A Recessive Mutation in Desmoplakin Causes Arrhythmogenic Right Ventricular Dysplasia, Skin Disorder, and Woolly Hair

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OBJECTIVES
The goal of this study was to analyze the genetic disorder of a family with cardiomyopathy, skin disorder, and woolly hair.

BACKGROUND
Arrhythmogenic right ventricular dysplasia (ARVD) is a heart muscle disorder causing arrhythmia and sudden cardiac death. We report a patient with familial autosomal recessive ARVD, woolly hair, and a pemphigous-like skin disorder with a new mutation in the desmoplakin gene.

METHODS
Genomic deoxyribonucleic acid was extracted from the patient’s blood and 12 first- and second-degree family members, and was amplified by polymerase chain reaction. Linkage analysis with polymorphic microsatellites was performed for 11 genes that code for structural desmosomal proteins. The genetic locus of the disease in this family was mapped to the chromosomal region 6p24 that contains the desmoplakin gene. Exons of the desmoplakin gene were analyzed by single-strand conformational polymorphism and direct sequencing. Confirmation of the mutation was carried out by restriction enzyme analysis.

RESULTS
We identified in the patient a homozygous missense mutation in exon 24 of the desmoplakin gene, leading to a Gly2375Arg substitution in the C-terminal of the protein where the binding site to intermediate filaments is located. Eight of 12 family members without hair or skin abnormalities were heterozygous for this mutation. The remaining 4, as well as 90 unrelated healthy control individuals of the same ethnic origin, were homozygous for the normal allele.

CONCLUSIONS
We have described a new mutation in the desmoplakin gene that causes familial ARVD. These findings suggest that desmosomal proteins play an important role in the integrity and function of the myocardium. Dysfunction of these proteins can lead to the development of cardiomyopathies and arrhythmias. (J Am Coll Cardiol 2003;42:319–27) © 2003 by the American College of Cardiology Foundation

Arrhythmogenic right ventricular dysplasia (ARVD) is an idiopathic myocardial disease, characterized by fatty or fibrofatty replacement of the ventricular myocardium (1–3). A combined European Society of Cardiology/International Society and Federation of Cardiology (ESC/ISFC) task force has proposed diagnostic criteria for ARVD (4); ARVD is clinically characterized by ventricular arrhythmia of right ventricular origin (5), sudden death, and, occasionally, right heart failure (1). It is a major cause of sudden death in the young, especially during physical activity (6,7).

In about one-third of patients, ARVD is familial and mostly inherited as an autosomal dominant trait, with incomplete penetrance and variable degrees of clinical expression (8,9). Autosomal dominant forms of ARVD have been mapped by linkage analysis to chromosomal loci at 14q23 (10), 1q42 (11), 14q12 (12), 2q32 (13), 3p23 (14), and 10p12-14 (15). In one of these loci (1q42), mutations in the cardiac ryanodine receptor gene have been identified (16). Recently, Rampazzo et al. (17) reported a mutation in human desmoplakin gene causing a dominant form of ARVD.

A syndrome of autosomal recessive ARVD, skin disorder, and woolly hair has been reported in families in the island of Naxos. Genetic analysis has shown that Naxos disease is caused by a mutation in the gene for plakoglobin (18,19). Another familial syndrome affecting heart, skin, and hair has been described in families from Ecuador. In these families the heart disease was characterized as dilated cardiomyopathy of the left ventricle, and the cause is a recessive mutation in the gene for desmoplakin (20).

Both plakoglobin and desmoplakin are components of desmosomes (21). Desmosomes are major cell adhesion junctions prominent in the epidermis and cardiac tissue that are important for rigidity and strength of cells (21,22). We identified a new recessive mutation in the desmoplakin gene leading to a Gly2375Arg substitution in the C-terminal of the protein, where the binding site to intermediate filaments (IFs) is located in a family with a syndrome of ARVD, pemphigous-like skin disorder, and woolly hair.
METHODS

Subjects and clinical material. The family members studied are of Muslim-Arab origin from Jerusalem. Detailed questioning and complete physical examination of 12 available family members obtained a description of the family pedigree, manner of inheritance, and clinical features, especially for skin and hair abnormalities. Resting 12-lead electrocardiogram (EGC) and two-dimensional transthoracic echocardiography was performed on three subjects. The patient underwent computed tomography (CT) scan and electrophysiologic study. The diagnosis of ARVD was based on ESC/ISFC guidelines (4). A dermatopathologist reviewed slides of a skin biopsy from a deceased affected sibling, performed during evaluation of skin lesions at the age of 7 years.

