Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is associated with fibrofatty replacement of cardiac myocytes, ventricular tachyarrhythmias and sudden cardiac death. In 32 of 120 unrelated individuals with ARVC, we identified heterozygous mutations in *PKP2*, which encodes plakophilin-2, an essential armadillo-repeat protein of the cardiac desmosome. In two kindreds with ARVC, disease was incompletely penetrant in most carriers of *PKP2* mutations.

Individuals with ARVC may present with palpitations or ventricular tachyarrhythmias that originate from the right ventricle and may lead to syncope or sudden cardiac death, a relatively common and the most feared clinical manifestation of the disease[1–3]. Localized or diffuse atrophy and progressive replacement of right ventricular myocytes with fatty or fibrofatty tissue is the pathological hallmark of ARVC. Although right ventricular involvement predominates, the left ventricle also can be affected. Heart failure of the right and, less commonly, the left ventricle may occur later in the disease process. The exact prevalence and incidence of ARVC is unknown. Familial disease accounts for an unknown proportion of ARVC, with estimates ranging from 30% to 80%. Eight genomic loci and rare mutations in plakoglobin (*JUP*), desmoplakin (*DSP*) and the cardiac ryanodine receptor (*RYR2*) have been reported in nonsyndromic and syndromic cases[2].

Desmosomes are complex multiprotein structures of the cell membrane and provide structural and functional integrity to adjacent cells (*e.g.*, epithelial cells and cardiomyocytes[4]). Desmosomal proteins also have a role in cell signaling[5,6]. At least three groups of molecules contribute to the formation of desmosomes: desmosomal cadherins,

armadillo-repeat proteins and plakins[7]. The plakophilins, which are armadillo-related proteins, contain ten 42–amino acid armadillo-repeat motifs and are located in the outer dense plaque of desmosomes linking desmosomal cadherins with desmoplakin and the intermediate filament system[8]. Like other armadillo-repeat proteins, plakophilins are also found in the nucleus, where they may have a role in transcriptional regulation[9]. Plakophilin-2 exists in two alternatively spliced isoforms (2a and 2b), interacts with multiple other cell adhesion proteins and is the primary cardiac plakophilin[8,10].

On the basis of findings of a lethal defect in cardiac morphogenesis at embryonic day 10.75 in mice homozygous with respect to a deletion mutation of *Pkp2* (ref. 11), we hypothesized that mutations in human *PKP2* may account for ARVC. A total of 120 unrelated probands of western European descent (101 males and 19 females) were admitted to tertiary referral centers and diagnosed with ARVC in accordance with clinical criteria proposed by a Task Force[12]. We directly sequenced all 14 *PKP2* exons, including flanking intronic splice sequences (**Supplementary Methods** online), and identified 25 different heterozygous mutations in 32 probands (27 males and 5 females). Of the 25 *PKP2* mutations, 12 were insertion-deletion mutations, 6 were nonsense mutations, 4 were missense mutations and 3 were splice site mutations (**Table 1** and **Supplementary Figs. 1** and **2** online). Although most mutations were detected in the C-terminal half of the molecule, mutations were located throughout the gene. We observed one *PKP2* splice acceptor site mutation (2146–1G→C) and two nonsense mutations (235C→T and 2203C→T) in several unrelated individuals with ARVC (**Table 1**). Genetic analysis of microsatellite DNA markers at or near the *PKP2* locus on chromosome 12p11 did not identify shared haplotypes between the ARVC cases with identical mutations (data not shown). Because parental genotypes were not available, however, we could not carry out formal segregation analyses. Therefore, ancient founder mutations cannot be excluded with certainty. Six instances of a nonsense mutation in *PKP2* (235C→T) indicated that nucleotide residue 235 might be a mutational hot spot. None of the 25 *PKP2* mutations were observed in 500 control chromosomes.

