Reduced desmoplakin immunofluorescence signal in arrhythmogenic cardiomyopathy with epicardial right ventricular outflow tract tachycardia

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Introduction

Arrhythmogenic cardiomyopathy (ACM), formerly known as arrhythmogenic right ventricular dysplasia, is a primary myocardial disorder characterized by ventricular arrhythmias and sudden cardiac death (SCD).[1] Myocardial desmosomes are cell–cell junctions that reside within the intercalated disc. They consist of members of the cadherin family (desmocollin-2, desmoglein-2), which span the membrane mechanically coupling adjacent cells, and members of the plakin and armadillo families: plakoglobin (*PKG*), plakophilin-2 (*PKP2*), and desmoplakin (*DSP1*), which connect the cadherin complexes to the intermediate cytoskeleton filaments. A reduction in the *PKG* immunoreactive signal has been shown at intercalated discs of cardiomyocytes in ACM regardless of the underlying pathogenic mutation.[1,2] Herein we report 2 unusual cases of ACM with digenic heterozygosity showing normal immunoreactive *PKG* distribution but reduced *DSP1* signal at myocardial cell–cell junctions without known pathogenicity in the *DSP* gene.

Case report

Two unrelated male patients, aged 28 and 31 years old at time of first symptoms, complained of syncope during strenuous exercise. They had no known comorbidities and no family history of SCD. Both practiced high-intensity sports. Figure 1A shows the resting electrocardiograms of both cases. Despite some minor anomalies, there was no sign suggestive of ACM. The echocardiogram, coronary angiogram, and laboratory work-up were normal. Cardiac magnetic resonance imaging (CMR) in patient 1 showed a limited (1-segment) nontransmural area of late gadolinium enhancement (LGE) in the inferolateral left ventricle (LV) wall and a local hypokinesia of the right ventricular outflow tract (RVOT) on the cine short axis as well as a discrete right ventricle (RV) dilation considered borderline in a sportive patient (Supplemental Figure 1A–C). The CMR in patient 2 was normal (Supplemental Figure 1E–F). A metabolic FDG^{18} PET scan showed no focal activity. Signal-averaged electrocardiogram as well as higher precordial or specific Fontaine leads were not obtained at time of referral, which might have revealed epsilon waves suggestive of a subclinical ACM.

An electrophysiological study in patient 1 and a treadmill test in patient 2 revealed a monomorphic ventricular tachycardia (mVT) originating from the RVOT, as suggested by the inferior axis and the late transition occurring in lead V_4 (Figure 1B). An ablation was attempted in both cases using a 3-dimensional electroanatomic system (Carto 3, Biosense Webster, Inc, Diamond Bar, CA). A limited area of reduced bipolar voltage suggestive of a scar was observed on the anterolateral RVOT (Figure 2, endocardial mapping sections). Importantly, these reduced potentials preceded the ventricular tachycardia (VT) by 30 ms and were successfully ablated with noninducibility at the end of the procedures.

The initial diagnosis was idiopathic RVOT VT, and both patients were initiated on beta-blockers. After 2 and 9 months, respectively, both patients were referred again to our center for syncope during exercise. Symptomatic VT with a different morphology than the index arrhythmia in case 1, and VT of different cycle lengths in case 2 (Supplemental Figure 2), prompted a second endocardial ablation, deemed successful in case 1 and unsuccessful in case 2 because of inducibility at the end of the procedure. Amiodarone was then introduced. In view of early recurrence

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KEY TEACHING POINTS

- Some arrhythmogenic right ventricular dysplasia/cardiomyopathy might present as sustained ventricular tachycardia (VT) without overt structural heart disease.
- Recurrent VTs suggest an epicardial disease that can be further suspected by biopsies.
- Normal plakoglobin but reduced desmoplakin immunofluorescence staining signals on cardiac biopsies may be indicative of arrhythmogenic cardiomyopathy.

