Prevalence of Desmin Mutations in Dilated Cardiomyopathy

Matthew R.G. Taylor, MD, PhD; Dobromir Slavov, PhD; Lisa Ku, MS; Andrea Di Lenarda, MD; Gianfranco Sinagra, MD; Elisa Carniel, MD; Kurt Haubold, PhD; Mark M. Boucek, MD; Debra Ferguson, RN, MS; Sharon L. Graw, PhD; Xiao Zhu, BS; Jean Cavanaugh, MS, PA-C; Carmen C. Sucharov, PhD; Carlin S. Long, MD; Michael R. Bristow, MD, PhD; Philip Lavori, PhD; Luisa Mestroni, MD; for the Familial Cardiomyopathy Registry and the BEST (Beta-Blocker Evaluation of Survival Trial) DNA Bank

Background—Desmin-related myofibrillar myopathy (DRM) is a cardiac and skeletal muscle disease caused by mutations in the desmin (DES) gene. Mutations in the central 2B domain of DES cause skeletal muscle disease that typically precedes cardiac involvement. However, the prevalence of DES mutations in dilated cardiomyopathy (DCM) without skeletal muscle disease is not known.

Methods and Results—Denaturing high-performance liquid chromatography was used to screen DES for mutations in 116 DCM families from the Familial Dilated Cardiomyopathy Registry and in 309 subjects with DCM from the Beta-Blocker Evaluation of Survival Trial (BEST). DES mutations were transfected into SW13 and human smooth muscle cells and neonatal rat cardiac myocytes, and the effects on cytoskeletal desmin network architecture were analyzed with confocal microscopy. Five novel missense DES mutations, including the first localized to the highly conserved 1A domain, were detected in 6 subjects (1.4%). Transfection of DES mutations in the 2B domain severely disrupted the fine intracytoplasmic staining of desmin, causing clumping of the desmin protein. A tail domain mutation (Val459Ile) showed milder effects on desmin cytoplasmic network formation and appears to be a low-penetrant mutation restricted to black subjects.

Conclusions—The prevalence of DES mutations in DCM is between 1% and 2%, and mutations in the 1A helical domain, as well as the 2B rod domain, are capable of causing a DCM phenotype. The lack of severe disruption of cytoskeletal desmin network formation seen with mutations in the 1A and tail domains suggests that dysfunction of seemingly intact desmin networks is sufficient to cause DCM. (Circulation. 2007;115:1244-1251.)

Key Words: cardiomyopathy ■ desmin ■ genetics ■ heart failure

Desmin-related myofibrillar myopathy (DRM) is a rare heritable myopathy affecting skeletal and cardiac muscle (OMIM #601419),1 caused by mutations in the desmin (DES) gene. Skeletal muscle weakness starting in the lower limbs and progressing to involve truncal, neck flexor, bulbar, and respiratory muscles without cardiac involvement has been reported most commonly.2–5 Cardiac manifestations in other families include restrictive cardiomyopathy, dilated cardiomyopathy, conduction system diseases, arrhythmias, and sudden death.6–7 The majority of the >40 DES mutations reported cause DRM and are localized to the central rod domain of the desmin protein (Figure 1). Selected skeletal and cardiac muscle biopsies from affected patients have shown cytoplasmic aggregations of intermediate filaments, which are presumed to reflect a disruption of the assembly of desmin protein into a filamentous network.4,6–14 Mutations in α-B-crystallin (CRYAB), dystrophin (DMD), and myotilin (TTID) cause similar morphological changes to skeletal muscle. Transfection of various DES mutations into cellular models has largely confirmed in vitro aggregation of desmin protein and disruption of the cytoplasmic filamentous desmin network.4,5,8,9,11,13–15

