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Background—Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by progressive myocardial atrophy with fibrofatty replacement. The recent identification of causative mutations in plakoglobin, desmoplakin (DSP), and plakophilin-2 (PKP2) genes led to the hypothesis that ARVC is due to desmosomal defects. Therefore, desmoglein-2 (DSG2), the only desmoglein isoform expressed in cardiac myocytes, was screened in subjects with ARVC.

Methods and Results—In a series of 80 unrelated ARVC probands, 26 carried a mutation in DSP (16%), PKP2 (14%), and transforming growth factor-β3 (2.5%) genes; the remaining 54 were screened for DSG2 mutations by denaturing high-performance liquid chromatography and direct sequencing. Nine heterozygous DSG2 mutations (5 missense, 2 insertion-deletions, 1 nonsense, and 1 splice site mutation) were detected in 8 probands (10%). All probands fulfilled task force criteria for ARVC. An endomyocardial biopsy was obtained in 5, showing extensive loss of myocytes with fibrofatty tissue replacement. In 3 patients, electron microscopy investigation was performed, showing intercalated disc paleness, decreased desmosome number, and intercellular gap widening.

Conclusions—This is the first investigation demonstrating DSG2 gene mutations in a significant number of ARVC-unrelated probands. Cardiac phenotype is characterized clinically by typical ARVC features with frequent left ventricular involvement and morphologically by fibrofatty myocardial replacement and desmosomal remodeling. The presence of mutations in desmosomal encoding genes in 40% of cases confirms that many forms of ARVC are due to alterations in the desmosome complex. **(*Circulation*. 2006;113:1171-1179.)**

Key Words: arrhythmogenic right ventricular dysplasia ■ cell adhesion molecules ■ genetics ■ pathology ■ sudden death

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a myocardial disease characterized by progressive myocyte loss and fibrous and fatty tissue replacement of the right ventricular free wall, which is the substrate for reentrant arrhythmias and sudden death.[1–5] Familial occurrence is common.[6,7] Five disease genes have been identified thus far[8–12] that encode desmosomal proteins, except for the cardiac ryanodine receptor 2 (RyR2) gene reported in arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2)[9] and the transforming growth factor-β3 (TGFβ3) gene in arrhythmogenic right ventricular cardiomyopathy type 1 (ARVD1).[12] A deletion in plakoglobin has been proven to cause a recessive form of ARVC associated with palmoplantar keratosis and woolly hair, ie, Naxos disease.[8] More recently, mutations of desmoplakin (DSP) and plakophilin-2 (PKP2) genes have been found in ARVC, in the absence of skin and hair abnormalities.[10,11,13,14] Of note, a homozygous desmoplakin mutation has also been reported in Carvajal syndrome, which is characterized by hair and skin disorders associated with cardiac disease.[15,16] DSP, plakoglobin, and PKP2 are proteins of the intercellular junctions (fascia adherens and desmosome), which are responsible for the mechanical coupling of the myocytes and provide a continuous cell-to-cell connection to sarcomeric actin and intermediate filaments. The involvement of genes encoding desmosomal proteins in ARVC suggested that impaired cell adhesion might be among primary molecular defects.[17] Desmosomal adhesion is mediated by calcium-dependent cell adhesion glycoproteins (cadherins) of the desmocollin (DSC) and desmoglein (DSG) types, which interact later-

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TABLE 1. PCR Primers and Conditions for DHPLC Analysis of DSG2 Gene

Exon	bp	T°C DHPLC	Forward 5′ 3′	Reverse 5′ 3′
1	314	67/69,1	CCAGGGAGGAGCCGAGTG	ACCTGGGGCTTCGGAAAATC
2	391	54,8/55,1/57,5	AGATTTCTCCTCGGGCACT	ATCCAGGGGTATTCAGTGCA
3	463	Direct sequencing	GCCTCATAGGAAATACGAAGCA	GATTCTCTTTCCCATTCCGG
4	393	53,1/55	GGCTTTTGGCTAAGATCAAATC	ACAGGTTACGCTTTGGATGC
5	491	Direct sequencing	TTCTTGATCGAGAAGAAACACCA	GGACTTTACATGCCATTGCTT
6	490	53,8/55/55,8	CCCATTCACGCTTATGTCCT	TTTTAGGGATGTGCTGTCCA
7+8	700	Direct sequencing	TCTACAACTCCCGAGGCTTTTCT	TTTGAGCCCTGAACACTTAAAA
9	a 343	54,7/55,7/56,7	TGCTGCTATATTTCCTGTGCAT	GCAAAGGCCAAATAATTGGA
	b 355	54,8/56,9	CCTACACCCATTCCCATCAA	GATGAGGGGGAATTTTCACC
10	373	53,7/54,3	GAGAGGGGCTTTTAGGATGG	TTCCAACATCAGAAACCATTGA
11	466	55,4/56,8/59	TTAGTACCTTCTCCACTCCAAA	ATGAAATGAGGTCCTGAACC
12	362	Direct sequencing	GCAATGAAAGAACATTTGTGGA	GCTTTTGGGAATCTGAGTGC
13	289	55/57,5/59,5	GACAAGTCCAGGAAGGGACAT	TAAAGGGGCCTAGGGAAGTG
14	a 292	60,1	CTGGCCTCAGTGGAAATAGC	GGAAGAACACAGAAGCCTGC
	b 299	60,6	TCATTTCTGCCAGTGGATCA	GCTGTTGCACTGAACGAAGA
	c 225	62,8	GATGGAAGGTGGGAAGAACA	TTATGTATTGGTGGTGGGGG
	d 286	Direct sequencing	TGACCACTGAAACCACGAAG	AGGAAAGAGAAATGGGACCC
15	a 273	53,8/54,5/57,5	TTTCCCTGATGGTTCCTTGT	AAGGAGAGCTAGATGACCGC
	b 331	57,2	CGGCCTCTTACACTGAGGAA	TCTGGCAGTAGCTTCCCAGT
	c 377	56,5/57,9	TCGCTGAATGCTTCTATTGG	CCTGACCCAATGGCTTCTAG
	d 263	60,1	TCTGGCAGTAGCTTCCCAGT	GAGAGGGTGTATGCTCCAGC
	e 261	59.8	CACCTCTTCCTGACCCAATG	GGAAGGCACTCAGCATCTTC
	f 412	58/59/60	CCTTGGTAGATCAGCCTTATGC	TCCACCAGAGTTACCAAGCATAGCA
	g 305	57,2/58,5	ACGGTGTCTGGAGCTGGA	GACCACACTGTCTCTGCTTCC
	h 230	57,2	GCCACAAACTGACCCAGAGT	CTGCTGCTTAGGTGCCTTTT

