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Intermediate Filament Diseases: Desminopathy

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Abstract

Desminopathy is one of the most common intermediate filament human disorders associated with mutations in closely interacting proteins, desmin and alphaB-crystallin. The inheritance pattern in familial desminopathy is characterized as autosomal dominant or autosomal recessive, but many cases have no family history. At least some and likely most sporadic desminopathy cases are associated with de novo *DES* mutations. The age of disease onset and rate of progression may vary depending on the type of inheritance and location of the causative mutation. Typically, the illness presents with lower and later upper limb muscle weakness slowly spreading to involve truncal, neck-flexor, facial and bulbar muscles. Skeletal myopathy is often combined with cardiomyopathy manifested by conduction blocks, arrhythmias and chronic heart failure resulting in premature sudden death. Respiratory muscle weakness is a major complication in some patients. Sections of the affected skeletal and cardiac muscles show abnormal fibre areas containing chimeric aggregates consisting of desmin and other cytoskeletal proteins. Various *DES* gene mutations: point mutations, an insertion, small in-frame deletions and a larger exon-skipping deletion, have been identified in desminopathy patients. The majority of these mutations are located in conserved alpha-helical segments, but additional mutations have recently been identified in the tail domain. Filament and network assembly studies indicate that most but not all disease-causing mutations make desmin assembly-incompetent and able to disrupt a pre-existing filamentous network in dominant-negative fashion. AlphaB-crystallin serves as a chaperone for desmin preventing its aggregation under various forms of stress; mutant *CRYAB* causes cardiac and skeletal myopathies identical to those resulting from *DES* mutations.

Introduction

Desminopathy is associated with mutations in desmin, alphaB-crystallin and possibly other proteins that interact with desmin. Identification of pathogenic mutations in *DES* and *CRYAB* genes, analysis of the underlying human disease phenotypes and successful modeling of these conditions in cell cultures and transgenic mice helped to understand the critical events involved in the pathogenesis of desminopathy. Widespread abundant desmin-immunoreactive deposits in the cardiac and skeletal muscles and aggregation of granulofilamentous material seen at the ultra-structural level are the morphological hallmarks of desminopathy.^{1,2} Transfected into cell cultures, many but not all mutant desmins are incapable of generating an intracellular filamentous network^{3–5} and disrupt pre-existing endogenous intermediate filament structures.⁶ Disease-associated *DES* mutations expressed in transgenic mice cause an accumulation of chimeric intracellular aggregates containing desmin and other cytoskeletal proteins. Misfolded desmin resists turnover by the cellular enzymatic machinery.⁷

Identification of multiple causative mutations in the DES gene^{6,9–11} helped to establish desminopathy as a distinct disease. The second genetically independent subset of desminopathy is myopathy associated with mutations in *CRYAB*, a chaperone that normally stabilizes proteins including desmin and prevents their irreversible aggregation.^{8,12} Genetic linkage of clinically and pathologically confirmed desminopathy to other loci has also been demonstrated.^{13,14} A more inclusive group of disorders named myofibrillar myopathy includes conditions associated with mutations in *SEPN1*, myotilin, *ZASP* and *Filamin C*.¹⁵

Genetic mechanisms influence desminopathy phenotype in several ways: (a) dominant, recessive and de novo mutations cause somewhat distinct syndromes; (b) desmin is expressed in skeletal, cardiac and smooth muscles, hence, combinations of damage in various tissues result in diverse phenotypes; and (c) the type and location of the mutation in *DES* or *CRYAB* may produce additional phenotypic modifications.

During the several years since previously published reviews on desminopathy, many new patients and affected families have been identified and studied. The range of clinical and myopathological manifestations has widened. As a result, the diagnostic criteria of desminopathy and interrelations with other disorders require a reevaluation.

Intermediate Filaments of the Muscle

Intermediate filament (IF) proteins are expressed in a cell-type specific manner and form 10-nm diameter filaments, which are intermediate in thickness between the 6–8-nm diameter microfilaments and the 25-nm diameter microtubules.¹⁶ At least 65 different IFs have been identified in humans.¹⁷ IFs are known to play a structural role in eukaryotic cells by participating in formation of the cell cytoskeleton and providing mechanical stability to the cells.¹⁸

The main muscle IF is desmin, a 53-kDa protein expressed in cardiac, skeletal and smooth muscles. In mature skeletal muscle, Desmin links adjacent myofibrils at the level of the Z disc and binds myofibrils to the sarcolemma at the level of the costameres, possibly through plectin.¹⁹ This allows the formation of a continuous cytoskeletal network that maintains a spatial relationship between the contractile apparatus and other structural elements of the cell,¹⁸ providing maintenance of cellular integrity, force transmission and mechanochemical signaling. In the heart, desmin is increased at intercalated discs and is the major component of the Purkinje fibers.²⁰ In smooth muscle, desmin is located in the cytoskeletal region at the dense bodies and dense plaques.