Clinical Perspective p 1251

Only 1 reported mutation (Ile451Met), located in the tail domain of the desmin protein and found in a study of 44 subjects with dilated cardiomyopathy (DCM), has been linked to DCM without skeletal muscle disease.16 The Ile451Met mutation is either a recurrent or founder mutation because a screen of 265 unrelated Japanese DCM cases for this specific mutation detected 3 additional cases (1.1%).12 Ile451Met mutations were also found in a family with classic DRM4 and in another family with slowly progressive skeletal
myopathy with no apparent cardiac involvement and incomplete penetrance evidenced by 3 adult healthy mutation carriers. Thus, the contribution of DES mutations to isolated DCM and whether mutations located outside the desmin tail region can cause DCM are incompletely understood. The largest previous population study of 63 cases of DCM did not identify any DES mutations. The aim of the present study was to comprehensively screen a large population of DCM cases for DES mutations with the use of 2 unique DCM cohorts: (1) subjects from 115 DCM families with familial and nonfamilial (sporadic) forms and (2) 309 DCM subjects from the Beta-Blocker Evaluation of Survival Trial (BEST). Genetic screening was done with the use of denaturing high-performance liquid chromatography elution profiles and tail domains. Mutations showing abnormal desmin aggregation in cellular studies are underlined. Mutations reported are identical in current study in boldface italics. Arrows indicate whether the mutations found by genetic screening had cellular phenotypes. Accordingly, we developed constructs for cellular expression in (1) SW13 cells (ATCC, American Tissue Culture Collection, Manassas, Va), which express desmin and vimentin, and (2) neonatal rat cardiac ventricular myocytes cultured as previously described. In the case of the myocyte experiments, cotransfection of desmin construct and green fluorescent protein (GFP) constructs were done (pmaxGFP; Amaxa, Inc). Briefly, ventricular cells of 1- to 3-day-old rats were isolated by trypsin digestion, and 2×10⁶ cells were transfected with 2 μg total DNA (1 μg GFP construct and 1 μg of each desmin construct) with the use of a Nucleofector device (Amaza, Inc) according to the manufacturer’s instructions and with Rat Cardiomyocyte–Neonatal Nucleofector transfection kits (Amaza Inc). Briefly, cells were pelleted and suspended in 100 μL of the appropriate buffer. DNA was added to the solution, and cells were electroporated with the neonatal rat ventricular myocyte–specific program. Cells were plated in a 10-cm² slide coated with 1% gelatin. Media solutions were supplemented with HEPES (pH 7.5) to a final concentration of 20 mmol/L to buffer the media pH. Metabolic studies were conducted in DES cDNA with a modified overlap extension approach, with the use of oligonucleotides containing the DES mutations. Polymerase chain reaction (PCR) fragments containing full-length desmin cDNA were inserted into pcDNA3.1/V5-His-Topo T/A cloning vector (Invitrogen, Carlsbad, Calif). Oligonucleotides with single-nucleotide mutations in combination with flanking primers (primers available on request) were used to generate PCR fragments from desmin cDNA clone (Gene Bank accession No. BC032116). PCR fragments without a mutation that overlapped mutagenized PCR products (overlap range, 243 to 613 nucleotides) were amplified with separate primers, and then

**Methods**

**Patient Cohorts**

The family-based cohort drew subjects from the Familial Cardiomyopathy Registry, a multicenter DCM study. Affected subjects from 116 DCM families were screened for DES mutations (1 subject from a recessive-appearing pedigree was unaffected). The diagnosis of DCM was made according to published criteria; probands and available relatives were evaluated by the investigators, an evaluation that included a detailed history, physical examination, 12-lead ECG, and echocardiography; medical records were also reviewed in the case of unavailable or deceased relatives. Informed consent was obtained from Familial Cardiomyopathy Registry subjects under the institutional review board policies of the participating institutions. The 148 subjects were predominantly white and comprised 90 men and 58 women with an average age at study entry of 44 years. A second group of DCM subjects was screened from the BEST cohort, which was cosponsored by the National Heart, Lung, and Blood Institute and the Department of Veterans Affairs Cooperative Studies Program. BEST was a multicenter study of moderate to severe congestive heart failure (New York Heart Association class III-IV and ejection fraction of ≤35% at entry) comparing bucindolol with placebo. A subset of BEST subjects participated in a DNA bank, and we were granted approval to study 309 anonymous samples from DCM subjects. This cohort had an average age of 56 years and was divided into 213 men and 96 women, with 240 and 69 nonblack and black (non-Hispanic) subjects, respectively.

**DES Mutation Screening**

Genetic screening was done with the use of denaturing high-performance liquid chromatography with a Transgenic WAVE Fragment Analysis System (Transgenomic Inc, Omaha, Neb) followed by selective DNA sequencing of variants with abnormal denaturing high-performance liquid chromatography elution profiles on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, Calif). The 9 DES exons and intron boundaries and the 5’ upstream enhancer sequence that modulates expression of the desmin protein were studied (primer sequences and conditions available on request).