ally and transcellularly.[18] Both desmosomal cadherins types are encoded by a small multigene family on chromosome 18q12.1, each of which consists of cell type–restricted members. Unlike other cadherins that are restricted to stratified epithelial tissues, DSG2 and DSC2 are expressed in all desmosome-possessing tissues, including myocardium and epithelia.[19]

We report here for the first time mutations of the DSG2 gene associated with ARVC and clinicopathological data of the 8 probands in whom such mutations were detected in the course of a systematic mutation screening.

Methods

Clinical Evaluation

The study was approved by the University of Padua Medical School–Azienda Ospedaliera ethical committee. Informed consent was obtained from all participating individuals. Clinical evaluation consisted of a detailed personal/family history, physical examination, 12-lead ECG, 2-dimensional echocardiogram, signal-averaged ECG (SAECG), and stress test ECG, performed according to previously reported methods.[7] Invasive studies including angiography and right ventricular endomyocardial biopsy were performed in selected cases when deemed necessary for the diagnosis. In 80 probands of Italian descent, a clinical diagnosis of ARVC was made on the basis of the established European Society of Cardiology/International Society and Federation of Cardiology Task Force major and minor criteria.[20]

Mutation Screening

The coding region of DSP, PKP2, and TGFβ3 genes was screened for mutations in all study subjects. Because none of the subjects had effort-induced polymorphic ventricular arrhythmias or gross skin and hair abnormalities, we chose not to screen RyR2 or plakoglobin. Thirteen probands (16%) carried a DSP mutation, 11 (14%) a PKP2 mutation, and 2 (2.5%) a TGFβ3 mutation. Fifty-four probands (33 males and 21 females) negative for mutations of these genes were considered for further investigation.

On the basis of previous findings on desmosomal genes involved in ARVC and on DSG2 expression in myocardial tissue,[21] we hypothesized that mutations in human DSG2 could account for ARVC. The 54 ARVC probands were screened for DSG2 mutations by denaturing high-performance liquid chromatography (DHPLC) and direct sequencing. Polymerase chain reaction (PCR) primers flanking each exon of the human DSG2 gene were designed by PRIMER3. PCR amplifications were performed in a final volume of 25 μL, containing 50 ng of genomic DNA, 1× PCR buffer II (Applied Biosystems), 1.5 mmol/L $MgCl_2$ (Applied Biosystems), 400 nmol/L each primer (Sigma Genosys), 100 μmol/L deoxynucleotide triphosphates (Invitrogen), and 0.8 U of *Taq* DNA polymerase (Taq Gold, Applied Biosystems). Cycling conditions (denaturation at 94°C for 30 sec, annealing at 60°C for 30 seconds, and extension at 72°C for 45 seconds) were repeated for 35 cycles. PCR primers and DHPLC conditions are reported in Table 1. DHPLC analysis was performed with the use of WAVE Nucleic Acid Fragment Analysis System 3500HT with DNASep HT cartridge (Transgenomic Ltd NE). Temperatures for sample analysis were selected with the use of WAVEMAKER software. The gradient mobile phase consisted of buffer A (0.1 mol/L triethyl ammonium

TABLE 2. Clinical, Pathological, and Genetic Findings in DSG2 Mutation Unrelated Carriers