with different VT morphologies or cycle lengths than the index arrhythmia, there was increasing concern of an incipient ACM.[3] An implantable cardioverter-defibrillator (ICD) was implanted and an epicardial ablation was programmed. Figure 2A and B shows an extended area overlying most of the RV epicardium (900 points) made of low-voltage electrograms depicted as a shade of colors from red (dense scar, <0.5 mV) to blue (<1.5 mV), and late abnormal ventricular activations (LAVA) and fragmented potentials (blue and pink dots, respectively). Ablation primarily targeted the "early" LAVA (Figure 2A), then whether mid and late LAVAs were still present was checked immediately, with noninducibility at the end of the procedure. Amiodarone was discontinued immediately after the epicardial VT ablation and beta-blocker treatment resumed. Septal RV biopsies, taken at the time of the second endocardial ablation, revealed no morphologic abnormality. Immunofluorescence staining (IF) of desmosomal proteins was performed on the biopsies and compared to a control sample. The following proteins were tested: *PKG*, *PKP2*, *DSP1*, desmin (*DES*), connexin 43, and N-cadherin. IF revealed in both cases a severely reduced signal for *DSP1* at cell–cell junctions but normal distribution of other proteins (Figure 3). Interestingly, negative T waves occurred in leads V_2 and V_3 over a 3-year follow-up period before any epicardial procedure (Figure 1C).

Repeat imaging studies, performed at 10 and 12 months after symptom onset, respectively (before ICD implant in patient 1 and after ICD implant in patient 2), provided a major ACM criterion for patient 1 (segmental akinesia to dyskinesia with RV dilation, no change in the LV at CMR) and a minor criterion in patient 2 (RV free wall akinesia with normal function at echocardiogram). In the presence of 3 major criteria in patient 1 (imaging, sustained mVT, and repolarization abnormalities) and 1 major (repolarization abnormalities) + 2 minor criteria in patient 2 (sustained mVT and imaging), the diagnosis of ACM was established.[4] Patients were advised to refrain from intense physical activity according to current guidelines.[5] Over a 3- and 5-year follow-up no VT recurrence occurred after epicardial ablation.

Genetic analysis was performed in both cases (details in Supplemental Data). In patient 1, mutational analysis revealed 3 heterozygous variants: 1 likely pathogenic alteration in the *DES* gene: NM_001927.3:c.638C>T (p.Ala213Val) and 1 likely pathogenic variant in the *CTNNA3* gene: NM_001127384.1:c.1603C>T (p.Arg535Cys); and an additional variant of unknown significance identified in the *DSG2* gene: NM_001943.3:c.877A>G (p.Ile293Val).

In patient 2, mutational analysis also revealed 3 heterozygous variants: a pathogenic mutation in the *PKP2* gene: NM_004572.3:c.2197_2202delCACACCinsG (p.His733AlafsTer8); and 2 variants of unknown significance in the *DSG2* gene: NM_001943.3:c.2759T>G (p.Val920Gly) and *DSP* gene: NM_004415.2:c.6208G>A (p.Asp2070Asn).

Discussion

The diagnosis of ACM remains challenging owing to the age-related progression, vast phenotypic variation, and reduced genetic penetrance.[1,6] We report here 2 young patients presenting with arrhythmias displaying the typical morphology of RVOT VT. VT occurred years before any structural disease was declared. Importantly, none of the initial VTs displayed the typical features of a right epicardial origin, such as Q waves in II, III, aVF or Q waves in I.[7] Cardiac sarcoidosis was reasonably excluded by multimodality imaging.[1,8]

Importantly, IF of myocardial biopsies showed reduced *DSP1* signal at junctional sites. Reduced *DSP1* signal at intercalated discs has been recently reported in the majority of cases of dilated cardiomyopathy (DCM).[9] In our cases, however, the normal RV and LV ejection fraction at the time of the biopsies and the lack of a clear DCM phenotype after several years of follow-up reasonably exclude DCM as the underlying causative disease. Interestingly, RV imaging abnormalities and T-wave inversion occurred more than 2 years after VT onset, confirming that some ACM might present with arrhythmic events in the absence of any structural heart disease.[2,6]