Wild-type control DNA was added to those samples from Familial Cardiomyopathy Registry families in which autosomal recessive inheritance or sporadic disease was suspected. This approach favors segregation among affected family members, and absence of the variant in a collection of 300 predominantly white control chromosomes from healthy subjects.

**Expression Studies**

The majority of DES mutations studied previously led to in vivo (muscle biopsies) or in vitro (cellular models) abnormal aggregation of cytoplasmic desmin protein. Because cardiac tissue was not available from our subjects, we used the in vitro approach to determine whether the mutations found by genetic screening had cellular phenotypes. Accordingly, we developed constructs for cellular expression in (1) SW13 cells (ATCC, American Tissue Culture Collection, Manassas, Va), which express desmin and vimentin; and (3) neonatal rat cardiac ventricular myocytes cultured as previously described. In the case of the myocyte experiments, cotransfection of desmin construct and green fluorescent protein (GFP) constructs were done (pmaxGFP; Amaxa, Inc). Briefly, ventricular cells of 1- to 3-day-old rats were isolated by trypsin digestion, and 2×10⁶ cells were transfected with 2 μg total DNA (1 μg GFP construct and 1 μg of each desmin construct) with the use of a Nucleofector device (Amaxa, Inc) according to the manufacturer’s instructions and with Rat Cardiomyocyte–Neonatal Nucleofector transfection kits (Amaxa Inc). Briefly, cells were pelleted and suspended in 100 μL of the appropriate buffer. DNA was added to the solution, and cells were electroporated with the neonatal rat ventricular myocyte–specific program. Cells were plated in a 10-cm² slide coated with 1% gelatin. Media solutions were supplemented with HEPES (pH 7.5) to a final concentration of 20 mmol/L to buffer the media pH. Putative mutants were created in DES cDNA with a modified overlap extension approach, with the use of oligonucleotides containing the DES mutations. Polymerase chain reaction (PCR) fragments containing full-length desmin cDNA were inserted into pcDNA3.1/V5-His-Topo T/A cloning vector (Invitrogen, Carlsbad, Calif). Oligonucleotides with single-nucleotide mutations in combination with flanking primers (primers available on request) were used to generate PCR fragments from desmin cDNA clone (Gene Bank accession No. BC032116). PCR fragments without a mutation that overlapped mutagenized PCR products (overlap range, 243 to 613 nucleotides) were amplified with separate primers, and then

Figure 1. Desmin protein represented with precoil domain (PCD) and rod domains separated by linkers and flanked by non-helical head and tail domains. Mutations showing abnormal desmin aggregation in cellular studies are underlined. Mutations reported are identical in boldface italics. Arrows indicate whether the mutations found by genetic screening had cellular phenotypes. Accordingly, we developed constructs for cellular expression in (1) SW13 cells (ATCC, American Tissue Culture Collection, Manassas, Va), which express desmin and vimentin; and (3) neonatal rat cardiac ventricular myocytes cultured as previously described. In the case of the myocyte experiments, cotransfection of desmin construct and green fluorescent protein (GFP) constructs were done (pmaxGFP; Amaxa, Inc). Briefly, ventricular cells of 1- to 3-day-old rats were isolated by trypsin digestion, and 2×10⁶ cells were transfected with 2 μg total DNA (1 μg GFP construct and 1 μg of each desmin construct) with the use of a Nucleofector device (Amaxa, Inc) according to the manufacturer’s instructions and with Rat Cardiomyocyte–Neonatal Nucleofector transfection kits (Amaxa Inc). Briefly, cells were pelleted and suspended in 100 μL of the appropriate buffer. DNA was added to the solution, and cells were electroporated with the neonatal rat ventricular myocyte–specific program. Cells were plated in a 10-cm² slide coated with 1% gelatin. Media solutions were supplemented with HEPES (pH 7.5) to a final concentration of 20 mmol/L to buffer the media pH. Putative mutants were created in DES cDNA with a modified overlap extension approach, with the use of oligonucleotides containing the DES mutations. Polymerase chain reaction (PCR) fragments containing full-length desmin cDNA were inserted into pcDNA3.1/V5-His-Topo T/A cloning vector (Invitrogen, Carlsbad, Calif). Oligonucleotides with single-nucleotide mutations in combination with flanking primers (primers available on request) were used to generate PCR fragments from desmin cDNA clone (Gene Bank accession No. BC032116). PCR fragments without a mutation that overlapped mutagenized PCR products (overlap range, 243 to 613 nucleotides) were amplified with separate primers, and then