Ablation of desmin expression in mice by gene targeting²¹ has demonstrated that desmin expression is crucial for maintaining the architectural and functional integrity of striated muscle. Mice lacking desmin develop numerous muscle architectural and ultrastructural defects, especially in extensively used muscles such as the heart, soleus and diaphragm. Structural abnormalities include loss of the lateral alignment of myofibrils, perturbation of myofibril anchorage to the sarcolemma and loss of nuclear shape and positioning.^{22,23}

AlphaB-crystallin has its own influence on the cytoskeleton as a molecular chaperone for desmin and other proteins. AlphaB-crystallin directly binds titin/connectin at the I-band region, more specifically at the N2B and I26/I27 domains, as demonstrated by both copurification and colocalization of alphaB-crystallin with titin/connectin.^{24–26}

Molecular Genetics

Desmin and the Desmin Gene

Human desmin is encoded by a single copy gene (*DES*) located in chromosome band 2q35;²⁷ it encompasses nine exons within an 8.4 kb region and codes for 476 amino acids.²⁸ The gene is highly conserved among vertebrate species. In accordance with its function, the desmin molecule is organized into three domains: a highly conserved alpha-helical core of 308 amino acid residues flanked by globular N- and C-terminal (“head” and “tail”) structures.²⁹ The alpha-helical core maintains a seven-residue (heptad) repeat pattern with a typical sequence of hydrophobic and hydrophilic amino acids. This heptad repeat structure guides two polypeptides into formation of a homopolymeric coiled-coil dimer, the elementary unit of the filament. The heptad periodicity within the helical rod is interrupted in several places resulting in four consecutive helical segments 1A, 1B, 2A and 2B connected by short nonhelical linkers.

Structural analysis of the 2B helix attracted maximum attention because more than 50% of the known *DES* mutations have occurred in this region. The 2B segment located at the C-terminal part of the desmin core domain contains a discontinuity in the heptad repeat pattern, a “stutter”, which is equivalent to an insertion of four extra residues at the end of the 2B eighth heptad.³⁰ The “stutter” is an obligatory feature of all IF proteins and its position is absolutely conserved.³¹ Experimental “straightening out” of the stutter by inserting three “missing” amino acids to restore a continuous heptad repeat leads to inability of this “stutterless” molecule to anneal into longer filaments.³² As a compensation for the stutter, the coiled coil slightly unwinds in the stutter vicinity. The local unwinding caused by the stutter modifies the assembly of the protein and its interaction properties.

Another thoroughly examined structure is the 405-YRKLEGEESRI-416 motif at the C-terminal end of the 2B helix. Starting with several amino acids preceding the YRKLEGEESRI motif and through the YRKLL peptide, the coiled-coil structure loosens and the alpha-helices gradually separate eventually bending away from each other at the EGEE level.³³ In vitro data demonstrate that this motif directs the proper formation of tetramers and controls the number of subunits per filament cross section.

The desmin “tail” domain contains ~30% beta-sheet with the remainder of the domain having predominantly random structure and lacking the heptad repeat pattern. The desmin tail is involved in the longitudinal head-to-tail tetramer assembly³⁴ and control of lateral packing, stabilization and elongation of the higher order filament structures.³⁵ However, the tail’s major function seems to be interacting with other cytoskeletal proteins to establish a cytoplasmic intermediate filament network.³⁶

Desmin normally interacts with many other structural proteins (Fig. 1), including intermediate filament-associated proteins (IFAPs) which cross-link desmin filaments into a network that anchor the cytoskeleton.¹⁶ The inability of desmin to interact with these proteins may trigger disease development.

Nucleotide sequencing of 163 control individuals in the NIH laboratory (Goldfarb, unpublished) detected 17 DNA polymorphisms (Table 1) and several reported by others have been included in the NCBI database. c.914T>C, c.1100C>G and c.1190A>G are the most frequent. The p.Ala213Val and p.Val459Ile substitutions have been seen in unaffected control individuals, but both are thought to influence the disease phenotype (see below).

DES Mutations

The number of disease-causing *DES* mutations has reached 42 (Table 2 and Fig. 2): 37 missense mutations; 3 small in-frame deletions, deletion of exon 3 caused by mutations in splice

donor or acceptor sites flanking exon 3 and an insertion of a single nucleotide resulting in premature translation termination. Thirteen mutations are recurrent and the others private. Five mutations have occurred in the “head” domain, five in the 1B segment, the most—22 mutations—are located in the 2B alpha-helix and 10 in the tail domain. No mutations have so far been identified in the 1A and 2A segments.

Mutations in the 1B Segment of Desmin

A homozygous deletion of 21 nucleotides predicting an in-frame loss of seven amino acids from Arg173 through Glu179 (Arg173_Glu179del) in the 1B helix caused a severe clinical syndrome and compromised the ability of desmin to assemble into intermediate filaments in cell culture.¹⁰ Although the p.Ala213Val substitution was seen in four control individuals of 199 tested⁴⁰ and 2 of 86 analysed for another study,³⁸ the information generated so far supports the idea that this may be a modifying functional polymorphism. Although p.Ala213Val desmin created a filamentous network in SW13 cells and preserved the existing network in the C2C12 cells,⁴⁰ filament assembly experiments showed it aggregated in the viscometer; in the BMGE +H cells, the p.Ala213Val filaments were bundle-like, suggesting that the pathomechanisms of this mutation probably involve subtle but critical interactions with non-IF components in muscle cells.⁶¹

A heterozygous single-nucleotide (adenine) insertion mutation occurring at the third position of codon 241 causes a frameshift leading to serial amino acid replacements: Val242Glu, His243Ser, Glu244Ala and eventually a premature termination signal at codon 245 (numbering according to the updated sequence, GenBank submission # AF167579). This mutation is predicted to create a truncated desmin molecule with molecular weight of 27 kDa.⁴² Transfection studies confirmed that this mutation induces collapse of the preexisting desmin cytoskeleton. It also alters the subcellular distribution of mitochondria and affects biochemical properties of mitochondria in affected skeletal muscles.