Although not yet part of the current guidelines for the diagnosis of ACM,[4] IF staining appears to be a promising tool giving insights into tissue characterization up to the desmosomal subunits.[9] Intercalated disc anomalies occur in many diseases, including severe heart failure, but they are always accompanied by histologic abnormalities.[6] The occurrence of an abnormal intercalated disc IF signal in histologically unaffected regions of the myocardium appears typical of ACM.[1,2,6] IF of endomyocardial biopsies can reliably identify ACM even in the absence of genetic mutations.[9,10] The main intercalated disc culprits included *PKG*[9] and connexin 43.[10] While both were highly sensitive in the setting of ACM,[9,10] only reduced *PKG* signal was highly specific for the diagnosis of ACM.[9] Our patients showed a selective reduction of desmosomal *DSP1* expression, while the distribution of *PKG* appeared normal. IF bears some limitations that were addressed here to limit the risk of false-negatives. All biopsies were fixed within minutes after

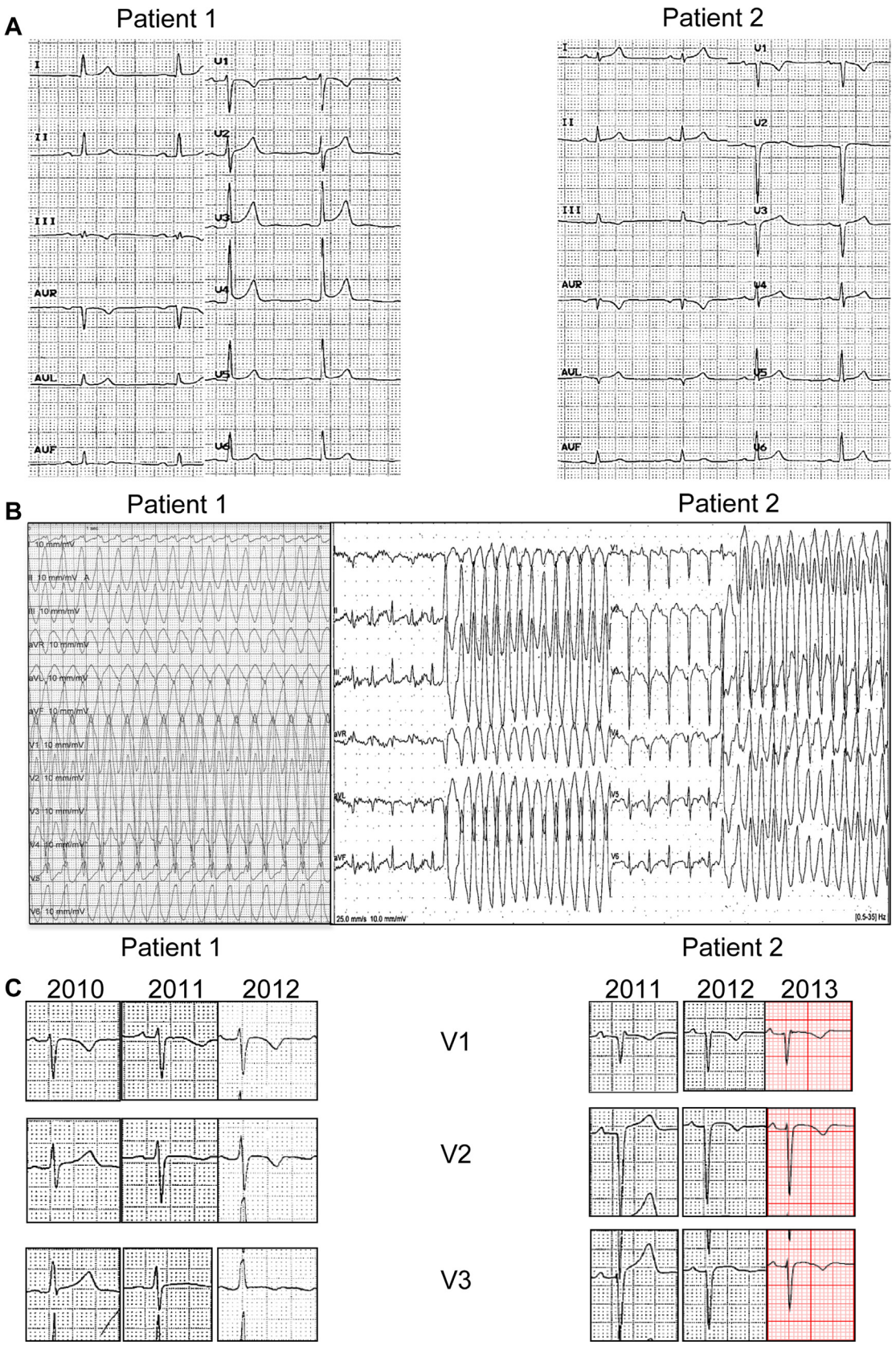

Figure 1 **A:** Baseline 12-lead electrocardiogram showing normal sinus rhythm with 1.5 mm J-point elevation in V_3–V_5 in patient 1 and normal sinus rhythm with late R-wave progression in precordial leads and incomplete right bundle branch block in V_1 not fulfilling the criterion for an epsilon wave in patient 2. **B:** Left: Sustained monomorphic ventricular tachycardia (heart rate 214 beats/min) induced during an electrophysiological study in patient 1. Right: Nonsustained ventricular tachycardia (heart rate 230 beats/min) occurring during an exercise stress test in patient 2. **C:** Progressive occurrence of negative T waves up to V_3 over a 3-year follow-up.

