Desmin Mutation Responsible for Idiopathic Dilated Cardiomyopathy

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Background—Idiopathic dilated cardiomyopathy, of which ≈20% of cases are familial (FDCM), is a primary myocardial disorder characterized by ventricular dilatation and impaired systolic function. It is a common cause of heart failure and the need for cardiac transplantation. Although 6 chromosomal loci responsible for autosomal dominant FDCM have been mapped by linkage analysis, none of these genes have been identified. By use of the candidate-gene approach, actin was identified recently as being responsible for dilated cardiomyopathy. Considerable evidence suggests desmin, a muscle-specific intermediate filament, plays a significant role in cardiac growth and development.

Methods and Results—To determine whether a defect of desmin induces dilated cardiomyopathy, 44 probands with FDCM underwent clinical evaluation and DNA analysis. Diagnostic criteria, detected by echocardiography, consisted of ventricular dimension of $2.7 \text{ cm/m}^2$ with an ejection fraction $\leq 50\%$ in the absence of other potential causes. After amplification by polymerase chain reaction, the exons of the desmin gene were sequenced. A missense desmin mutation, Ile451Met, which cosegregates with FDCM without clinically evident skeletal muscle abnormalities, was identified in a 4-generation family but was not detected in 460 unrelated healthy individuals.

Conclusions—A novel missense mutation of desmin, Ile451Met, was identified as the genetic cause of idiopathic dilated cardiomyopathy. This finding is of particular significance because this is the first mutation detected in the desmin tail domain, and the function of the desmin tail remains unknown. Because this mutation leads to a restricted cardiac phenotype in the family studied in the present report, it suggests that the tail of desmin plays an important functional role in cardiac tissue.

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Key Words: cardiomyopathy • genes • desmin

Dilated cardiomyopathy (DCM) is the most common form of primary cardiac muscle disease; it is several times more common than the hypertrophic form. The estimated prevalence of DCM in the United States is 36.5 per 100,000 individuals. Clinically, DCM is characterized by ventricular chamber enlargement, systolic dysfunction, sudden death, and heart failure. Heart failure due to DCM is a lethal disease, with a 5-year mortality rate of 75%. Cardiac transplantation is the only cure for this disease. Approximately 50% of DCM cases are idiopathic, of which at least 20% are familial. Progress in unraveling the genetic causes of idiopathic DCM has been slow compared with that for hypertrophic cardiomyopathy. By use of genetic linkage analysis, 6 chromosomal loci have been mapped for autosomal dominant DCM, but none of the genes have been identified. In 1998, by use of the candidate-gene approach, actin was shown to be responsible for idiopathic DCM. In musculoskeletal disorders such as Duchenne muscular dystrophy, which are associated with DCMs, the responsible genes are dystrophin, $\alpha$-dystroglycan, and $\alpha$-sarcoglycan, all of which are cytoskeletal proteins. Desmin, a cytoskeletal protein that forms intermediate filaments specific for muscle, has been shown to be associated with cardiac and skeletal abnormalities. Accordingly, because the families with FDCM that we studied were too small to provide adequate power for linkage analysis, we adopted the candidate-gene approach and sequenced the DNA of 44 probands with FDCM for the actin and desmin genes. No actin mutation was found, but a missense mutation (Ile451Met) in desmin was found in the proband of family 20-032, which has DCM without any skeletal abnormalities. The remainder of the family of this proband was subsequently studied, and analysis of the data showed the mutation cosegregates with DCM as an autosomal dominant trait.

Methods

Clinical Evaluation

Probands with FDCM and family members were evaluated by detailed history and physical examination, 12-lead ECG, M-mode...
affected individuals and 2 others with the mutation without any clinical phenotype. The proband (III:10) had cardiomegaly and chronic cardiac failure for >5 years with a left ventricular ejection fraction of 40% and diffuse hypokinesis. The son of the proband (IV:2) has cardiomegaly and a left ventricular ejection fraction of 45%. The paternal grandfather of the proband had chronic cardiac failure for >2 decades and died at age 65 years. The 3 cousins of the proband (III:1, III:2, and III:3) and offspring of II:2 died between the ages of 15 and 37 years and had heart failure before death. There was no evidence to suggest II:2 and II:3 were affected with the disease, and thus they are referred to as obligate carriers.

There was no clinical evidence of skeletal muscle involvement on physical examination and no symptoms to suggest peripheral muscle involvement. Plasma creatine kinase activity in each individual was consistently in the normal range (<120 IU/L).