A series of mutations has been identified in the highly conserved donor and acceptor splice sites flanking exon 3 (Table 3). The 96-bp exon 3 sequence encodes 32 complete codons, therefore total deletion of exon 3 would not interrupt the reading frame and is predicted to result in synthesis of a desmin polypeptide that is lacking 32 residues from Asp214 through Glu245 (p.Asp214_Glu245del).³ This deletion disrupts the heptad repeat pattern and interferes with the coiled-coil structure. The presence of the deletion was confirmed on the mRNA level. Functional analysis indicates that desmin lacking the 32 amino acids was incapable of forming a filamentous network in SW13 (vim-) cells.³ Binding sites to nebulin⁶² and perhaps other interacting proteins are located within this segment.

Mutations in the 2B *DES* Segment

Current data show significant clustering of mutations and polymorphisms in exons 5 and 6 corresponding to the 2B helix. The cluster includes 22 mutations (Fig. 2, Table 2), or 52% of all known *DES* mutations within only 25% of the coding region. Ten missense mutations introduce proline. Proline is not normally present in the desmin helical rod and is known as a potent helix breaker; its dihedral angle is fixed at -65° and creates a kink in the protein structure.⁶³ In addition, proline destabilizes alpha-helix by its inability to form hydrogen bonds. In mutagenesis experiments, the introduction of proline residues resulted in production of short, thick and kinked abnormally assembled filaments.⁶⁴

The p.Arg350Pro mutant is incapable of forming a desmin IF network in BMGE+H, MCF7, or SW13 cells and disrupts the endogenous vimentin cytoskeleton in 3T3 fibroblast cells.⁴⁶ The filament assembly process of the p.Arg350Pro mutant is disturbed at the unit length filament level and the lateral packing taking place in the first phase of assembly ultimately

leading to abnormal protein aggregation.⁴⁶ Focal disturbances in the assembly may inhibit the proper interaction of desmin with other cellular binding partners.

A comparative study of pathogenic potentials of *DES* mutations based on their effects in cell lines indicated that the p.Ala213Val, p.Asn393Ile and even proline-inserting mutations p.Ala360Pro and p.Glu389Pro allowed *bona fide* filament formation when transfected separately.^{40,61} However, when the p.Ala360Pro and p.Asn393Ile mutations were cotransfected, this caused devastating effects in each cell line used in the experiment.⁴⁰ Indeed, in a family segregating both p.Ala360Pro and p.Asn393Ile mutations, a highly aggressive early onset cardioskeletal myopathy affected only those having both mutations in a compound heterozygous fashion but spared the carriers of either mutation.⁹ The p.Ala337Pro, p.Leu338Arg, p.Asp399Tyr, p.Glu401Lys and p.Arg406Trp mutations alone make desmin filaments dysfunctional and cause increasingly more severe disease that starts earlier and leads to the development of life-threatening dysphagia, cardiomyopathy, or respiratory weakness.⁴⁰

The severity of illness caused by the mutations located in the C-terminal part of the 2B alpha-helical domain can be explained by structural relationships these mutations potentially disrupt.⁴⁰ The p.Leu338Arg mutation replaces an apolar amino acid, thus disrupting the highly conserved (abcdefg)n repeat pattern. The p.Asp399Tyr, p.Glu401Lys and p.Arg406Trp mutations disrupt potentially critical intrahelical and interhelical salt-bridge interactions.⁴⁰ In addition, the p.Arg406Trp mutation identified in four unrelated West European patients disrupts the fine tuned arrangements within and around the highly conserved YRKLEGEESRI motif of the 2B helix.⁵⁴ A molecular image of a short peptide from this motif containing the p.Arg406Trp mutation shows that replacement of arginine with a larger tryptophan residue makes the peptide at least a half-turn longer and therefore changes the peptide structure and symmetry.⁵⁴ Filament assembly studies indicate that the p.Arg406Trp mutant has a reduced ability to support longitudinal annealing as well as radial compaction.⁵

The p.Glu413Lys mutation is positioned near the p.Arg406Trp mutation within the YRKLEGEESRI segment. Structural analysis indicates that desmin residue Glu413 is still part of the coiled-coil segment.⁵⁵ Apparently, any change in this highly conserved region can influence filament assembly by disrupting interactions within the dimer and/or above the dimeric level. The results of molecular modeling indicate that the p.Glu413Lys mutation alters electrostatic interactions which are important for the proper dimer-dimer interactions during the assembly process. In addition, the intrahelical salt bridge projected to appear as a result of the p.Glu413Lys mutation would increase stiffness of the coiled-coil structure thus multiplying the destructive effects of the mutation.