Genetic evaluation. After informed consent, venous blood samples from the patient and first- and second-degree family members were taken. Genomic deoxyribonucleic acid (DNA) was extracted using Puregene kit (Gentra system, catalog no. D-k50, Minneapolis, Minnesota). Genomic DNA was also extracted from 90 unrelated individuals from the same ethnic origin, to serve as controls. DNA was also extracted from 90 unrelated individuals from catalog no. D-k50, Minneapolis, Minnesota). Genomic (DNA) was extracted using Puregene kit (Gentra system, catalog no. D-k50, Minneapolis, Minnesota).

Samples from the patient and first- and second-degree available family members obtained a description of the family pedigree, manner of inheritance, and clinical features, especially for skin and hair abnormalities. Resting 12-lead electrocardiogram (ECG) and two-dimensional transthoracic echocardiography was performed on three subjects. The patient underwent computed tomography (CT) scan and electrophysiologic study. The diagnosis of ARVD was based on ESC/ISFC guidelines (4). A dermatopathologist reviewed slides of a skin biopsy from a deceased affected sibling, performed during evaluation of skin lesions at the age of 7 years.

Pedigree analysis of this family with a high rate of consanguinity suggested an autosomal recessive inheritance.

This allowed performing an efficient homozygosity mapping, based on a candidate gene approach (23). The homozygosity mapping was done in the patient and five first-degree family members for several suspected genes. Based on previous studies (19,20), we assumed that a defect in one of the desmosomal proteins was underlying the syndrome in the family studied. We constructed haplotypes using polymorphic microsatellites markers (dinucleotide [CA] repeats) that, according to known genetic maps (24,25), are linked to genes, encoding structural desmosomal proteins, and known to be expressed in the heart muscle and skin (22). We looked for homozygosity in the affected proband as compared with nonaffected siblings. The selected candidate genes and the informative markers are listed in Table 1.

Each marker was amplified by polymerase chain reaction (PCR) in a solution containing PCR buffer (1, 1.5 mM MgCl2, 200 mM—each deoxyribonucleoside triphosphate, 200 ng of template DNA, and 2.5 U of Taq DNA polymerase. Reaction was done with 30 cycles at 94°C for 30 s, annealing for 60 s at 58°C, and extension at 72°C for 60 s. Primers were designed according to sequence of both sides of the markers; PCR products were analyzed by electrophoresis on 8% urea, 8% polyacrylamid gel (500 V, 20 h), and were visualized by silver staining, using the protocol recommended by Promega (Madison, Wisconsin).

After the linkage analysis, the 24 exons of the desmoplakin gene were amplified by PCR. Primers were designed to amplify the entire coding region of the gene; PCR products were analyzed by single-strand conformational polymorphism (SSCP) gel electrophoresis (6% acrylamide, 5% glycerol, 1.5 W, 20 h) and by direct sequencing; PCR products were also subjected to restriction analysis using SmaI restriction enzyme.

In order to examine conservation of this genetic region, a BLAST search for related proteins was performed with the deduced protein sequence (CAAA19927) against the protein nonredundant database (26). The results of the search are available at the BLink (BLAST Link) section of the protein.

Table 1. Results of Haplotype Mapping for 11 Genes and 10 Informative Microsatellite Markers for the Patient and First-Degree Family Members

<table>
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<th>Gene</th>
<th>Genetic Locus</th>
<th>Marker</th>
<th>No. of Alleles</th>
<th>P</th>
<th>M</th>
<th>B1</th>
<th>B2</th>
<th>S1</th>
<th>S2</th>
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*The interval between the markers D18S456 and D18S457 contains the cluster of six genes: Desmocollin 1,2,3 and Desmoglein 1,2,3.