			Family History		12-Lead ECG					Arrhythmias			RV Size/Function						
Proband (Family)	Sex	Age at Diagnosis/ Symptom Onset	Major	Minor	Negative T Waves Precordial Leads	Negative T Waves Inferior Leads	QS in II, III, aVF	Epsilon Wave	SAECG	PVCs >1000/24 hours NSVT	LBBB SVT	VF	Major	Minor	LV Involvement	Diagnostic Criteria	Histology	Nucleotide Change	Amino Acid Change
1	M	42	−	−	−	−	III	−	+	−	+	−	+	−	+	2 major/2 minor	+	298G→C	G100R
2	M	14	−	−	V_1 through V_3	−	−	+	+	+	−	−	+	−	+	3 major/2 minor	+	1253_1257 insATGA	E418fsX419
3	M	31	−	−	V_1 through V_3	III,aVF	−	−	+	+	−	−	−	+	−	4 minor	NP	2036delG	G678sX681
4	M	32	−	+	V_1 through V_4	III, aVF	−	−	−	+	−	−	−	+	−	4 minor	NP	260A→G	Y87C
5 (172)	F	63	−	+	V_1 through V_5	II,III, aVF	−	+	+	−	+	−	+	−	−	3 major/3 minor	+	991G→A 1881–2A→G	E331K Mutant splice product
6 (169)	F	55	−	+	V_1 through V_5	−	−	+	+	−	+	−	+	−	+	1 major/3 minor	NP	797A→G	N266S
7	F	58	−	+	V_1 through V_4	−	−	−	+	+	−	−	−	+	+	1 major/4 minor	+	877A→G	K294E
8	M	11	−	−	V_1 through V_3	−	−	−	+	+	−	−	+	−	−	1 major/3 minor	+	1672C→T	Q558X

LBBB indicates left bundle-branch block; LV, left ventricular; NP, not performed; NSVT, nonsustained ventricular tachycardia; RV, right ventricular; SVT, sustained ventricular tachycardia; and VF, ventricular fibrillation.

acetate, pH 7, Transgenomic Ltd) and buffer B (0.1 mol/L triethyl ammonium acetate, pH 7, and 25% acetonitrile). Buffers were mixed to produce a linear gradient varying buffer B over a 2-minute period at 1.5 mL/min. After each analysis the cartridge was cleaned for 0.2 minutes with 75% acetonitrile and equilibrated at 5% buffer B below the gradient starting concentration to allow for sample loading. Samples showing a change in DHPLC pattern were directly sequenced with the use of the BIG DYE dideoxy-terminator chemistry (Perkin Elmer) on an ABI 3730XL DNA sequencer (PE Applied Biosystems). Chromas 1.5 software (Technelysium) and LASERGENE package computer programs (DNASTAR) were used to edit, assemble, and translate sequence. DSG2 sequences were compared with reference sequence BC099656.

A control group of 280 healthy and unrelated subjects (560 alleles) from the Italian population was used to exclude that detected mutations could be common DNA polymorphisms. All the controls were matched to the probands by ancestry.

Mutation screening was performed in all available family members of index cases in which a DSG2 mutation was detected. For further analysis of the splice site mutation, mRNA was isolated from 2.5 mL whole blood of the patient, with the use of the PAXgene Blood RNA kit (Qiagen) following the supplier's protocol. The corresponding cDNA was obtained from a reverse transcriptase (RT)–PCR reaction with the use of 1 μg total RNA. The following primers were used in the first-round PCR to amplify the fragment corresponding to exons 12 to 15: 5′CAGTTTGTGAGTGTCTGCATGG3′ and 5′ACTGGGAAGCTACTGCCAGA3′. DSG2 cDNA products were amplified by the use of nested PCR oligonucleotides (5′CACAGCATGACTCCTATGTGG3′ and 5′GCGGTCATCTAGCTCTCCTT3′). The nested PCR products were size separated by agarose gel electrophoresis, gel isolated, and directly sequenced.

Identified mutations altering restriction sites were confirmed by restriction digest, accordingly to the manufacturer's protocol (New England Biolabs).

Light Microscopy

A right ventricular endomyocardial biopsy was obtained in 5 probands via the femoral vein, by the long sheath technique (disposable Cordis bioptome). The samples were obtained at the junction between the ventricular septum and the anterior right ventricular free wall. Biopsy specimens were fixed in 10% phosphate-buffered formalin (pH 7.35) and then processed for histology. Seven-micrometer-thick paraffin-embedded sections were serially cut and stained by hematoxylin-eosin and Heidenhain trichrome. Histomorphometric analysis was performed on samples stained with Heidenhain trichrome with the use of an image analyzer system and commercially available software (Image-Pro Plus Version 4.0) accordingly to a previously described method.[22] A diagnosis of ARVC was made on the basis of a significant amount of myocardial atrophy and fibrofatty tissue replacement.

Ultrastructural Investigation

In 3 patients, an endomyocardial biopsy sample was routinely fixed in 2.5% glutaraldehyde in 0.1 mmol/L phosphate buffer (pH 7.3) and postfixed in buffered 1% osmium tetroxide for 1 hour. Samples were then dehydrated in a series of ethanol and embedded in Epon. Semithin sections were first evaluated under light microscopy before we proceeded with the ultrathin sections. Thin sections were stained with uranyl acetate and lead citrate and examined under a Hitachi H-7000 electron microscope equipped with a digital camera.

Intercalated discs were assessed at a final magnification of $\times$30 000 and $\times$60 000 accordingly to a standardized method.[23] By histomorphometric analysis, intercalated disc convolution index, desmosome length, desmosome number per 10 μm unity length of intercalated disc, and gap sizes were calculated. Values were compared with those obtained in endomyocardial biopsies from 10 sex- and age-matched donor hearts before cardiac transplantation.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Main clinical, pathological, and genetic findings of DSG2 mutations carriers are reported in Table 2.