Small in-frame deletions in the 2B alpha-helix have been identified in three families.⁴⁹ Molecular modeling indicate that the disease mechanism in these cases is associated with the disturbance of the dimer coiled-coil structure. The p.Glu359_Ser361del mutation creates a second stutter adjacent to the normally existing stutter (Fig. 3). This is very likely to cause additional local unwinding of the coiled coil. The p.Asn366del mutation also occurring in the vicinity of the wild-type stutter converts the latter into a heptad repeat stammer, which is expected to cause overwinding of the coiled coil.⁴⁹ Both mutations are thus expected to result in an altered coiled-coil geometry within segment 2B. As a consequence, the angular position of the tail domain with respect to the dimer axis is changed and dimer-dimer interactions and ultimately the filament assembly are very likely to be compromised. Functional studies conducted by expression of full length mutant cDNAs in SW13 and BHK21 cells confirmed the pathogenicity of these mutations.

The p.Ala357Pro mutation also destroys the stutter by introducing a proline residue and results in accumulation of desmin-positive aggregates in human muscle and evident perturbation of the intermediate filament network in transfected cells.⁴⁸

Tail Domain Mutations

The disease mechanism in patients with the “tail” domain mutation is distinct from the alpha-helical rod mutations. The nonhelical tail domain lacks the heptad repeat pattern and is involved mainly in interacting with other cytoskeletal proteins to establish a cytoplasmic intermediate filament network. The inability to interact with these proteins may trigger disease development. Expression of tail mutants in SW13 cells led to formation of an apparently normal filament network, indicating that at least some of the tail mutations did not prevent normal filament assembly and network formation.^{56,58} Furthermore, the p.Thr442Ile, p.Lys449Thr, p.Ile451Met and p.Val469Met mutants (but not p.Arg454Trp and p.Ser460Ile) left intact the pre-existing intermediate filament network when transfected into C2C12 cells,^{56,58} suggesting that the tail mutations do not have the dominant negative effects shown for almost every alpha-helical mutation. Nevertheless, desmin tail domain mutations cause severe cardiac and skeletal myopathy similar to syndromes associated with desmin alpha-helical domain mutations. Further studies are needed to fully understand disease mechanisms associated with tail mutations.

Incomplete Penetrance

As some *DES* mutations are less pathogenic than others,⁴⁰ members of families carrying the least pathogenic mutations never develop the disease. The phenomenon of incomplete penetrance was first established in the DCM 20-032 family in which some but not all p.Ile451Met mutation carriers expressed the cardiomyopathy phenotype.⁵⁷ In “family B” with progressive skeletal myopathy and no evidence of cardiac involvement three members carrying the p.Ile451Met mutation were also clinically asymptomatic in their 50s and 60s.⁵⁸

Modifier Genes

Five patients showed a combination of mutations in *DES* with mutations or functional polymorphisms in *DES* or other “neuromuscular” genes, *alpha-glucosidase*, *ZASP*, *MYOT*, or *Lamin A/C*. A compound heterozygous carrier of two substitutions in the *alpha-glucosidase* gene: a c.271A>G (p.Asn91Asp) substitution inherited from his mother and a c.781G>A (p.Ala261Thr) change inherited from the father had also a heterozygous p.Ala213Val *DES* mutation inherited from the mother.⁴⁰ None of his parents or other relatives was affected with any neuromuscular disorder. There is no indication that alpha-glucosidase interacts in any way with desmin, but some kind of synergetic influence can be expected since both Pompe disease and desminopathy cause muscle pathology.

A combination of *lamin A/C* p.Arg644Cys and *DES* p.Val469Met mutations was observed in another family.⁶⁰ The patients developed muscle weakness and complete heart block. Immunohistochemistry of the explanted heart and biopsied skeletal muscle showed desmin aggregates and granulofilamentous electron dense material on EM. Desmin and *lamin A/C* proteins are believed to be indirectly connected via other intermediate filament proteins.¹¹

CRYAB and AlphaB-Crystallin

CRYAB is mapped to chromosome 11q22.3-q23.1 and is composed of three exons highly conserved in a variety of species.⁶⁵ AlphaB-crystallin belongs to the “small heat shock protein” family, which includes hsp20, hsp22, hspB2, B3, hsp25, hsp27 and the myotonic dystrophy kinase binding protein. Although it was originally discovered and classified as a lens protein,

alphaB-crystallin is found in nonlenticular tissues and is abundant in cardiac and skeletal muscle.⁶⁶

AlphaB-crystallin is a chaperone that responds to stressful conditions by binding to unfolded proteins and preventing their denaturation and aggregation;⁶⁷ it binds to desmin and cytoplasmic actin and helps to maintain cytoskeletal integrity.⁶⁸ When a cell is subjected to stress, alphaB-crystallin protects muscle fibers from the effects of ischemia.⁶⁹ AlphaB-crystallin participates in a number of other cellular processes, helping to modulate correct protein-folding, compartment-targeting, degradation and signaling.