AF = alleged father; BPAG1 = bullous pemphigoid antigen I; B1, B2 = brothers; M = mother; P = patient; S1, S2 = sisters. Mapping results for the markers linked to the desmoplakin gene (bolded).
sequence entry (CAA19927) at the National Center for Biotechnology Information protein database.

RESULTS

Clinical features. This clinical syndrome consists of sudden death with hair and skin abnormalities. Family medical history was significant for eight members who died suddenly during young adulthood (ages 15 to 30 years). All of them had woolly hair and exceptionally dry skin in all parts of the body beginning at birth. During childhood, they presented with vesicular lesions on the extremities, particularly on the knees, palms, and soles. Biopsy from a lesion taken from the deceased patient’s brother, who died at the age of 23, demonstrated histology similar to pemphigous foliaceous (Fig. 1). The affected family members died before we carried out this study. They did not have major cardiac symptoms before their sudden death and did not undergo any cardiac evaluation.

We identified one living affected female with similar skin and hair abnormalities as described above. She presented with recurrent syncope at the age of 16. Ventricular tachycardia (VT) of right ventricle origin was documented (Fig. 2). The origin of the VT is in the midseptal area, which is not the typical origin in ARVD; however, ventricular arrhythmia can originate from different parts of the right ventricle in ARVD patients (5). Resting 12-lead ECG demonstrated T-wave inversion with localized prolongation of the QRS complex in the right precordial leads (V1 to V3) and normal QT interval (Fig. 2).

Transthoracic echocardiography (two-dimensional) revealed a mildly dilated right ventricle without regional wall motion abnormalities or aneurysmatic dilation, a normal left ventricle, and no significant valvular defects or pulmonary hypertension; magnetic resonance imaging was not performed because of prior implantable cardiac defibrillator (ICD) implantation. Cardiac CT scan was not informative because of artifacts from the ICD electrodes, located in the right ventricle. Based on the ESC/ISFC criteria (4), a diagnosis of ARVD was made (one major and four minor criteria; typical arrhythmia, ECG changes, echocardiographic changes, and family history of sudden death). Ventricular arrhythmia was not inducible on electrophysiologic study, and the patient was treated with an ICD. Antiarrhythmic medication was added to prevent recurrent VT.

No skin and hair abnormalities were found in 12 of the patient’s first- and second-degree relatives. Cardiac assessment of two heterozygous subjects for the disease mutation (the patient’s mother and cousin) revealed no abnormalities.

In summary, this family presented with “Naxos-like” syndrome with ARVD and sudden death, woolly hair, and pemphigous-like skin disorder. Constructed pedigree (Fig. 3) showed a high rate of consanguinity with various affected individuals, suggestive of an autosomal-recessive inheritance pattern. The available heterozygous individuals demonstrated normal cardiac (two subjects) and skin (eight subjects) phenotype.

Genetic analysis. Haplotyping with 20 microsatellite markers was performed in the patient, her mother, and four siblings; eight of them were informative. The results are summarized in Table 1. Homozygosity in the affected individual compared with heterozygosity in the unaffected family members was demonstrated in the marker D6S296, mapping to chromosomal region 6p24. This marker is located 0.6cM from the desmoplakin gene. No other genetic loci showed similar results, and homozygous state of the marker alleles in the patient exclude the possibility of a relevant homozygous mutation in these loci.
To confirm these findings, two additional microsatellite markers were genotyped. We chose the markers Des.mic.1 and Des.mic.3 that are located inside the introns of the desmoplakin gene (20). Haplotyping of these markers demonstrated homozygosity only in the affected individual (Table 1). These results suggest that the desmoplakin gene might be the mutated gene in this family.

The desmoplakin gene contains 24 exons and 9,588 bases messenger ribonucleic acid coding for a 2871 aa protein; PCR products of all exons were analyzed by SSCP and direct sequencing. Sequence analysis of exon 24 revealed a point mutation in position 7402 causing substitution of G in the wild type to C in the patient leading to a Gly2375Arg substitution in the protein (Fig. 4). Sequence analysis of the rest of the gene did not reveal other mutations.