Mutations in Desmoglein-2

Nine DSG2 mutations have been identified in 8 (5 males and 3 females; mean age at diagnosis/symptom onset, 38$\pm$20 years; range, 11 to 63 years) of 80 ARVC index cases (10%).

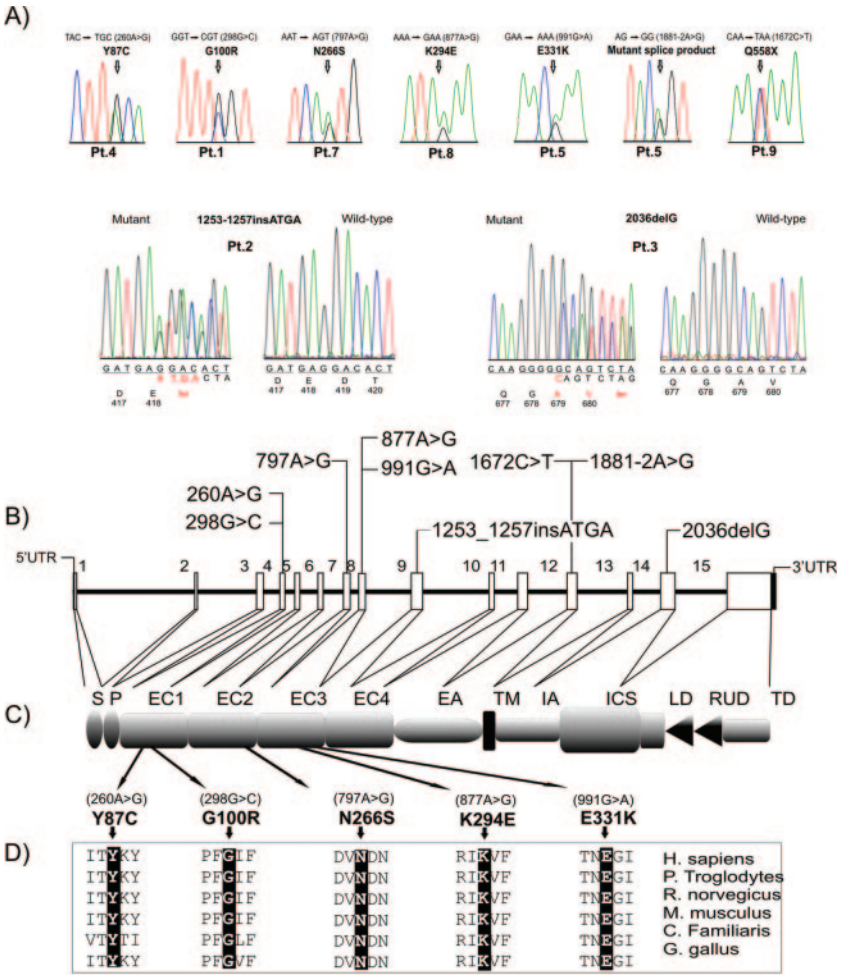

Figure 1. A, DSG2 mutations associated with ARVC. Top, Sequence electropherograms show the 5 DSG2 missense mutations (260A→G, 298G→C, 797A→C, 877A→G, 991G→A), 1 nonsense mutation (1672C>T), and 1 splice site (1881 to 2A→G). Bottom, Sequence electropherogram showing the 2 insertion-deletions mutations of DSG2 (1253 to 1257insATGA, 2036delG) compared with the wild types. Numbering of the nucleotides starts at ATG and refers to Genbank Accession number BC099657. B, Genomic organization of the human DSG2. In DSG2 structures, exons are represented by vertical white boxes, and introns are represented by horizontal lines. The gene consists of 15 exons, spanning ≈48.6 kb of genomic DNA. Positions of the 9 DSG2 mutations are indicated. C, The encoded protein, DSG2, comprises a signal (S) domain and a preprotein (P) domain followed by 5 extracellular domains (EC1, EC2, EC3, EC4, and EA), a transmembrane domain (TM), an intracellular anchor domain (IA), an intracellular cadherin-typical segment domain (ICS), a linker domain (LD), a repeat unit domain (RUD) containing 6 repeats, and a terminal domain (TD). D, Evolutionary conservation of the 5 DSG2 missense mutations (Y86C, G100R, N266S, K294E, and E331K) among 6 species: *H sapiens* (AAH9965), *P troglodytes* (XP_512079), *R norvegicus* (XP_574106), *M musculus* (NP_031909), *C familiaris* (XP_547622), and *G gallus* (XP_426083). The mutated amino acids are marked by arrows, and the identities across species are indicated by a black background.

Overall, DSP, PKP2, TGFβ3, and DSG2 account for 42.5% of genotyped ARVC probands.

Of the 9 DSG2 mutations, 5 were missense, 2 insertion-deletions, 1 a nonsense, and 1 a splice site mutation (Figure 1). None of the detected nucleotide changes was found in 560 control chromosomes. Identified mutations altering restriction sites were confirmed by restriction digest. The PCR products were submitted to digestion with the following restriction enzymes: *HpyCH*4V for the Tyr87Cys, *Pfl*mI for Gly100Arg, *Hpy*AV for Glu331Lys, *Alw*NI for Gln557Ter, and *Bsm*AI for 1253_1257insATGA.