CRYAB sequence variants not associated with any disease and their frequencies studied in Caucasian population⁷⁰ are shown in Table 4. No studies regarding functional consequences of the two amino acid altering variants have been carried out, but it is reasonable to assume that polymorphisms in the promoter region may influence gene expression.

***CRYAB* Mutations**

Most of the currently known *CRYAB* mutations occurred in highly conserved regions; they generate adverse reactions when expressed in cell cultures⁸ and a mouse model revealed a collapse of the desmin network.¹² Disease phenotypes associated with *CRYAB* mutations are clinically heterogeneous, including four mutations causing cardioskeletal syndrome with cataract present or absent and two mutations associated with cataracts alone.

A heterozygous A-to-G transition at *CRYAB* codon 120 resulting in p.Arg120Gly mutation⁸ was identified in the original multigenerational French family with autosomal dominant desminopathy.⁷¹ The Arg120 residue is located in the most conserved region shared by other small heat-shock proteins. Structural and functional studies indicate that the mutant alphaB-crystallin had reduced or completely lost chaperone function.^{72,73} AlphaB-crystallin lost the ability to interact with alphaA-crystallin, but strongly interacted with wild type alphaB-crystallin,⁷⁴ suggesting a mechanism for dominant negative effect. In transiently transfected cells, mutant alphaB-crystallin accumulated in inclusion bodies (aggresomes) that may be due to misfolding of the mutant protein.⁷³

Muscle cell lines transfected with p.Arg120Gly cDNA also show the presence of intracellular aggregates that contain desmin and alphaB-crystallin.⁸ Transgenic mice expressing mutant alphaB-crystallin have abnormal desmin and alphaB-crystallin immuno-positive aggregates in cardiomyocytes.¹² This compromises cardiac muscle function and results in cardiac hypertrophy.

A 2-bp deletion (c.464_465CTdel) in the C terminus of alphaB-crystallin resulting in a truncated protein of 162 amino acids, instead of the normal 175, was present in a patient with myofibrillar myopathy.⁷⁵ The mutation was predicted to impair the ability of alphaB-crystallin to inhibit heat-induced protein aggregation of unfolded and denatured proteins, resulting in aberrant accumulation of proteins in muscle fibers. A c.451C>T transition resulting in a p.Gln151X substitution was also identified in a patient with myofibrillar myopathy.⁷⁵ The mutation results in a truncated protein of 150 amino acids and is predicted to be functionally deficient. Immunoblots under nondenaturing conditions showed that the mutant protein forms lower than normal molecular mass multimeric complexes with the wild type protein and exerts a dominant-negative effect.

The missense *CRYAB* p.Arg157His mutation occurring in an evolutionary conserved amino acid residue is associated with a late onset dilated cardiomyopathy. This mutation reduced the binding of alphaB-crystallin to the N2B domain of titin/connectin.²⁶

Other *CRYAB* mutations have been associated with nonsyndromic autosomal dominant congenital cataracts (ADCC). A single nucleotide deletion in *CRYAB* (c.450Adel) causing termination at codon 150 was identified in a 4-generation English family with a posterior polar ADCC.⁷⁶ A p.Pro20Ser mutation of *CRYAB* cosegregated with ADCC in a large Chinese family.⁷⁷

Pathogenesis

DES mutations are responsible for inadequate supply of normal functional desmin and toxic effects of aggregates containing mutant misfolded desmin and debris of other myofibrillar and ectopically expressed proteins that accumulate in the myofibers and eventually destroy them.^{7,78} Additionally, mutant desmin hampers normal interactions with other cytoskeletal proteins. The amount of desmin in affected muscle fibres of desminopathy patients is increased as demonstrated by immunoblotting.^{46,83} The pathological process in the myofibrils starts with the disintegration of the Z-disk, the functionally important site of tension transmission between the sarcomeres.¹⁵

Filament and Network Assembly Studies

Interference of *DES* mutations in filament-formation was tested in in-vitro filament formation experiments, generation of ordered cytoskeletal arrays and assays for the ability to integrate into an existing cytoskeletal network in various cell lines.⁶¹ Analysis of filament assembly behavior demonstrates that some desmin mutants do not prevent filament formation (p.Ala213Val, p.Glu245Asp, p.Ala360Pro, p.Glu389Pro, p.Asn393Ile, p.Asp399Tyr). Some others interfere with the assembly process at distinct stages and these are classified into three groups: (1) mutations compromising longitudinal annealing properties (p.Leu385Pro, p.Arg406Trp); (2) mutants with enhanced adhesiveness leading to filament aggregation (p.Ala337Pro, p.Asn342Pro, p.Ala357Pro); and (3) mutants showing rapid disintegration of assembly precursors (p.Leu345Pro, p.Arg350Pro, p.Leu370Pro).⁸³

It has been conclusively shown that pathogenic mutant desmin disrupts a preexisting filamentous network in a dominant-negative fashion.^{6,53,84} But some mutations located in the non-alpha-helical tail domain of the desmin molecule do not interfere with the initial IF assembly steps. Thus, the p.Thr442Ile, p.Lys449Thr, p.Ile451Met and p.Val469Met mutants, but not p.Arg454Trp and p.Ser460Ile, formed a filamentous network in SW13 cells. Furthermore, all tested tail domain mutants, including p.Arg454Trp and p.Ser460Ile, were incorporated into IF arrays of transfected C2C12 cells, suggesting that cultured myoblasts are able to neutralize the “poisonous” effect of the mutated protein.⁵⁶ Most likely, *DES* tail mutations affect multiple interactions with other cellular proteins resulting in distinct cellular malfunctions.