Downloaded from http://circ.ahajournals.org/ by guest on April 13, 2017
### Characteristics of DES Mutation Carriers

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age at Diagnosis, y</th>
<th>Race</th>
<th>Gender</th>
<th>NYHA Class</th>
<th>Conduction System Disease</th>
<th>Muscle Disease</th>
<th>EF, %</th>
<th>LVEDD, cm</th>
<th>Status (Comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu108Lys</td>
<td>60</td>
<td>White</td>
<td>M</td>
<td>3</td>
<td>LAFB</td>
<td>None</td>
<td>33</td>
<td>NA</td>
<td>Living</td>
</tr>
<tr>
<td>Ser298Leu</td>
<td>45</td>
<td>White</td>
<td>F</td>
<td>3</td>
<td>LBBB</td>
<td>None</td>
<td>27</td>
<td>NA</td>
<td>Living</td>
</tr>
<tr>
<td>Asp312Asn</td>
<td>35</td>
<td>Black</td>
<td>M</td>
<td>3</td>
<td>None</td>
<td>None</td>
<td>17</td>
<td>NA</td>
<td>Dead (age 45 y at last follow-up)</td>
</tr>
<tr>
<td>Arg350Trp</td>
<td>55</td>
<td>White</td>
<td>M</td>
<td>2</td>
<td>None</td>
<td>None</td>
<td>29</td>
<td>7.1</td>
<td>Living (EF 19% at 68 y)</td>
</tr>
<tr>
<td>Val459Ile*</td>
<td>44</td>
<td>Black</td>
<td>M</td>
<td>3</td>
<td>None</td>
<td>None</td>
<td>34</td>
<td>7.6</td>
<td>Living</td>
</tr>
<tr>
<td>Val459Ile*</td>
<td>34</td>
<td>Black</td>
<td>F</td>
<td>3</td>
<td>1° AVB</td>
<td>None</td>
<td>17</td>
<td>NA</td>
<td>Living</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association; EF, ejection fraction; LVEDD, left ventricular end-diastolic diameter; LAFB, left anterior fascicular block; LBBB, left bundle-branch block; AVB, atrioventricular block; and NA, not available.

*Allele frequency of 1% of black (ethnically matched) controls.

full-length desmin cDNA was generated by extension of overlapping PCR fragments. All mutations were confirmed by sequencing.

Plasmid DNA constructs were transfected into Escherichia coli (top10; Invitrogen Inc), isolated, and sequenced from single colonies to confirm the presence of the mutations. Transformation was performed with the FuGene 6 transfection reagent (Roche Diagnostics Corp, Indianapolis, Ind) according to the manufacturer’s instructions (3 µL). FuGene was diluted in 97 µL Opti-MEM (Invitrogen), and 1 µg plasmid DNA was added; DNA-FuGene complex was formed at room temperature for 30 minutes and added to cell culture. Cells (SW13 and coronary artery smooth muscle cells) were grown on 8-cm² slides to ~90% confluence and then transfected with 1 µg of plasmid DNA. After incubation for 48 hours, the slides were washed 3 times, for 10 minutes each, with PBS, and cells were fixed with an ice-cold mixture of 70% methanol and 30% acetone for 10 minutes. Slides were washed 3 times, for 10 minutes each, with PBS.

Immunostaining with primary monoclonal mouse anti-desmin antibody (D1033; Sigma Inc, St Louis, Mo) at 1:1000 dilution in PBS overnight (16 hours) at 4°C was followed by washing 3 times with PBS and incubation with secondary anti-mouse antibody FITC conjugate (F5387; Sigma) at 1:1000 dilution for 1 hour at room temperature.

Slides were washed 3 times with PBS, and coverslips were mounted in drop of Mowiol. Fluorescent confocal microscopy with the use of an Olympus IX81 inverted motorized spinning-disk confocal microscope (Olympus America Inc, Center Valley, Pa) was used to evaluate the transfected cells for expression of desmin protein and its abilities to form an intracellular filament network. The Gln389Pro mutation reported by Goudeau et al.25 to cause severe desmin network disruption was used as a positive control for our experiments.