The G2375R mutation in the desmoplakin gene creates a new recognition site for the restriction enzyme SmaI that facilitates rapid screening for the mutation in other family members. We amplified by PCR a 722 base pair (bp) fragment from exon 24 of the desmoplakin gene that contains the mutation. After digestion with SmaI, the mutant allele was cleaved to two fragments of 365 and 357 bps, while the wild type allele did not. We analyzed the patient, 12 unaffected family members, and 90 unrelated control individuals. Only the patient was homozygous for the mutation, while eight unaffected family members were heterozygous for the mutation, and the remaining four were homozygous for the normal allele (Fig. 5). The 90 control subjects were homozygous for the normal allele (data not shown), excluding the possibility of a polymorphic site in the mutation region.

We also examined the conservation of the mutation genetic region with a blast search (26). The search reveals that the Homo sapiens desmoplakin I sequence is almost identical to that of Mus musculus (XP_138583), and significantly similar to those of Rattus norvegicus (CAA42169) and Cricetulus griseus (AAF70372) and the available 5' sequence of the Xenopus laevis (AAD48849) gene. In particular, the region of the described mutation (Fig. 4B) is highly conserved.
DISCUSSION
We report a new recessive mutation in the desmoplakin gene causing familial ARVD, woolly hair, and an epidermolytic skin disorder. The genetic work-up in this large family with a high rate of consanguineous marriage utilizes a strategy of homozygosity mapping to examine loci based on a candidate gene approach. This efficient method led to the identification of a new mutation, despite the relatively small number of analyzed subjects.

The G2375R mutation in the desmoplakin gene creates a
new recognition site for the restriction enzyme Sma1. Using restriction enzyme analysis, we performed rapid screening for the mutation in the family members, and eight heterozygous subjects were identified. This method can facilitate the diagnosis of ARVD and genetic counseling in this family.

Mutations in desmosomal proteins causing a syndrome with cardiac, hair, and skin abnormalities have been described. Naxos disease is an autosomal recessive condition where the phenotype is ARVD, woolly hair, and palmo-plantar keratoderm, and the affected gene is plakoglobin.

Figure 4. (A) The G2375R mutation in the desmoplakin gene (bold) is a g to r substitution. (B) The protein sequence alignment of the mutated region in the human desmoplakin (HS DSP), in desmoplakin of other species (Mus musculus, Rattus norvegicus, and Cricetulus griseus) and in other human plakin family members (PLE = plectin, EPP = epiplakin, BPA = bullous pemphigoid antigen1). The glycine in position 2375 of the human desmoplakin (bold) is extremely conserved.

Figure 5. Gel electrophoresis of the mutation containing polymerase chain reaction product after digestion with Sma1. The mutant fragment is cleaved to two smaller fragments in similar size demonstrated by a single band, 365/357 base pairs (bp). The wild-type fragment is resistant to digestion and demonstrated by the higher single band, 722 bp.
Mutations in the desmoplakin gene have been shown to underlie some cases of the autosomal dominant skin disorder (striate palmoplantar keratoderma) without cardiac involvement (28–30), a dominant form of ARVD without skin disease (17) and an autosomal recessive condition characterized by dilated cardiomyopathy, woolly hair, and keratoderma (20). Both desmoplakin and plakoglobin are important components of desmosomes (22). Desmosomes are symmetrical disc-shape intracellular junctions found primarily in epithelial tissue. They mediate adhesion between cells and link the IFs of neighboring cells, thus establishing an integrated framework across the entire tissue (30,31).

Desmoplakin is a member of the plakin family of IF binding proteins. The primary structure of desmoplakin contains three functional domains: the N-terminal, which binds to the desmosome via connection with plakoglobin and plakophilin; the rod segment, which is predicted to form a dimeric coil; and the C-terminal domain, which binds IF's (32) (Fig. 6). The C-terminal contains three segments A, B and C; each expresses 4.5 copies of the plakin repeat domain (PRD). The G in position 2375 resides at the end of PRD3 of segment B and is extremely conserved among other plakin family members (32). The PRDs form a globular structure with a basic groove, which is predicted to represent the IF's binding site. The crystal structure of segment B revealed that the Glycine residues at the end of PRD1 and PRD3 adopt a backbone conformation only available to the Glycine residue, which produces a sharp turn. Thus, the mutation described here of substitution of the Glycine in position 2375 to a positively charge arginine is very likely to alter the B segment and disrupt the desmoplakin-IF's interaction. A similar mechanism was proposed by Norgett et al. (20) who described a mutation that causes truncation of desmoplakin and the lack of the C segment of the IF's binding site (Fig. 6).