In cultured satellite cells taken from a patient carrying the p.Leu345Pro mutation, desmin created a fully normal network in early cell passages, however, in further passages an increasing number of cells showed abnormal accumulation of desmin-positive material with one of three patterns: perinuclear, spot-like or subsarcolemmal.⁸⁵ Nestin colocalised with the abnormal desmin deposits; alphaB-crystallin was only present in cells with a disrupted desmin network.

Animal Models

Although desmin knockout mice (*Des*^{-/-}) develop normally and are fertile, lack of a desmin filament network prevents organizing the cellular components spatially.^{21,23,81} Some cell architecture defects such as misaligned muscle fibers, abnormal sarcomeres, swollen mitochondria and unusual distribution of myosins are seen in earlier stages of development.⁸⁶ After birth, irregularities in the myofibrillar organization are predominantly observed in the extensively used skeletal muscles such as the tongue, diaphragm and the soleus muscle.^{21,23,}

⁸¹ Mitochondria exhibit an increase in size and number, a loss of correct positioning and finally degeneration, which was especially pronounced after exercise overload.²³

Cardiac muscle is most susceptible to the lack of desmin. Mice develop cardiomyopathy early in postnatal life manifested by lysis of individual cardiomyocytes, invasion of macrophages, varying degree of calcification and finally fibrosis.⁸¹ Older animals show fully characteristic morphology of muscle dystrophy.²³ Disorganized, distended and non-aligned fibers are observed in the diaphragm. Muscle fibres are gradually lost and replaced by fibrosis. The lack of desmin in growing and adult knock-out mice results in a multi-organ disorder involving severe disruption of skeletal and cardiac muscle architecture. These experiments show that desmin is essential for the structural integrity of skeletal muscle, but not for myogenic commitment, differentiation, or fusion of myoblasts.⁸⁷

Analysis of transgenic mice has provided insights into the mechanisms of intracellular protein aggregation. In transgenic mice expressing a human mutation p.Arg173_Glu179del, examination of the myocardium reveals an accumulation of chimeric intracellular aggregates containing desmin and other cytoskeletal proteins.⁷ Such inclusions are not seen in knockout mice, supporting the hypothesis that mutant misfolded proteins may act as seeds in the formation of these protein aggregates in desminopathies and likely in other myofibrillar myopathies. These aggregates clearly disrupt the continuity and overall organization of the desmin network throughout the cell.⁷ The protein aggregates appear as electron-dense granulofilamentous structures proximal to the nucleus and in the inter-myofibrillar space. Misfolded desmin protein escapes proteolytic breakdown and attracts other cytoskeletal proteins into high molecular weight insoluble chimeric aggregates⁸² that grow and become toxic.⁸⁸ Toxic effect of the aggregates may depend on sequestering of essential cellular proteins. Numerous fragmented filaments were found in the immediate area surrounding the aggregates.

Role of AlphaB-Crystallin

Chaperones assist normal protein folding and, if necessary, enhance ubiquitination and proteasomal degradation of abnormally constructed proteins. They help to restore proteins to their native conformation after these proteins have been misfolded by heat, ischemia, chemotoxicity, or other cellular stresses.⁷² An in vitro chaperone assay demonstrated that the mutant p.Arg120Gly alphaB-crystallin becomes functionally deficient.⁸⁹ Expression of the mutant alphaB-crystallin in SW13 and BHK21 cells leads to formation of abnormal aggregates that contain both desmin and alphaB-crystallin.⁸ Transgenic mice expressing mutant alphaB crystallin show the presence of abnormal desmin and alphaB-crystallin aggregates in the cardiomyocytes; formation of aggregates is the result of the loss of protection from alphaB-crystallin.^{12,90}

Aggregation of amyloid-like material was recently demonstrated to be a typical feature of many human cardiomyopathies, especially those caused by mutations in *CRYAB*.⁹¹ Physiologically, the inclusion of misfolded proteins into aggregates was proposed to be a protective mechanism in alphaB-crystallinopathy,⁹² linking desminopathy to a broad class of conformational neurodegenerative diseases. Remarkably, desmin knock-out and transgenic mice show less severe pathology as compared to the *CRYAB* transgenic mice.¹²

Evidence that kinases are involved in desminopathies has come from observations of CDC2 and CDK2 overexpression in the abnormal intracytoplasmic aggregates,^{93,94} but the specific role kinases may play in desminopathy has not yet been determined.