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

### Results

#### Mutation Screening

Mutation screening detected 4 novel variants in 4 DCM subjects that predicted missense changes and met criteria for pathogenic mutations: (Glu108Lys:c.408.G→A; Ser298Leu:c.979.C→T; Asp312Asn:c.1020.G→A; Arg350Trp:c.1134.C→T; reference NM_001927.3) (Figure 1 and Table). The Glu108Lys mutation is the first pathogenic mutation reported in the highly conserved 1A rod domain. The mutations were absent in 300 control chromosomes and were unique among all the alleles screened in the study subjects. We also found a mutation in the tail domain (Val459Ile:c.1461.G→A) in 2 black BEST subjects of a total of 69 black subjects in the BEST cohort. This mutation is only 8 amino acids away from the only other mutation (Ile451Met) linked to isolated DCM, which is also a recurrent mutation in unrelated families.5,5,12,16 The Val459Ile mutation was also absent in our standard controls but was heterozygous in 2 of 100 samples (200 chromosomes) from a population of black controls (Coriell Institute; human variation panel HD100A; http://www.coriell.org/).

A sixth DES mutation of interest was found in our study at position 213. An alanine to valine (Ala213Val:c.730.T→C; reference NM_001927.3) substitution was detected in a large familial dilated cardiomyopathy pedigree and in 6 unrelated BEST subjects. Three other groups have reported finding Ala213Val mutations, which have been proposed as low-penetrant mutations.3,26,27 To determine whether this was a pathogenic mutation in our study, we analyzed DNA samples from 12 additional members of this pedigree. The 213Val mutation did not segregate with the disease phenotype because it was present in 4 healthy individuals and absent in 1 affected individual. We further analyzed 86 DNA samples from healthy controls without cardiomyopathy and detected the Arg213Val mutation in 2 samples. These data suggest that the Ala213Val is most likely a benign polymorphism (allele frequency ~1%), making it currently the only known amino acid polymorphism in the rod domain of desmin.

#### Phenotype Analysis

The carriers of DES mutations in our study all had a pure DCM without any detected involvement of skeletal muscle disease (Table). Elevated creatinine kinase levels have been reported in some DRM patients.5,15,25 The creatinine kinase level was normal in the 1 subject for whom that information was available (Arg350Trp mutation). In contrast to typical observations in DRM, severe conduction system disease and arrhythmias were absent. The single mutation from the Familial Cardiomyopathy Registry cohort occurred in a male DCM subject who developed DCM at age 55 years. He had no evidence of skeletal myopathy and was the only affected member of his family (left ventricular ejection fraction 19% at age 68 years in 2004). His 2 children were clinically evaluated and were found to be normal; no DNA was available from the children. In this patient, the following DCM genes had already been screened negative: LMNA, MYH7, MYH6, TMPO, TNNI3, and SGCD.
Expression Studies

Immunofluorescence microscopy analysis of transfected SW13 and human coronary artery smooth muscle cells and neonatal rat cardiac myocytes expressing our desmin mutations revealed obvious cellular phenotypes for mutations in the 2B rod domain (Ser298Leu, Asp312Asn, and Arg350Trp) (Figures 2 and 3) that mirrored the desmin disruption seen in the Gln389Pro-positive control. For these mutations, severe disruption of the normal desmin cytoskeletal architecture occurred in the majority of studied cells with clumping and aggregation of antibody-positive staining cytoplasmic protein. The mutations had a dominant phenotype in the human coronary artery smooth muscle cell lines in which the constitutively expressed desmin was unable to compensate for the presence of the introduced desmin mutations. Interestingly, the Glu108Lys mutation, located in a highly conserved region of the 1A rod domain, did not disrupt the assembly of desmin in SW13 or human coronary artery smooth muscle cells. Mutations in the 1A domain have not been reported previously, even mutations in the neighboring head domain are rare, and the effects on desmin network assembly have not been studied.
The Val459Ile mutation in the tail domain showed an intermediate phenotype with modest impairment of the desmin filamentous network affecting approximately half of the cells observed. This result is similar to another reported tail domain mutation (Ile451Met) in which only subtle in vitro effects on desmin assembly were noted.4 Our Val459Ile mutation was found in 2 unrelated black patients; similarly, the Ile451Met tail mutation has been reported in at least 6 unrelated families.4,5,12,14 The Val459Ile mutation was found in 2 apparently healthy black control subjects; likewise, the Ile451Met tail mutation has also been reported in clinically unaffected adults, suggesting that the penetrance is incomplete.5 Overall, cellular studies and clinical findings suggest that the Val459Ile and the neighboring Ile451Met tail domain mutations are low-penetrant mutations that exert more modest pathogenic effects than the rod-domain mutations found in DRM.5,28