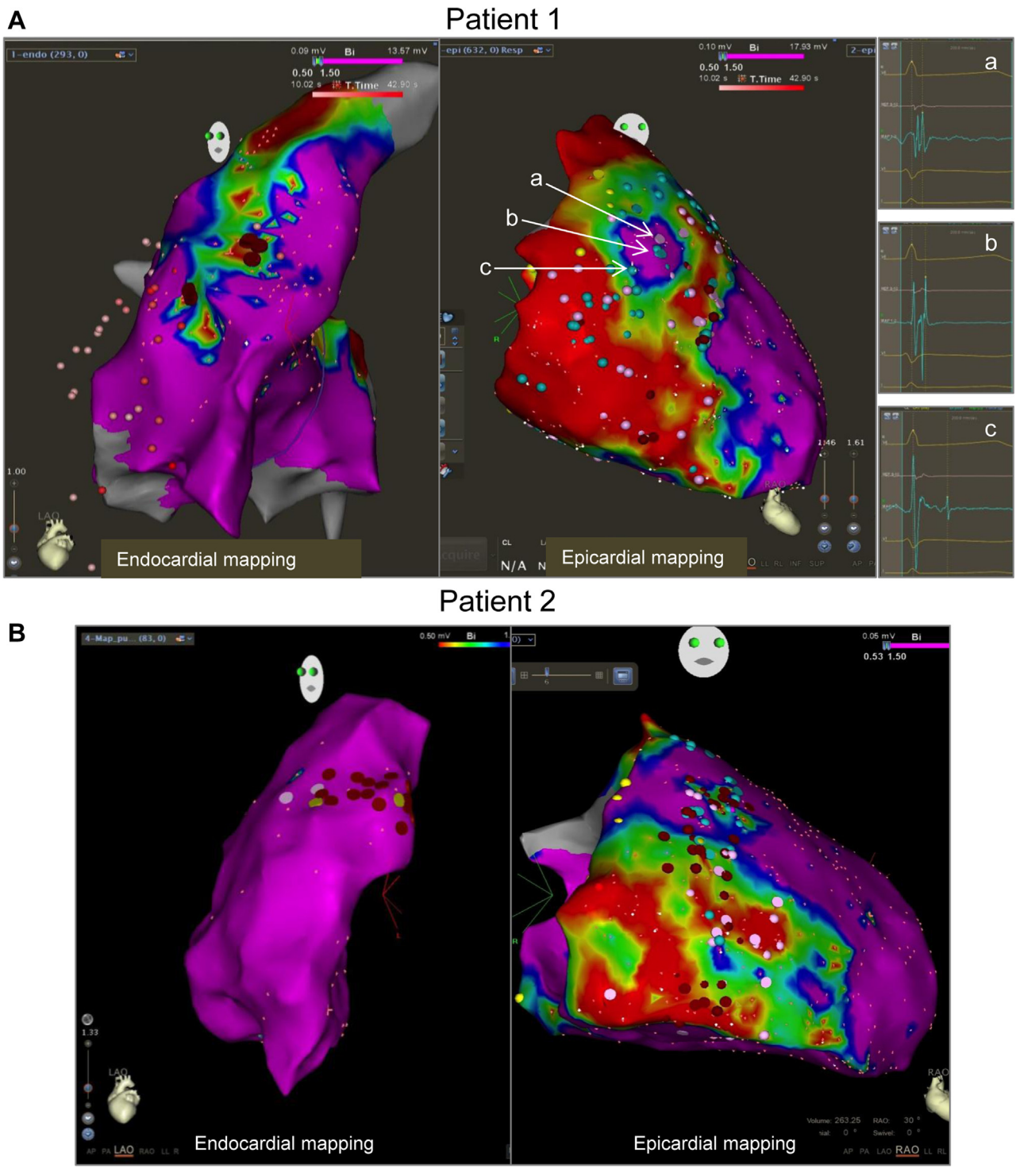

Figure 2 **A:** Left: Endocardial bipolar map based on a 3-dimensional electroanatomic system in patient 1. Note the presence of a limited area of low-voltage potentials in the anterolateral right ventricular outflow tract (RVOT). Right: Epicardial bipolar map showing an extended area of low-voltage potentials overlying most of the right ventricular (RV) epicardium. Examples of early (a), mid (b), and late (c) late abnormal ventricular activations are shown on the right-hand side. **B:** Left: Endocardial bipolar map in patient 2. A limited number of fragmented potentials in the anterolateral RVOT are shown as *pink dots*. Right: Epicardial bipolar map showing an extended area of low-voltage potentials overlying most of the RV epicardium.

harvesting and compared to a control to ensure that the reduced *DSP* IF signal stemmed from the disease and not from the technique. *DSP1* is the dominant isoform in cardiac tissue.[6] Traditionally, loss of *DSP1* was described by Alcalai and colleagues[11] and Uzumcu and colleagues[12] as part of a cardiocutaneus syndrome. In 2005, Norman and colleagues[13] identified an ACM mutation in *DSP1* with subsequent protein truncation,[2,6] which mainly involved the LV. To our