Clinical Manifestations

Definitions of Desminopathy

Desminopathy was originally described as skeletal and cardiac myopathy characterized by bilateral weakness in distal leg muscles spreading proximally, or in proximal muscles spreading distally and leading eventually to wheelchair-dependence.⁹⁵ Weakness may or may not involve upper extremities, trunk, neck flexors and facial muscles. In disease variants marked with early onset cardiomyopathy, patients experienced dizziness, syncopal and fainting episodes associated with conduction blocks requiring a permanent pacemaker. Respiratory muscle weakness is a frequent component; bulbar signs appear in the later stages of illness.

Many new affected families were identified and reported within the 10-year period since desminopathy has been classified as an independent entity; this review represents 65 published families with 98 patients. New knowledge allows defining more precisely the clinical manifestations and diagnostic criteria of desminopathy and relationships between desminopathy and other disorders.

The pattern of inheritance was reliably established in 52 desminopathy families. It was autosomal dominant in 32 families and autosomal recessive in three. Seventeen patients had sporadic disease, in seven of them both parents were available for genetic testing and evidence was obtained that the mutations have occurred *de novo*. The age of disease onset in patients with autosomal dominant disease is between 14 and 48 years, while patients with recessive mutations develop the disease in their childhood or adolescence. Analysis of clinical/pathological characteristics of desminopathy outlined several sometimes overlapping clinical forms.⁹⁶

Progressive Skeletal Myopathy

Uncomplicated progressive skeletal myopathy was observed in 13 patients from 6 families (Table 2). The disease onset in members of the p.Asn342Asp family, mother and son, was at age 30 and 23 years, respectively.¹¹ Initial symptoms were bilateral distal muscle weakness in the lower extremities that later spread to proximal leg muscles, upper extremities and neck flexors. ECG was normal as was the serum creatine kinase level (CK). Muscle biopsy showed abnormal accumulation of desmin-immunoreactive deposits in muscle fibers and the presence of red-rimmed vacuolated fibers. The p.Glu359_Ser361del mutation was identified in two Polish families⁴⁹ sharing a disease-associated haplotype. Eight patients were fully characterized. The age of disease onset was between 31 and 46 years. Initial symptoms were bilateral muscle weakness first in the lower and later in the upper extremities. Electrocardiogram (ECG) and echocardiogram (EchoCG) showed no abnormalities. Sections of skeletal muscle demonstrated the presence of cytoplasmic inclusions that were immunoreactive for desmin and contained electron-dense coarse granular and filamentous aggregates on EM.

Muscle CT scan studies performed in desminopathy patients carrying various *DES* mutations have identified a recognizable pattern of muscle involvement. At the thigh level, early involvement of the semitendinosus and sartorius is followed by the gracilis muscle and then the hip adductors. In advanced illness, quadriceps femoris and other muscles of the posterior thigh compartment also become affected. At the mid-calf level, initial changes occur in the peroneal group, followed by involvement of the anterior tibialis and later the posterior group⁵⁰ (Fig. 4).

Respiratory Dysfunction

Respiratory insufficiency can be a major cause of disability and death. Respiratory dysfunction causes nocturnal hypoventilation with oxygen desaturation and, eventually, daytime respiratory failure. The diaphragm, unlike other skeletal muscles, is functioning in an environment in which forces can be transmitted both in longitudinal and transverse directions during each respiratory cycle; desmin is the only known molecule having dual orientation and therefore serving as a viscoelastic element that dissipates mechanical energy in both planes. Reflecting on this critical significance of desmin in respiratory function, its content in the diaphragm is 38% higher than in the biceps femoris muscle.⁹⁸

Progressive skeletal myopathy with early respiratory muscle involvement, but no cardiac disease, was observed in 10 patients from 5 families. In a family carrying the missense p.Ala357Pro mutation, the father and his son and daughter developed at age 35 to 45 years slowly progressive muscle weakness in the lower and upper extremities but no signs of cardiac involvement.⁴⁸ Respiratory function tests revealed progressive reduction of respiratory muscle strength that became clinically detectable between approximately the 3rd and the 8th years of illness. A gradient between vital capacity in the upright and supine position of more than 30% is suggestive of preferential diaphragmatic involvement. The patients had modest (4-times normal) elevation of serum CK and normal ECG and EchoCG studies. Muscle biopsy showed variation in fibre size, intracytoplasmic eosinophilic patches immunocytochemically identified as desmin deposits and deposits of dense granular material between myofibrils and in the subsarcolemmal space on EM.

Cardiomyopathy

Primary cardiomyopathies are classified pathophysiologically as dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC).⁹⁹ DCM is characterized by an increased ventricular chamber size and reduced systolic output.⁵⁷ RCM results from processes that stiffen the myocardium by infiltration or fibrosis leading to impaired ventricular filling and reduced diastolic volume of either or both ventricles with the cavity size, wall thickness of the ventricles and ejection remaining near normal.¹⁰⁰ Of 34 patients with cardiomyopathy in which EchoCG was performed, DCM was diagnosed in 18, RCM in 10 and HCM in 6.