Alternative splicing of the protein produces two isoforms, desmoplakin I and desmoplakin II, which differ in the length of the intermediate rod domain (33). The mutations described are in the N or C terminal of the desmoplakin (Fig. 6); therefore, they will affect both isoforms.

The pathology of ARVD is characterized by fibrofatty replacement of right ventricular myocytes (1). The mechanism could be disruption of desmosomal function caused by a mutated structural protein, such as desmoplakin or plakoglobin. Under conditions of mechanical stress, the desmosomal dysfunction can lead to detachment of the myocytes at the intercalated disc, with progressive myocyte apoptosis (19,34). As regeneration of cardiac myocytes is limited, fibrofatty replacement takes place and provides the anatomical basis for progressive cardiac failure, arrhythmia, and sudden death. Impairment of cell-cell adhesion could also be a mechanism common to other familial cardiomyopathies (35).

Nonsense mutations leading to desmoplakin haplo insufficiency caused skin disorders (27–29); mutation in the N-terminal caused ARVD (17), while mutation in the C-terminal as described here and by Norgett et al. (20) caused cardiac, skin, and hair syndrome (Fig. 6). The genotype-phenotype relationships are subjects for further investigation.

Defects in desmoplakin, and autoantibodies to desmoplakin, have been described in various skin disorders, particularly palmo-plantar keratodermas (21), skin blistering disorders (19).
diseases such as autoimmune pemphigous and other pemphigous-related disorders (36,37). Desmosomal involvement was described in infectious skin diseases as in bolus impetigo and in staphylococcal scalded-skin syndrome (38).

In our family the skin disease is characterized clinically and histologically as the autoimmune blistering skin disease pemphigous foliaceous. Antibodies to desmoplakin in a patient with pemphigous foliaceous have been previously described (37). This finding supports the hypothesis that this skin disease is a result of dysfunction of the desmosome, and particularly of the desmoplakin. It is likely that the same mechanism that causes disruption of IF’s interaction with the desmosome in the heart muscle impairs binding of keratin to the desmosome in the epidermis thereby causing skin lesions under conditions of stress. Areas of the skin that are less mechanically stressed such as the arm and back demonstrate normal histology. This theory could underline the tissue-specific phenotype seen here and in the previously described desmosomal syndromes, as the heart muscle, and in some areas in the skin are tissues that are most exposed to constant mechanical stress.

The large spectrum of skin phenotypes related to dysfunction of the desmosomal and IFs is probably due to multiple genetic and environmental factors that have yet to be determined.

The defect in desmoplakin could also lead to fragility at desmosomal junctions in hair cells leading to hair-shaft dysplasia that causes the woolly hair phenotype as described in other desmosomal molecules (22,39).

The major limitation of this study was that we analyzed only a single patient. Most members of this family reside in the Palestinian Authority and Jordan. Due to political unrest in the area, we have been unable to locate and evaluate other affected family members. Nevertheless, the possibility that the mutation identified is a rare polymorphism rather than a disease-causing mutation is extremely unlikely. First, we demonstrated homozygosity for the desmoplakin locus only in the patient. Second, we excluded the possibility of some other candidate genes to be the disease-causing gene in this family. Third, the mutation we found results in a substitution from the highly conserved glycine residue, which adopts a backbone conformation, to a positively charged amino acid. This is likely to alter the configuration of the tail region of the protein. Fourth, by restriction enzyme analysis in multiple family members and 90 healthy controls, we demonstrated that this Gly2375Arg substitution exists only in this family and the patient is the single homozygous, supporting the conclusion that this is a mutation site rather than a polymorphic site.

Our finding underlines the importance of cellular junctions in maintaining tissue integrity, especially in the heart and skin. The exact role of desmosomal proteins in the pathogenesis of heart failure and cardiac arrhythmia will require further studies.

REFERENCES