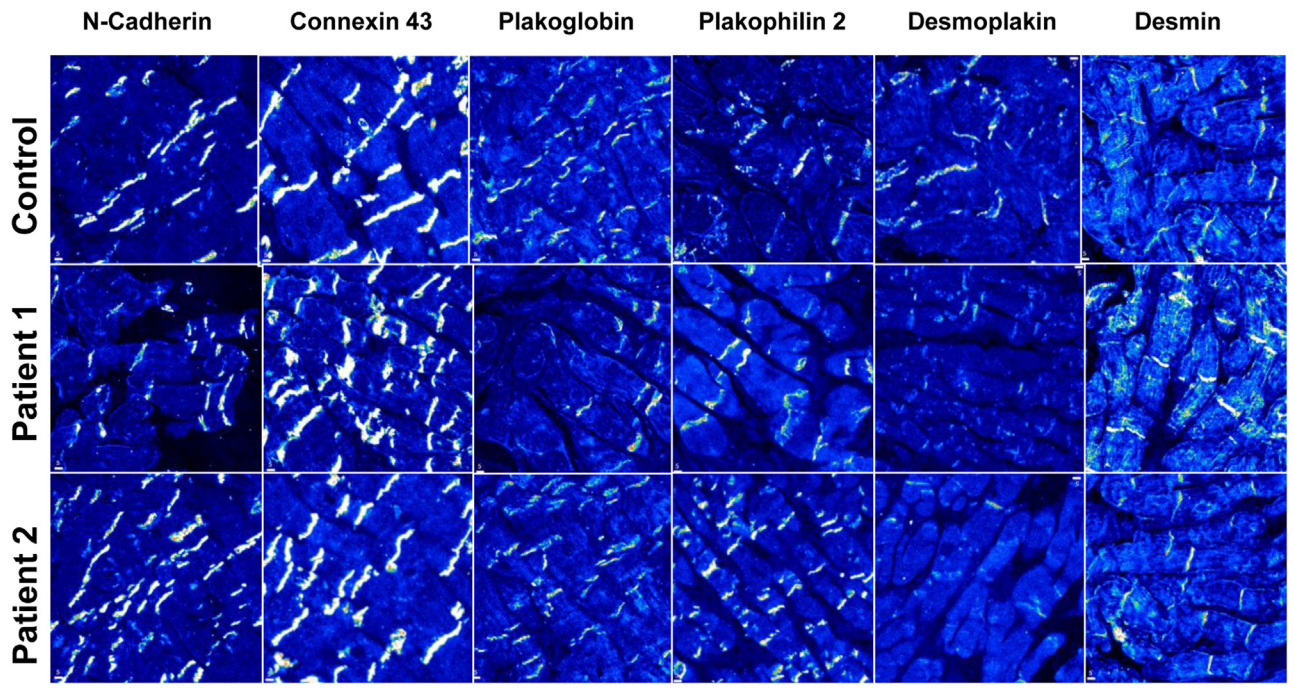

Figure 3 Immunofluorescence staining of intercalated disc proteins in a control subject and in patients 1 and 2.

knowledge, we report here the first cases of isolated reduced *DSP1* signal that mainly affects the RV without associated skin and hair disease.

The discovery of causative mutations in desmosomal genes has shed light into ACM pathogenesis.[2] Nevertheless, much less is known about how the mutant proteins actually cause the disease. A mutation in a single desmosomal protein can affect the localization of other proteins not genetically mutated.[2] They appear to play a role in cell death through abnormal cell–cell adhesion and as signaling proteins. Interestingly, redistribution of *PKG* and *DSP1* are involved in abnormal transcriptional activity and protein trafficking and in increased expression of fibrogenic and adipogenic genes.[2] Our first case exhibited a mutation in the *DES* and catenin genes and the second case a mutation in the *PKP2* gene, all encoding for proteins tightly connected to *DSP1*. Hence, it is possible that these abnormal proteins, although correctly expressed at the intercalated disc, might have limited the trafficking of *DSP1*. Another finding is the presence of multiple heterozygous gene variants, which carry a worse prognosis in terms of survival and disease progression.[1,14]

Antiarrhythmic drugs are prescribed in ACM to reduce the arrhythmic burden, but they have no impact on preventing SCD.[5] Catheter ablation has become an option for refractory VT.[15] Substrate-based ablation has improved the procedural outcome by targeting multiple epicardial circuits typically seen in ACM.[5,15] Further studies are warranted to determine whether desmosome imaging techniques could predict the presence of an epicardial substrate suitable for ablation in suspected cases of ACM.

Conclusion

We report 2 ACM cases with RVOT VTs displaying an isolated reduced *DSP1* signal at intercalated discs from histologically normal myocardial tissue. Although the RV might look unremarkable at histologic examination, the ability to sustain recurrent VT suggests a complex epicardial substrate that can be further suspected by IF studies of endomyocardial biopsies.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrcr.2018.06.013.

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