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

Arhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease associated with significant genotype and phenotype heterogeneity.1 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.2

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 al11 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.6–8

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.

In particular, Kirchner et al show, by a combination of in vitro assays and in silico predictions, that PKP2-C796R mutant protein is intrinsically unstable and undergoes protein degradation. Although the aberrant expression of the mutant protein did not disrupt the localization of other endogenous junctional components, the authors showed that mutant PKP2-C796R protein was not able to interact with DSP to ensure the assembly at the junction plaque, indicating the need of functional PKP2 for DSP integration into the desmosome.

To validate these findings, the authors evaluated other putatively 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.5 Moreover, 16% of healthy individuals without

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

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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


© 2012 American Heart Association, Inc.

Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org

DOI: 10.1161/CIRCGENETICS.112.964163
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 PKP2, DSP, and JUP at the intercalated disks of the “spared” myocardial tissue were unchanged, whereas in areas next to massive fibrosis were markedly reduced, thus suggesting a regional process of expression alterations typically adjacent to myocardial tissue remodeling. This observation is in contrast to what has been previously reported by Asimaki et al. In fact, although myocardial degeneration and fibrofatty replacement occur preferentially in the right ventricle in patients with ARVC, Asimaki et al have observed a diffuse reduction in the level of JUP, not only in areas of the right ventricle showing typical fibrofatty pathological features but also in the left ventricle and interventricular septum, which otherwise appeared to be structurally normal and in subendocardial myocytes, which are usually spared in ARVC. On the basis of these findings, it was suggested that to diagnose ARVC, in contrast with traditional histological assessment, it is no longer necessary to perform biopsy of the heart in areas with structural changes.

The discrepant findings observed in the study by Kirchner et al, together with recent reports from other groups both in humans and experimental animal models and the possibility of false-negative and false-positive results such as in sarcoidosis and giant-cell myocarditis, highlight that additional validation studies are required before this immunohistochemical assay can be used as routine clinical practice.

It is worth mentioning that different anti-JUP antibodies, which are likely to recognize different epitopes in the N- or C-terminal of JUP, have been used by the investigators. Another possible explanation is that the macroscopic and histologic phenotype might develop via different molecular pathways at the subcellular level thus explaining the discordant immunohistochemical findings.

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.

Acknowledgments
Dr Pilichou is supported by a Grant of Pricard Conacuore, Modena, Italy.

Disclosures
None.

References


**KEY WORDS:** Editorials ◼ arrhythmogenic right ventricular cardiomyopathy ◼ diagnosis ◼ pathology ◼ autopsy ◼ genetics
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, Gaetano Thiene and Cristina Basso

doi: 10.1161/CIRCGENETICS.112.964163

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/5/4/384

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/