### Missense Mutation in Desmin Gene

A missense mutation, Ile451Met, in exon 8 of the desmin gene was found in the proband of family 20-032 by sequence analysis. The mutation results from a cytosine (C) substitution for guanine (G) at nucleotide (nt) 1353 (GenBank accession number AF137053) (Figure 2A). This base change created a restriction site for NcoI, which was used to screen all available members. The mutation cosegregated with all affected individuals. DNA analysis of 460 unrelated control whites (920 chromosomes) failed to show this mutation.

Isoleucine, replaced by the mutation, is highly conserved in the desmin gene across species including human, mouse, rat, chicken, golden hamster, and amphibians. This isoleucine is also a part of a 9-amino-acid motif (IKTIETRDG) that is highly conserved from humans to fish and between different type III intermediate filaments.9

Isoleucine is a nonpolar hydrophobic amino acid, whereas methionine is a polar neutral amino acid. The isoleucine-to-methionine substitution induces a dramatic change in the secondary structure of the desmin tail region based on the Garnier-Osguthorpe-Robson prediction; specifically, the mutation removes a predicted turn and replaces a probable $\beta$-sheet with an extension of the $\alpha$-helix in the preceding region (Figure 2B.)

### Results

Clinical Findings

The pedigree (Figure 1) consisted of 28 white individuals extending through 4 generations. Nine individuals had died before identification of the family, leaving only 2 living

**Figure 1.** Pedigree of DCM family 20-032. Squares indicate males; circles, females. Open symbols represent normal subjects; solid symbols, affected individuals; semisolid symbols, carriers; symbols with dots, obligate carriers; and slanted bars, deceased individuals.
Discussion

We identified a cytosine-to-guanine substitution in exon 8 of the desmin gene in a 4-generation family with affected members having DCM manifested by left ventricular dilatation, heart failure, and sudden death. This change in the DNA sequence substitutes methionine for isoleucine at codon 451 (Ile451Met) in the tail domain of human desmin. The altered DNA sequence is considered a disease-causing mutation (FDCM) rather than a rare polymorphism for the following reasons: (1) the mutation was present in all affected family members; (2) the mutation was not present on 920 chromosomes from 460 unrelated normal subjects; (3) the mutation replaces a highly conserved amino acid, isoleucine, at codon 451 (Ile451Met) in the tail domain of human desmin; and (4) the amino acid substitution caused by the mutation is predicted to significantly alter the secondary structure of desmin, as shown in Figure 2B. Furthermore, elimination of the desmin gene in the mouse has been shown to be associated with skeletal and cardiac myopathies. Penetrance was incomplete, with 2 female members (III:14 and III:16) having the mutation without obvious phenotype. It is of note that all of the individuals affected, living or dead, were male. Whether this reflects sex-related penetrance or is simply a chance observation remains to be determined.

Desmin is a muscle-specific 53-kDa subunit of the class III intermediate filament. It is a part of the cytoskeleton of all 3 muscle types and forms connections between the nuclear and plasma membranes. The tail domain undergoes posttranslational modifications by phosphorylation and glycosylation, regulating dynamic aspects of intermediate filament organization and structure during the cell cycle. Desmin is found at the Z lines and intercalated disk and is believed to play a role in the attachment or stabilization of the sarcomere. Recently, desmin mutations have been recognized as a cause of skeletal myopathies with cardiac involvement, manifested predominantly as conduction disorders and restrictive cardiomyopathy. The family in the present study, in contrast, has no discernible clinical involvement of the skeletal or smooth muscles but has a profound DCM. Furthermore, plasma creatine kinase activity, a highly sensitive marker of familial skeletal muscle disease, was normal, even for carriers. Interestingly, the previous mutations associated with skeletal muscle disorders are in the rod domain, whereas our mutation is in the carboxy tail domain of the protein, a region whose function is undetermined. This may reflect a distinct functional domain with a binding site specific for cardiac proteins. Additional study should provide insight into the pathogenesis of DCM and determine the specific function of the carboxy-terminal portion of desmin.
The actin mutations responsible for FDCM were located in the actin domains, which are immobilized and attached to the Z band or intercalated disc, and thus are involved with transmission of contractile force rather than affecting the myosin cross bridges and the generation of force. Similarly, desmin attaches to the sarcomere Z band, the nuclear membrane, and other organelles and is known to serve as a means for the transmission of force and other signals. This may be the common molecular basis whereby mutations in both actin and desmin induce a similar disease phenotype. It is reasonable to postulate that both desmin and actin provide a scaffolding role in the growth and maintenance of the sarcomere, and thereby the mutant leads to an impaired growth response, namely, DCM.

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