Missense mutations Y87C, G100R, N266S, K294E, and E331K occurred in residues that are highly conserved among species. Mutations Y87C, G100R, and N266S, which are also conserved in all the desmogleins, and K294E and E331K are located in the extracellular cadherin (EC) domains. Cadherin domains are important for homophilic intercellular associations and form Ca^{2+}-dependent rodlike structures. All the inherent amino acid changes may destabilize the rod structure and influence the homophilic binding. Moreover, mutation N266S is localized in a putative calcium binding site (DXNDN).

The 4-bp insertion in exon 9 (1253_1257insATGA) causes the addition of an amino acid residue before a premature stop signal is introduced (E418fsX419); the predicted truncated DSG2 molecule would lack transmembrane and cytoplasmic components.

The single base pair deletion in exon 14 (2036delG) results in a frameshift mutation that would leave the first 678 amino acids of the DSG2 protein intact, followed by addition of 2 novel amino acids (G678fsX681). The predicted truncated protein should maintain the transmembrane domain, but it would lack the intracellular cadherin-typical segment, thus precluding normal interaction with plakoglobin.

Mutation 1672C→T generates a termination codon (Q558X); therefore, the mutant should encode a short DSG2 molecule lacking transmembrane and cytoplasmic domains.

Mutation 1881 to 2A→G affects the acceptor splice site of intron 12. Sequencing analysis of the aberrant DSG2 transcript obtained from lymphocyte RNA of the proband showed that this mutation activates an alternative cryptic splice site in exon 13, located 38 bp downstream from the authentic 3′ splice acceptor site. This aberrant spliced mRNA contains a 38-bp deletion (Figure 2) and should code for a truncated protein of 646 amino acids in length, missing the cytoplasmic domain.

In 1 patient (patient 5), 2 different mutations (E331K and 1881 to 2A→G) were detected. Family members were available for molecular analysis (Figure 3, family 172).

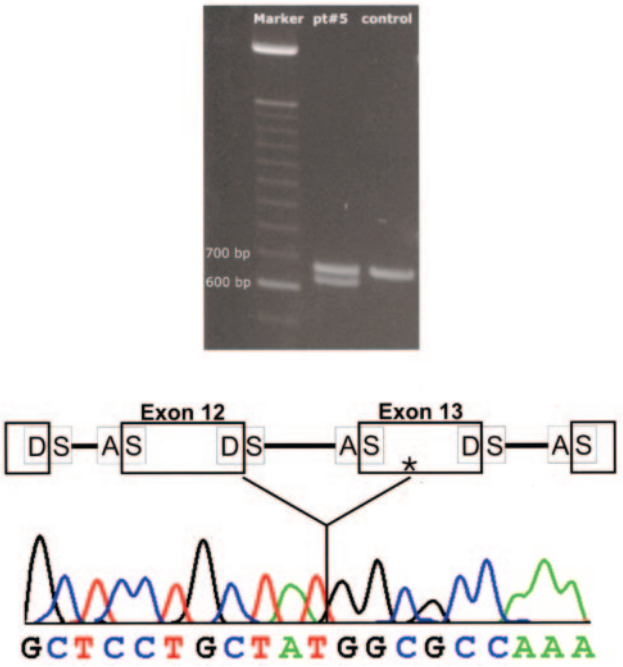

Figure 2. Gel electrophoresis showing normal (668 bp in length) and mutant (630 bp) RT-PCR fragments caused by the DSG2 splice-site mutation (1881 to 2A→G). Patient 5 shows both the wild-type and the aberrant spliced fragment (top). Sequence electropherogram of the aberrant fragment is shown. Mutation 1881 to 2A→G affects the acceptor splice site (AS) of intron 12 and activates an alternative cryptic splice site (asterisk) in exon 13. DS indicates donor splice site (bottom).

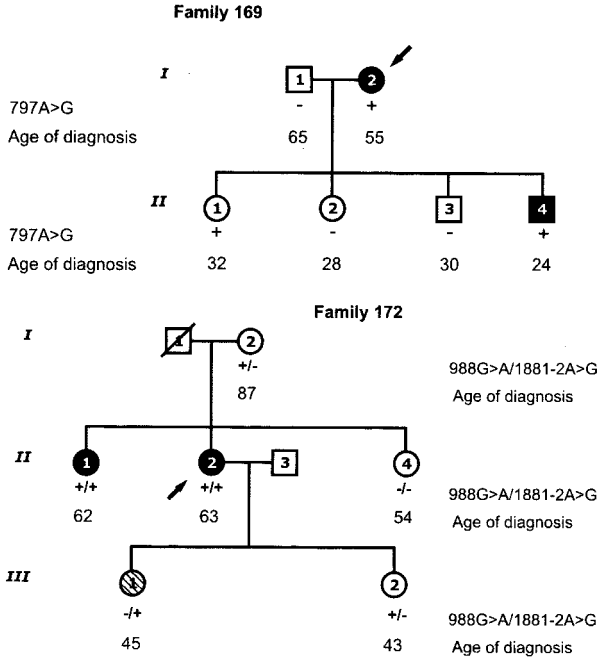

Figure 3. Pedigrees of probands 169 (patient 6) and 172 (patient 5) with ARVC. Black, white, and hatched symbols represent clinically affected individuals, unaffected individuals, and individuals of unknown disease status, respectively. Presence (+) or absence (−) of the DSG2 mutation is indicated. Arrows indicate index cases.

