosis of ARVC in this family, despite the fact that 2 cases suffered sudden death and underwent autopsy and 1 case had endomyocardial biopsy. This underlies the need for a qualified pathology assessment so that the clinicians should not hesitate to request a second opinion to referral centers with expertise in cardiovascular genetics.

Finally, the article by Kirchner et al brings back the focus to the significance of immunohistochemical assay for desmosomal proteins in ARVC diagnosis. To further determine changes in the expression level and pattern of desmosomal proteins, the authors performed immunoblot and immunofluorescence analysis in both EMB and autopic specimens. It is interesting to note that the signal and localization of proteins, the authors performed immunoblot and immuno-fluorescence analysis in both EMB and autopic specimens. It is interesting to note that the signal and localization of proteins, the authors performed immunoblot and immuno-fluorescence analysis in both EMB and autopic specimens.

Conclusions
The PKP2 ARVC family reported by Kirchner et al underlies the potential pitfalls existing in the ARVC diagnostic workup. The correct interpretation of the genetic screening and histopathologic findings currently represents a crucial step in family investigation, besides the other clinical invasive and noninvasive tools.

The complexity involved in the interpretation of missense mutations suggests that, although ARVC clinical testing is accessible to many patients and cardiologists, significant skill is required to properly interpret mutation test results and distinguish pathogenic mutations from background noise. Basic research needs to complement the clinical approaches. Until the specificity of molecular genetic tests becomes robust and well understood, these tests should be performed at referral centers with expertise in cardiovascular genetics.

At the same time, the need for an expert cardiac pathology service is advocated, not only for in vivo tissue characterization and new immunohistochemical assays, but particularly for postmortem investigation because autopsy findings can drive specific pathways for cardiac evaluation of first-degree relatives to identify potentially inherited cardiac diseases. Accurate interpretation of the autopsy findings is crucial. Misdiagnoses may misguide familial evaluation with unnecessary examinations and therapeutic interventions or, conversely, wrongly reassure relatives and stop familial screening, with potentially serious implications in terms of sudden death prevention.

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Disclosures
None.

References

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