**Discussion**

Our screening of DES in 425 probands with DCM, the largest number to date, indicates that DES mutations account for 1% to 2% of DCM. Before our study, only 1 mutation linked to isolated DCM (tail domain, Ile451Met) had been reported.16 It had been suggested that the tail domain might be functionally important in cardiac tissue.29 Our results indicate that rod domain mutations are also capable of leading to isolated DCM. The low prevalence of DES mutations is in agreement with 2 smaller studies in DCM in which a screen of a combined 107 families yielded 1 mutation (Ile451Met).16,17 Another study of 265 Japanese DCM cases in whom only exon 8 was studied found 3 instances (1.1%) of the Ile451Met mutation.12 The detection of DES mutations in DCM is not restricted to familial forms of DCM, as evidenced by the 4 different mutations found in the BEST cohort, which was not a family-based study. Our study further demonstrates that DCM due to DES mutations can present without clinically recognizable skeletal muscle involvement. In addition, our data show that mutations in the rod domain can result in a DCM phenotype. Finally, the Ala213Val variant reported previously3,27,28 is likely a rare nonpathogenic polymorphism.

Overall, DES mutations can cause a spectrum of phenotypes including skeletal myopathy, mixed skeletal-cardiac disease (“desmin-related myopathy”), and cardiomyopathy (DCM as well as hypertrophic or restrictive cardiomyopathies). Cardiomyopathy may also be found in some cases. Of the 28 DES mutations we found in the literature in which cardiac status was clearly reported, only the Ile451Met mutation is described as causing an isolated DCM phenotype, although others have linked this mutation to DRM.4,5,16 The 5 novel mutations reported here had an apparently cardiac-restricted phenotype. Mild conduction disease was present in 2 of the 4 mutation carriers, and none of the carriers had evidence of clinical skeletal muscle disease, suggesting that the recognition of DES mutation carriers in the cardiology clinic setting on the basis of clinical data alone is a difficult task.

Desmin is expressed early in cardiac development, is the major muscle-specific intermediate filament protein, and is highly expressed in heart tissue, accounting for 2% of cellular protein in myocytes.2,26,29 Desmin filament assembly is a complex process that proceeds stepwise, with desmin monomers first associating into parallel coiled-coil homodimers. After this, antiparallel staggered tetramers form, leading next to laterally associating unit length filaments that ultimately interact end to end to form the final desmin filament.26 The process of filament assembly depends on the central rod domain, whereas the tail domain is believed to be important for interactions between tetramers and elongation of higher-order filament structures.2,30,31 The collection of morpholog-

---

**Figure 3.** Immunofluorescence microscopy of transfected neonatal rat cardiac myocytes, which constitutively express desmin. Cotransfections were done with GFP and mutant desmin constructs, as described in Methods. The desmin cytoskeletal network for the Glu108Lys mutation did not appear different from the wild-type cells. The remaining 4 mutations all show evidence for aggregation of intracytoplasmic desmin protein, although the phenotype of Val459Ile was less severe. The Gln389Pro mutation reported by Goudeau et al24 served as a positive control. Left column, GFP images; middle column, desmin images; and right column, merged images. Magnification ×60.
ically homogeneous disorders in which desmin architecture is perturbed is referred to as myofibrillar, desmin-related myopathies and is due to mutations in desmin as well as myotilin, dystrophin, and α-B-crystallin. Haploinsufficiency for desmin may potentially be less deleterious than single-point mutations (the bulk of reported mutations); this model is supported by observations in mice heterozygous for desmin-null mutations that have unremarkable skeletal and cardiac phenotypes.32

Three of the DCM-causing DES mutations we found (Ser298Leu, Asp312Asn, and Arg350Trp) are located in the 2B rod domain and caused severe disruption of desmin filament assembly. This domain of desmin is home to the majority of known DES mutations and is believed to be important for dimer-dimer interactions within the mature desmin filament as well as with correct filament assembly.3,33 The clustering of mutations in the 2B domain means that genetic analysis in suspected desmin-related myopathy could be initially targeted to this region as well as to the 1B rod region, where another clustering of mutations occurs. Cardiac conduction abnormalities are prominent features of mutations in the 2B domain, being reported in 7 of 10 previously described 2B mutations.4,6–9,11,14,15,34 A left bundle branch block was present in the Ser298Leu carrier in our study, and normal conduction was found for the Asp312N and Arg350Trp carriers.