Although familial disease was not systematically evaluated in all probands, members of two kindreds (kindreds A100 and EPF; **Fig. 1**) were available for a detailed clinical and molecular analysis. A 2-bp deletion in *PKP2* exon 10 (2076_2077delAA) is predicted to cause the addition of 48 amino acid residues before a premature stop signal is introduced (C693fsX741); we observed this mutation in two clinically affected members of kindred A100 (individuals 18 and 161; **Fig. 1** and **Supplementary Table 1** online). We carried out western-blot analysis

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Table 1 Clinical status and *PKP2* mutations of 32 index individuals with ARVC

Individual	Sex	Clinical status	Exon	Nucleotide change	Amino acid change
1	M	VT, RV, LV, H	1	145_148delCAGA	S50fsX110
EPF17	F	RV, H	1	216insG	Q74fsX85
3	M	VT, RV, H	2	235C→T	R79X
4	M	VT, RV	2	235C→T	R79X
5	M	S, CA, VT, RV	2	235C→T	R79X
6	M	CA, VT, RV, LV	2	235C→T	R79X
7	M	VT, RV	2	235C→T	R79X
8	M	VT, RV	2	235C→T	R79X
9	F	VT, RV	3	419C→T	S140F
10	M	VT, RV	3	534_535insCT	C179fsX190
11	M	VT, RV, LV	5	1369_1372delCAAA	Q457X
12	M	VT, RV	7	1631_1632insTT	L544fsX563
13	M	S, VT, RV, LV	7	1642delG	V548fsX562
14	M	S, VT, RV, LV	9	1844C→T	S615F
15	M	S, VT, RV	9	1912C→T	Q638X
16	M	S, VT, RV, LV, H	9	1948delA	V650fsX655
17	M	VT, RV, LV, H, HTX	9	1951C→T	R651X
18	M	S, VT, RV	9	1960A→C	K654Q
A100/161	M	VT, RV, H	10	2076_2077delAA	C693fsX741
20	M	S, RV	10	2088insA	K696fsX742
21	M	S, VT, RV	10	2095C→T	Q699X
22	M	CA, VT, RV, H	11	2146–1G→C	Mutant splice product*
23	M	S, VT, RV, LV	11	2146–1G→C	Mutant splice product*
24	M	VT, RV	11	2176C→T	Q726X
25	F	VT, RV, LV, H	11	2203C→T	R735X
26	M	S, VT, RV	11	2203C→T	R735X
27	M	VT, RV	12	2386T→C	C796R
28	F	VT, RV	12	2393_2401delCATTGAACA	N798fsX879
29	M	S, SCD, VT, RV, LV, H	12	2424insA	E809fsX826
30	F	VT, RV	12	2489+1G→A	Mutant splice products*
31	M	S, VT, RV, LV	13	2490–1G→C	Mutant splice products*
32	M	VT, RV, LV	13	2509delA	V837fsX930

M, male; F, female; RV, right ventricular involvement; LV, left ventricular involvement; H, positive family history; S, syncope; CA, cardiac arrest; HTX, heart transplantation. *See **Supplementary Figure 2** online. Numbering of the nucleotides starts at the ATG codon of *PKP2*.

of a right ventricular biopsy specimen obtained from individual A100/161 but did not detect the truncated plakophilin-2 protein (expected size of truncated PKP2, 82.1 kDa; normal PKP2, 97.4 kDa). The specimen did contain less wild-type plakophilin-2 than control myocardium (**Supplementary Fig. 3** online), implicating haploinsufficiency as the operant mechanism in this *PKP2* mutation.

Clinical evaluation of first-degree relatives of kindred A100 identified two individuals (individuals 18 and 161; **Fig. 1** and **Supplementary Table 1** online) who are affected by ARVC in accordance with the Task Force criteria[12]. Although four other members of kindred A100 also carried the 2-bp deletion mutation in *PKP2*, they have normal or mild disease phenotypes, even at more advanced ages (*e.g.*, individual 1). Two members of another family with ARVC (EPF) died suddenly at ages 16 years and 15 years (individuals 11 and 12; **Fig. 1**). An autopsy study showed that both individuals had ARVC. Of the five surviving mutation carriers in kindred EPF, only one showed clinical manifestations diagnostic of ARVC (individual EPF/17; **Supplementary Table 1** online). The reason for this incomplete penetrance in kindreds A100 and EPF is unknown, but it may be related to gender, genetic or epigenetic modifiers or other unknown factors (*e.g.*, viral infection as trigger for disease). Although transmission of disease in kindreds A100 and EPF is compatible with autosomal dominance,

linkage analysis would have been difficult due to incomplete penetrance of disease in both kindreds (<0.5). ARVC in kindred EPF has been previously reported to be linked to chromosome 2q32.1–q32.3 (ref. 13). The identification of mutation 216insG in *PKP2* in this kindred, coupled with the absence in any individual family of statistical significance for the original linkage to chromosome 2, implies that assignment of ARVC to chromosome 2 on the basis of aggregation of lod scores from three small families should possibly be considered erroneous.