Subject II,1 carried both mutations, subjects I,2 and III,2 carried the missense mutation, and subject III,1 carried the splice site mutation, thus demonstrating that the 2 mutations are in *trans* orientation. Although both mutations are potentially pathogenic, the possibility that the missense mutation is a rare polymorphism cannot be ruled out.

Clinical Findings

Clinical Presentation

The first symptom consisted of sustained ventricular tachycardia (VT) in 3 patients, palpitations in 3, and chest pain with increased serum markers of myocardial necrosis (creatine phosphokinase/MB 363/45 U/L and troponin I 11.9 μg/L) in 1 who had angiographically normal coronary arteries. One patient was asymptomatic and was examined because ECG abnormalities were detected at preparticipation screening for sport activity. Skin and hair were grossly normal at physical examination in all. Mean age at symptom onset/diagnosis was 26±13 years in males versus 58±4 years in females (P=0.001).

ECG/SAECG Findings

All patients showed ECG abnormalities (Table 2) (Figure 4A), consisting of complete right bundle-branch block in 1, incomplete right bundle-branch block in 3, negative T waves in the precordial leads in 7, negative T waves in inferior leads in 3, QS aspect in inferior leads in 1, epsilon wave in 3, low voltages of QRS in 1, and ST segment elevation in 1. Moreover, 3 patients showed a PQ prolongation (PQ >200 ms).

Late potentials were detected in 7 subjects and were evidenced with 25-, 40-, and 80-Hz filters in 4 cases and with 40- and 80-Hz filters in 3 cases.

Ventricular Arrhythmias

Ventricular arrhythmias were recorded in all probands and ranged from sustained VT with left bundle-branch block morphology (3 cases) to nonsustained VT (3 cases) and isolated monomorphic PVCs (2 cases).

Echocardiographic Findings

Abnormal echocardiographic findings were present in all probands, with right ventricular kinetic abnormalities involving only 1 region in 1 patient and ≥2 regions in the remaining 7 (Figure 4B). A left ventricular involvement was present in 4 patients, with normal left ventricular ejection fraction in 1 (57%) and mildly decreased left ventricular ejection fraction in 3 (ranging from 45% to 48%); left ventricular kinetic abnormalities were localized in 1 and diffuse in 3 patients.

Familial Study

A family history of premature sudden death due to suspected ARVC was present in 1 proband and of clinically proven ARVC (based on diagnostic criteria) in 3 probands.

Family members of 2 index cases (patient 5, family 172 and patient 6, family 169) were available for clinical and genetic testing (Figure 3). In family 172, subject II,1, who

