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Original Article

Molecular changes in the heart of a severe case of arrhythmogenic right ventricular cardiomyopathy caused by a desmoglein-2 null allele

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Abstract

Introduction: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic disorder caused by mutations in desmosomal genes. It is often associated with life-threatening arrhythmias. Some affected individuals develop progressive heart failure and may require cardiac transplantation. **Methods:** The explanted heart of a young adult with end-stage heart failure due to a null allele in desmoglein-2 was studied at macroscopic, microscopic, and molecular level. Myocardial samples were probed for junctional localization of desmosomal components and the gap junction protein connexin43 by immunohistochemical staining. In addition, the protein content of desmosomal and adherens junction markers as well as connexin43 was assessed by Western blotting. **Results:** Histological analysis confirmed ARVC. Despite the loss of specific immunoreactive signal for desmosomal components at the cardiac intercalated disks (shown for plakoglobin, desmoplakin, and plakophilin-2), these proteins could be detected by Western blotting. Only for desmoglein-2, desmocollin-2, and plakoglobin were reduced protein levels observed. Adherens junction proteins were not affected. Lower phosphorylation levels were observed for connexin43; however, localization of the gap junction protein displayed regional differences. At the molecular level, disease progression was more severe in the right ventricle compared to the left ventricle. **Conclusion:** Our data suggest that, in the ARVC heart, plakoglobin is mainly redistributed from the junctions to other cellular pools and that protein degradation only plays a secondary role. Homogenous changes in the phosphorylation status of connexin43 were observed in multiple ARVC samples, suggesting that this might be a general feature of the disease.

Keywords: Arrhythmogenic right ventricular cardiomyopathy; Desmosomal mutation; Desmoglein-2; Plakoglobin; End-stage heart failure; Cardiac transplant; Connexin43; Gap junction

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1. Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heart muscle disorder often associated with life-threatening arrhythmias [1]. Appropriate management of these arrhythmias [e.g., by implantable cardioverter–defibrillators (ICDs)] can reduce the high risk of sudden cardiac death in patients [2]. However, a small percentage of individuals affected by ARVC will develop progressive congestive heart failure and eventually require cardiac transplantation [3].

Familial disease is frequent in ARVC, and mutations in five major desmosomal genes have been identified as causes of the disease [4]. Desmosomes are membrane-bound structures that span tissues, thereby offering mechanical stability [5]. In the heart, desmoglein-2 (DSG2) and desmocollin-2 (DSC2) establish a mechanical link of neighboring cardiomyocytes. These two membrane proteins are intracellularly anchored to plakoglobin (PG) and plakophilin-2 (PKP2), which link to the desmin intermediate filament system via desmoplakin (DSP). Beyond this structural function, desmosomal proteins are also involved in signaling pathways. In particular, PG has been suggested to compete with beta-catenin in Wnt signaling pathways [6,7].

Although mutations in each cardiac desmosomal component can cause ARVC, a reduction or loss of PG immunoreactivity from the cardiac junctions has been observed in the vast majority of ARVC cases, irrespective of the underlying genetic mutation(s) [8]. This feature has been reported for several cases [9–11] and has been suggested as a diagnostic test. While it points at a final common pathway, the underlying molecular principles are not completely understood. In particular, the molecular triggers of the event and the fate of PG are yet to be identified. It is currently unclear whether PG is redistributed to other cellular pools (as suggested by work in animal models [6,12]) or what the contribution of protein degradation might be.

Redistribution of the main ventricular gap junction protein connexin43 (Cx43) has been observed in many cases of ARVC; however, similar changes have also been observed in other cardiac diseases (e.g., end-stage heart failure) [8]. Nevertheless, conduction slowing as a consequence of gap junction remodeling may contribute to the arrhythmias observed in ARVC. However, the molecular mechanisms linking desmosomal dysfunction to gap junction remodeling remain to be elucidated.

Here we present a case of severe ARVC in a young adult with a *DSG2* null allele and progressive congestive heart failure requiring cardiac transplantation. The explanted heart was sampled at multiple sites and analyzed for regional differences in disease progression at the macroscopic, microscopic, and molecular level. The material gave a unique opportunity to study the molecular fate of PG and Cx43 in a heart severely affected by ARVC.

2. Materials and methods

2.1. Clinical evaluation and genetic testing

The study was performed in accordance with the 1964 Declaration of Helsinki, and the study protocol was approved by the local Ethics Committee. The patients gave written informed consent for genetic testing and for analysis on the explanted heart or of cardiac biopsies. Clinical evaluation included history, physical examination, 12-lead electrocardiogram (ECG), signal-averaged ECG, two-dimensional echocardiography, and 24-h ECG monitoring. Mutational screening of *DSP*, *JUP* (coding for PG), *PKP2*, *DSG2*, and *DSC2* by direct sequencing was performed as previously described [11].

2.2. Histological and immunohistochemical analysis of myocardial samples

Formalin-fixed, paraffin-embedded material from several regions of the explanted heart was stained with Masson's trichrome for histological examination (four samples of the right ventricle [RV], one sample of right atrium [RA], and one sample of the left ventricle [LV]). Unstained slide-mounted sections were immunostained for N-cadherin, PG, PKP2, DSP, and Cx43 as previously described [13]. Myocardial specimens obtained at autopsy (within very few hours of death) from three age-matched individuals with no clinical history or pathological evidence of heart disease (for details, see legend of Table S1) were subjected to the same staining protocols and served as controls.

2.3. Western blotting

Two LV and two RV samples of the explanted heart, as well as RV biopsy samples obtained during a right heart catheterization (Table S1), were snap-frozen in liquid nitrogen. Total protein extracts, which contained both cytosolic and membrane-bound protein fractions, were prepared by adding 2× sodium dodecyl sulfate (SDS) sample buffer to the tissue (ground to powder in liquid nitrogen), followed by incubation at 65°C for 15 min; identical extracts of nonfailing (NF) rejected donor hearts and explanted hearts from patients with dilated cardiomyopathy (DCM) served as control material (Table S1). SDS polyacrylamide gel electrophoresis and Western blotting were performed as previously described [14] using the antibodies listed in the Suppl. Methods section. Even loading was verified by Ponceau S stain (Sigma). Densitometric analysis was performed with ImageJ software.

2.4. Expression of DSG2 cDNAs in COS-1 cells

The M1I mutation was introduced into a full-length DSG2 GFP fusion construct [10] with the QuikChange