Plakophilins, together with the other desmosomal proteins, assemble to form cell adhesion complexes that carry out diverse structural and functional tasks. These include mechanically safeguarding cellular and organ architecture by transmitting force between cells and participating in several signal transduction pathways. Support for the idea that plakophilins have an essential role in desmosome formation and function comes from this and two other reports: (i) the identification of recessive mutations in *PKP1* that cause ectodermal dysplasia/skin fragility syndrome, a rare skin disorder characterized by small, poorly formed desmosomes and perturbed desmosome-keratin intermediate filament interactions[14]; and (ii) the ablation of mouse *Pkp2*, the main plakophilin in cardiac muscle, which results in defects of heart morphogenesis and junctional architecture with embryonic lethality at midgestation[11]. Therefore, plakophilin-1 and plakophilin-2 have essential roles in desmosome formation and in skin and heart development, respectively.

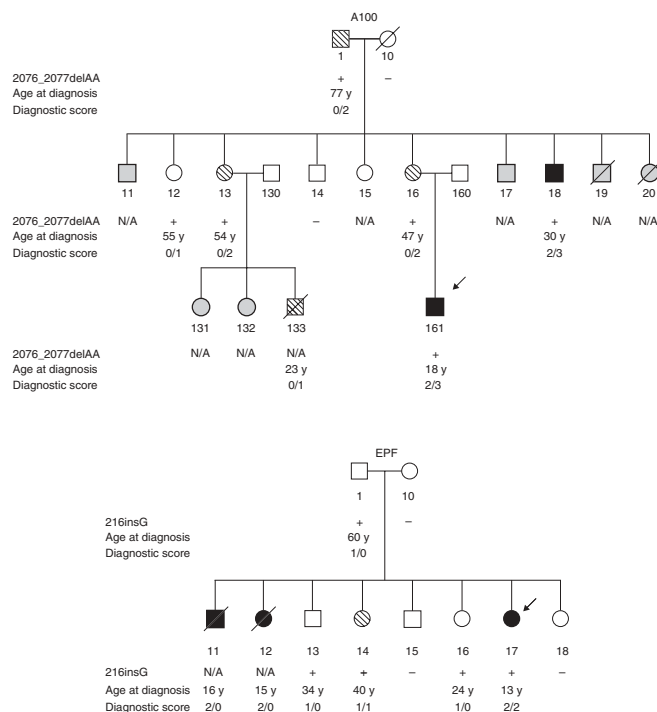How *PKP2* mutations perturb cardiac desmosome assembly and function in ARVC is unknown. We speculate that lack of plakophilin-2 or incorporation of mutant plakophilin-2 into cardiac desmosomes impairs cell-cell contacts and, as a consequence, disrupts adjacent cardiomyocytes, particularly in response to mechanical stress or stretch (thus providing a potential explanation for the high prevalence of the disorder in athletes, the frequent occurrence of ventricular tachyarrhythmias and sudden death during exercise and the predominant affection of the right ventricle). Intercellular disruption would occur first in areas of high stress and stretch: the right ventricular outflow tract, apex and inferobasal (subtricuspid) area, which are pathological predilection areas in ARVC (forming the 'triangle of dysplasia'[15]). The potential cellular mechanism for the initiation of ventricular tachyarrhythmias in ARVC is the intrinsic variation in conduction properties as a result of these patchy areas of fibrofatty myocyte degeneration.

Because mutations causing ARVC have been identified in *PKP2* (plakophilin-2), *JUP* (plakoglobin) and *DSP* (desmoplakin), ARVC may be considered as a disease of the desmosome.

All genetic analyses were done after informed consent was obtained from the participating individuals. The local ethical committees from Humboldt University Berlin, University of Münster, Weill Medical College of Cornell University and Harvard University approved the experimental plan.

Figure 1 Pedigrees of kindreds A100 and EPF with ARVC. Black, white, hatched and gray symbols represent clinically affected individuals, unaffected individuals, individuals of undetermined disease status and individuals of unknown disease status, respectively. Presence (+) or absence (–) of the *PKP2* mutation in those individuals available for mutation analysis is indicated. N/A, not available for genetic diagnosis. Arrows indicate index individuals. Diagnostic scores refer to proposed clinical criteria[12].

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