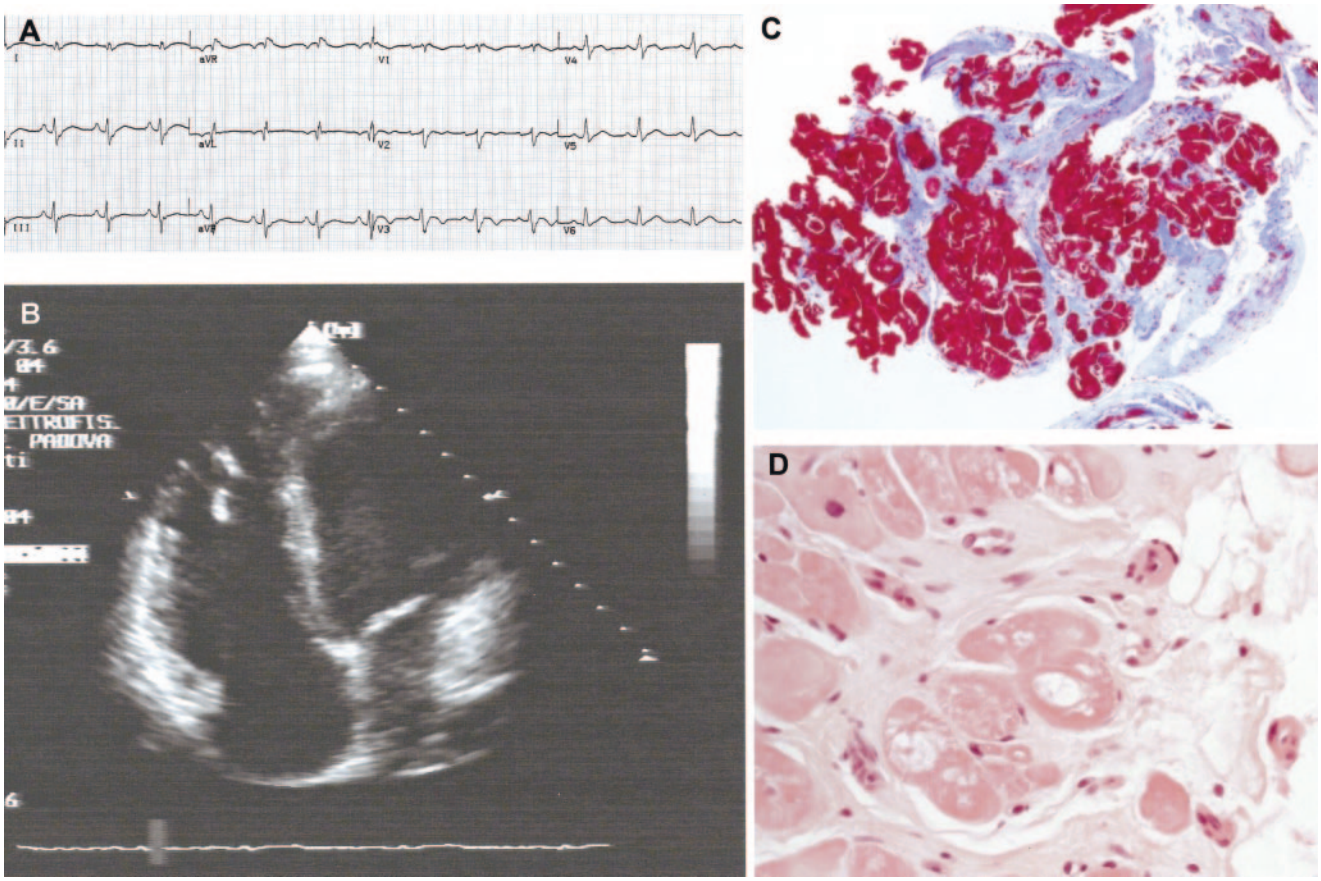

Figure 4. Proband 2, a 14-year-old boy who was diagnosed during preparticipation screening for sports eligibility. A, Twelve-lead ECG. Note the presence of complete right bundle-branch block, right atrial enlargement, negative T waves in V_1 through V_3, and epsilon wave. B, Apical 4-chamber view showing a right ventricular enlargement, apical aneurysm, and a hyperreflective moderator band. C, Biopsy sample showing myocardial atrophy with replacement-type fibrosis and mild fatty infiltration (trichrome Heidenhain, magnification ×20). D, At higher magnification, prominent degenerative changes of the myocytes close to fibrofatty tissue, consistent with cytoplasmic vacuolization, are evident (hematoxylin and eosin; magnification ×80).

was found to carry both mutations, showed negative T waves in V_1 through V_4 on 12-lead ECG, right ventricular enlargement and kinetic alterations on 2D echocardiogram, and premature ventricular beats (>1000/24 h) with left bundle-branch block morphology. Clinical investigation in subjects I,2 and III,2 was negative, whereas it was not feasible in subject III,1.

In family 169, the DSG2 mutation N266S was found in a daughter (subject II,1) and a son (subject II,4) of the index case. Clinical findings were negative in the former, whereas subject II,4 showed negative T waves in V_1 through V_2 on 12-lead ECG, right ventricular dilation together with kinetic abnormalities on 2D echo, and nonsustained ventricular arrhythmias.

All family members not carrying DSG2 mutations were negative at clinical investigation.

Light Microscopy and Electron Microscopy Findings

In the 5 patients who underwent endomyocardial biopsy (patients 1, 2, 5, 8, 9), histomorphometric evaluation revealed a mean area of residual myocardium 47±8%, fibrous tissue 24±11%, and fatty tissue 20±13%. Moreover, dysmetric and dysmorphic nuclei and prominent cytoplasmic vacuolization were evidenced in all (Figure 4C and 4D).

In 3 patients (patients 1, 2, 8), electron microscopy investigation was also performed. At the ultrastructural level, we found a decreased desmosome number per 10 μm unity length of intercalated disc (3.1±0.4 versus 5.5±3 in controls) and an increased desmosome gap (31.7±17.8 versus 21.7±3.4 nm in controls). Moreover, abnormal small junctions composed of series of repeating couplings, abnormally located desmosomes, and pale internal plaques were visible compared with controls (Figure 5).

Discussion

We identified DSG2 as a novel disease gene involved in ARVC, a genetically determined myocardial disease recognized as a common cause of sudden death among young adults.[1–5] No association of DSG2 mutations with inherited human diseases has been reported thus far. Desmosomes consist of 2 desmosomal-specific cadherin family members, DSGs and DSCs, as well as a collection of cytoplasmic plaque proteins including plakoglobin, DSP, and PKPs. All cadherins have tripartite functional domains: (1)

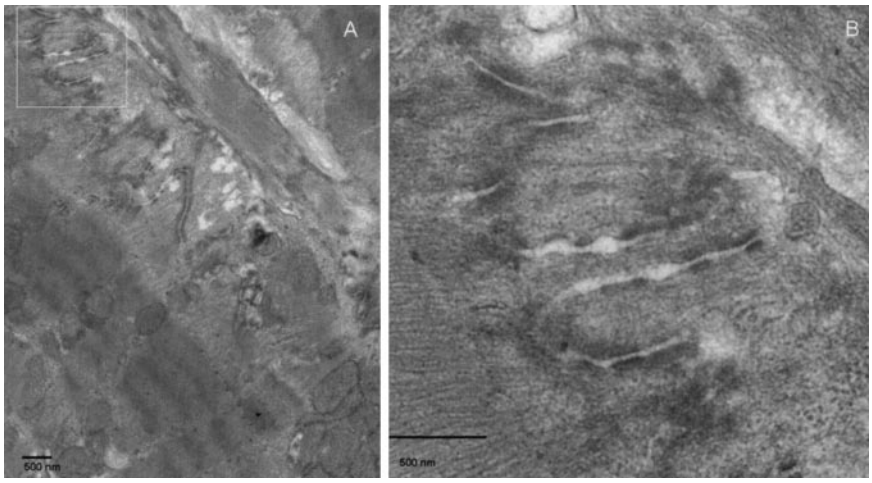

Figure 5. Electron microscopy findings in the same patient as shown in Figure 4. A, Panoramic view of cardiac myocyte intercalated disc showing multiple widened gaps of fascia adherens and desmosomes (magnification ×12 000); B, close-up of the boxed area in A (magnification ×30 000).