In our cellular assay, all 3 2B domain mutations caused the same phenotype of the positive control, Gln389Pro.25 Notably, the introduction of a proline, not normally present in the desmin rod and able to cause kinks in protein structure, was the mutant residue in 8 of the 13 previously reported 2B rod domain mutations causing DRM.4,6–9,11,14,15,34 On the contrary, the mutations we found are predicted to have less severe structural effects, an observation that might explain why overt muscle disease was absent in our subjects. The Arg350Trp mutation substitutes a nonpolar, hydrophobic residue for a basic hydrophilic arginine moiety in the conserved α-helical coil 2B domain of desmin. The GOR IV secondary structure analysis program states that a loss of α-helical structure in the 2B coil is predicted to occur because of this mutation.35 The same residue is involved in a family with skeletal, cardiac, and diaphragmatic muscle disease carrying a proline substitution at the 350 position.36 The Ser298Leu and Asp312Asn mutations introduce nonpolar amino acids and are predicted to result in additional α-helical structure by the nnpredict program.37

The Glu108Lys mutation found in our study is unique for 2 reasons. First, it is the only mutation found thus far in the 1A helix domain and in a location that is the most highly evolutionarily conserved region of desmin and of all intermediate filaments. The Glu108 position is fully conserved among desmin, vimentin, neurofilament L protein, cytokeratins 8 and 18, and nuclear lamin A and B1.31 The fourth position of the repeating heptad unit is a “core” position, located on the concave side of the 1A domain, and is believed to affect dimer-dimer interactions.33 This mutation predicts the introduction of α-helix structure at the position of a random coil by the GOR IV program.35 Second, the lysine substitution did not interrupt the in vitro desmin network assembly. No other 1A helical domain mutations are known to exist for comparison. We suggest that the pathogenic effect of this Glu108Lys mutation occurs beyond the stage of desmin filamentous assembly, perhaps affecting dimer-dimer interactions rather than a fatal interruption of the actual assembly process.

That not all DES pathogenic mutations will disrupt desmin network assembly has been suggested by others.3,5,26,38,39 The initial studies of the first DCM mutation, Ile451Met, were equivocal, and in a follow-up study it was reported that no effect on desmin filamentous network was observed.5 Most recently, the DRM mutations Ala213Val, Glu245Asp, Ala360Pro, Asn393Ile, Gln389Pro, and Asp399Tyr demonstrated normal filament assembly in SW13 cells.28,40 The authors of these studies concluded that the severity of the phenotype does not correlate directly with the in vitro cellular models. Other properties of desmin protein beyond mere network assembly could also be important in the expressed disease phenotype. Additional studies are needed to determine whether fundamental differences exist in desmin function between DCM and DRM DES mutations.

The Val459Ile mutation was recurrent in 2 of our 69 black subjects (allele frequency of 1.4%) as well as present in 2 of 100 black control subjects (1%), and it was restricted to samples having black ethnicity. This finding could be compatible with a polymorphic variant (Fisher exact test, \( P = 0.54 \)), although an intermediate phenotype was shown in the transfection studies. This tail-domain mutation is 8 amino acids away from the other DCM-causing DES mutation (Ile451Met) reported in several studies. The in vitro findings of both of these tail mutations are less striking and appear to represent a less severe effect on desmin assembly.4,5,41 The Ile451Met mutation has also been linked to DRM and isolated skeletal muscle disease and has been found in ostensibly asymptomatic adults, observations perhaps explained by other genetic or environmental modifiers of the phenotype.4,5 Thus, the balance of the data would indicate that these are rare mutations with low penetrance. The association of the Val459Ile mutation to blacks is similar to observations for hereditary amyloidosis due to transthyretin mutations. In this condition, also characterized by abnormal cellular protein aggregation, the Val122Ile TTR mutation has a frequency of 3% in blacks and is associated with reduced penetrance and late-onset disease.42,43