a calcium-inducible, extracellular amino terminal domain, important for homophilic intercellular associations, with 4 domains (EC1 to EC4), followed by an extracellular anchor domain (EA); (2) a single transmembrane domain; and (3) a cytoplasmic domain anchoring the cytoskeleton, an essential process for cell adhesion.[24,25]

Among the 9 mutations herein reported, 7 are located in the EC amino terminal domain, which directly participates in the adhesive interaction. Crystallographic and biochemical studies of classic cadherin ectodomains suggest that they form homophilic dimers in *cis* and in *trans* orientations.[26] Calcium binding to the EC domains is important for stabilizing their structure and function. Whereas *trans*-dimerization is likely required to form the adhesive interface in adjacent cells, it has been proposed that *cis*-dimerization is important for promoting adhesion via molecular clustering.[26] It is possible that even a single amino acid change could result in differences in molecular affinity and possibly in the abolition of the adhesive capacity of cadherins. In vitro functional studies are needed to evaluate this.

Effect of the DSG2 Mutations at the Cellular Level

Unlike PKP2 and plakoglobin, no data are available on cardiac abnormalities in the DSG2 knockout mouse because loss of DSG2 resulted in early embryonic lethality.[27] In vitro antisense experiments against DSC2,[28] expression of truncated DSG3 and DSG1 proteins,[29,30] and knockout mice of the DSG3 gene[31] all resulted in a decreased number of desmosomes, associated with increased asymmetry and detachment. These electron microscopy observations are in agreement with those obtained in 3 DSG2 mutations carriers herein reported, which show a decreased number of desmosomes as well as a widening of intercalated disc gap. On the basis of these ultrastructural findings and of the histological demonstration of myocardial atrophy in patients who underwent endomyocardial biopsy, we can postulate that in desmosomal cardiomyopathies (ie, Naxos syndrome, Carvajal syndrome, and ARVC),[8,10–12,14–16] impaired cell-to-cell adhesion leads to myocyte detachment, cell death, and fibrofatty repair.[17,32] Moreover, because TGFβ3 could modulate expression of genes encoding desmosomal proteins in different cell types,[33] the same pathogenic mechanism could be advocated in ARVD1 patients.[12]

From the clinical point of view, in our series of unrelated DSG2 mutation carriers, a typical form of ARVC was documented with left ventricular involvement in almost half of the cases. Genotyping of available family members of 2 index cases allowed us to demonstrate the transmission of the disease allele in all affected relatives. However, prospective studies of a larger number of family members are needed to evaluate the penetrance of DSG2 mutations and the entire clinical spectrum of the disease from the concealed to the overt forms. It is noteworthy that both sexes are represented equally, although the mean age at symptom onset/diagnosis was significantly higher in female than in male mutation carriers. These data confirm previous observations from clinical series and could be interpreted either as a gender-related penetrance or as the consequence of additional acquired and/or genetic factors involved in disease expression.[13]

None of the 8 probands carrying the pathogenic mutations showed gross skin/hair abnormalities. All DSG isoforms are expressed in the epidermal tissues, whereas DSG2 is the only one expressed in the myocardium.[21] We may hypothesize that compensation by other DSG isoforms might take place in the epidermis but not in the myocardium of DSG2 mutation carriers, thus accounting for the cardiac-specific phenotype.

Clinical diagnosis of ARVC is challenging, and the currently available diagnostic criteria, although acknowledged to be specific, are lacking in sensitivity. Because mutation detection is feasible in a significant proportion of patients, genetic screening could assume a pivotal role in the clinical evaluation of familial forms. In this setting, the benefits of detection of disease-gene mutations would include identification of asymptomatic carriers among family members and interpretation of borderline clinical phenotypes.

In conclusion, this is the first demonstration that mutations in DSG2 gene are associated with ARVC. On the basis of our data showing that 40% of ARVC probands carry a mutation in desmosomal proteins encoding genes,

we confirm that many forms of ARVC are due to alterations in the desmosome complex.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The recent identification of causative mutations in plakoglobin, desmoplakin, and plakophilin-2 genes in arrhythmogenic right ventricular cardiomyopathy (ARVC) led to the hypothesis that many forms of ARVC are due to alterations in the desmosome complex. In this article, the authors demonstrate for the first time that mutations in desmoglein-2, the only desmoglein isoform reported thus far as expressed in cardiac myocytes, are associated with ARVC. With this new discovery, mutation detection by genetic screening is now achievable in a significant proportion of probands affected by ARVC. In the clinical evaluation of family members, genetic screening is assuming a pivotal role, allowing the early identification of asymptomatic carriers and interpretation of borderline clinical phenotypes. On the other hand, the identification of a growing number of asymptomatic carriers with mild or subclinical disease expression leads to new problems in clinical management. When one takes into account that sudden death is not so rarely the first manifestation of ARVC, risk stratification remains a clinical challenge, and noninvasive markers able to predict the risk of life-threatening ventricular arrhythmias in mutation carriers should be prospectively evaluated in international registries.