Our study is limited by the possibility that some of the isolated DCM cases we studied will develop skeletal muscle involvement later in life. Formal neurological assessments were not done in the BEST cohort, none of the subjects in our study had indications for skeletal muscle biopsy, and we cannot exclude the possibility of mild skeletal muscle disease in the DES mutation carriers. In a previous report, a patient with a splicing site mutation predicting the deletion of amino acids 214 to 245 presented with second-degree atrioventricular heart block and DCM followed by muscle involvement developing over the next 10 years.11 We screened for mutations in the exons, promoter, and perieXonic portions of the introns, but it is possible that our screen could have missed other intronic mutations. We were also limited by the lack of cardiac or skeletal muscle biopsy material in our patients and
could not test whether our patients have desmin aggregation in muscle cells, as has been demonstrated by others.\textsuperscript{6,12} Finally, an estimate of the penetrance of \textit{DES} mutations is also not apparent from the literature because data are currently available predominantly from affected individuals, although we and others have found evidence of probable mutations in healthy adults.\textsuperscript{3,5} The effects of mutation status on lifetime penetrance as well as age-dependent manifestations of the disease will need to be explored in future family studies.

In summary, we found that \textit{DES} mutations can be implicated in 1\% to 2\% of DCM without skeletal muscle disease involvement. We have shown that DCM-causing mutations occur outside the tail domain and have reported the first mutation in the highly conserved region of the 1A rod domain. Disruption of desmin cytoskeletal networks was not reproducibly shown in the 1 previous \textit{DES} mutation linked to DCM (Ile451Met). Three of our mutations located in the 2B helical rod domain clearly interfere with desmin network assembly, suggesting that similar pathogenic mechanisms exist between DCM and DRM. One of our mutations, Glu108Lys, occurred in the most conserved domain of desmin and did not visibly disrupt the pattern of intracytoplasmic desmin filaments. It is possible that this mutation exerts its effect by perturbing the desmin dimer-dimer interactions in the assembled network. Our finding of a second desmin tail mutation that is recurrent and low penetrant indicates that mutations in the less-conserved tail domain are perhaps better tolerated and may require interactions with other genetic or environmental factors to exert an effect. Finally, because clinical screening of \textit{DES} is now available, clinicians evaluating patients with cardiomyopathy/myopathy need to be selective in their utilization of \textit{DES} testing and critical in their evaluation of novel variants detected in cardiomyopathy patients caused by introduction of proline residues in desmin c-terminal alpha-helical segment. \textit{Molecular Therapy.} 2003;7:669–705.


References


Desmin Mutations in Dilated Cardiomyopathy

Desmin-related myofibrillar myopathy is a cardiac and skeletal muscle disease caused by mutations in the desmin gene. Mutations cluster near the center of the protein (2B domain) and typically cause skeletal muscle disease, which usually precedes any overt cardiomyopathy. Previously, only 1 desmin mutation leading to a pure cardiac phenotype had been reported, and the overall prevalence of desmin mutations in dilated cardiomyopathy was unknown. We screened the desmin gene for mutations using denaturing high-performance liquid chromatography in 425 unrelated patients and detected 5 novel mutations in 6 individuals for an overall prevalence of 1.4%. None of the individuals had any overt signs of skeletal muscle disease. The Glu108Lys mutation we detected is the first reported pathogenic mutation in the highly conserved 1A domain of the desmin protein. Expression of the 3 2B domain mutations in cell lines, including neonatal rat cardiac myocytes, severely disrupted the normal cytoplasmic desmin network. One mutation in the tail domain had a milder effect on desmin architecture, and the mutation in the 1A domain did not visibly affect desmin staining, suggesting that dysfunction of seemingly intact desmin networks is sufficient to cause dilated cardiomyopathy. Overall, the results show that desmin mutations are a relatively rare cause of dilated cardiomyopathy, can present in the absence of overt muscle disease, and mechanistically disrupt desmin cytoskeletal architecture in the majority of cases.
Prevalence of Desmin Mutations in Dilated Cardiomyopathy

Matthew R.G. Taylor, Dobromir Slavov, Lisa Ku, Andrea Di Lenarda, Gianfranco Sinagra, Elisa Carniel, Kurt Haubold, Mark M. Boucek, Debra Ferguson, Sharon L. Graw, Xiao Zhu, Jean Cavanaugh, Carmen C. Sucharov, Carlin S. Long, Michael R. Bristow, Philip Lavori and Luisa Mestroni

for the Familial Cardiomyopathy Registry and the BEST (Beta-Blocker Evaluation of Survival Trial) DNA Bank

_Circulation_. 2007;115:1244-1251; originally published online February 26, 2007;

doi: 10.1161/CIRCULATIONAHA.106.646778

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/115/10/1244

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/