Atrioventricular conduction abnormalities requiring urgent implantation of a permanent pacemaker is a frequent feature in desminopathy patients attributed to the fact that the heart conduction system is rich in desmin. Desminopathy-associated atrioventricular conduction blocks may be associated with RCM, DCM and HCM.^{37,46,55}

Skeletal muscle weakness followed by cardiomyopathy was observed in 31 patients from 20 families (Table 2). A p.Leu345Pro mutation within the 2B helix⁶ was detected in a family that included 16 members suffering from gait disturbances caused by bilateral weakness in distal leg muscles that progressed to all limbs and bulbar, respiratory and facial muscles.¹⁰¹ Many of the surviving patients were confined to a wheelchair or using a walker 7 to 20 years after disease onset. Six of 8 studied patients were diagnosed with cardiac arrhythmias and conduction blocks about 12 years after the appearance of myopathic symptoms and developed congestive heart failure. Histopathologically, some skeletal muscle fibers were atrophic and contained abundant desmin-positive granulofilamentous deposits in the form of a reticular meshwork.

A phenotype characterized by cardiomyopathy with subsequently developing skeletal myopathy was seen in 15 patients from nine families. This group includes two brothers with a p.Asp214_Glu245del mutation.³ One patient developed DCM with recurrent left-sided cardiac failure, complete AV block and pulmonary hypertension.¹ Gait disturbance and weakness in

the legs appeared 10 years after the onset of cardiac illness and progressed to involve both hands. The patient died of cardiac failure at age 52 years. Skeletal muscle fibers showed accumulation of cytoplasmic bodies and patch-like lesions immunoreactive for desmin, alphaB-crystallin and dystrophin and granulofilamentous material in subsarcolemmal areas. His brother developed an AV block that required a pacemaker at age 41 years, but had no skeletal muscle weakness when last examined at age 50 years. He also died from cardiac complications.

Three patients from a family with p.Glu413Lys mutation experienced recurrent episodes of syncope requiring urgent cardiac pacemaker implantation at the ages of 30, 31 and 63 years.⁵⁵ Routine ECG showed atrioventricular block. On EchoCG, there was normal morphology and robust systolic function; atria were significantly enlarged and inferior vena cava distended. Doppler examination indicated restrictive pattern of left ventricular filling with deceleration time of E wave of mitral inflow shortened and annular velocity Em reduced, consistent with RCM. Two of the three patients developed muscle weakness and atrophy of the lower limb muscles starting 5 years after the cardiac disease onset in the index case and 16 years in the second case. Skeletal muscle biopsy showed multiple intrasarcoplasmic deposits reacting with anti-desmin antibodies and on EM granulofilamentous electron-dense material at the level of Z-lines.

The primary syndrome in seven patients from four families was RCM with atrio-ventricular block;³⁷ only two patients showed clinical evidence of skeletal myopathy. Desmin accumulation was demonstrated in the myocardial and skeletal muscle samples. The p.Ala213Val *DES* variant was detected in 12 unrelated desminopathy patients developing dilated⁴⁰ or restrictive³⁹ cardiomyopathy.

Cardiomyopathy alone with no signs of skeletal myopathy was seen in 19 patients from 15 families. Six members of an AD family bearing the p.Ile451Met mutation in the desmin tail domain developed cardiac failure between the ages of 15 to 37 years.⁵⁷ Two living patients, father and son, showed cardiomegaly and diminished left ventricular ejection fraction consistent with DCM. No signs of skeletal myopathy were observed. A cohort of six DCM patients with no skeletal muscle involvement caused by various *DES* mutations, including two patients with mutations in the tail domain, was recently reported.³⁸

Correlation between Genotype and Phenotype

Soon after routine genetic screening became available for diagnostic use, convincing evidence has emerged suggesting that different mutations result in somewhat distinct clinical phenotypes.⁹⁶ Cardiomyopathy, smooth muscle myopathy, neuropathy, respiratory dysfunction, facial paralysis or cataracts may be present in some cases and absent in others. With few exceptions, phenotypic manifestations in members of the same family were concordant, making it likely that the type and location of the mutation within the desmin molecule influences the phenotype. Analysis presented in Table 5 confirms that patients with mutations in the 2B segment tend to show primarily skeletal muscle pathology, while those carrying mutations in 1B and tail domains develop predominantly a more ominous cardiac disease. The difference between the frequency of cardiomyopathy in patients with 1B mutations vs. those with mutations in the 2B domain estimated by the Mantel-Haenszel Chi-Square criteria is at the level of $p = 0.0031$. The frequency of cardiomyopathy in patients with tail vs. 2B mutations is $p = 0.049$. We conclude therefore that the location of *DES* mutation exerts a significant influence on phenotypic characteristics. There is also clear tendency for the age of onset to be 4 to 10 years earlier and the disease progression faster in patients with cardiomyopathy as an early feature.

A discrepancy between *CRYAB* p.Arg120Gly mutation causing cardioskeletal myopathy and p.Arg157His mutation causing isolated cardiac muscle dysfunction was also investigated and found to be dependent on reduced binding of the p.Arg157His mutant to the heart-specific N2B domain, but not the I26/I27 domain of titin/connectin, while the p.Arg120Gly mutation decreased binding to both N2B and the striated muscle-specific I26/27 domains.²⁶ These observations suggest that the disease-causing mechanisms are different for these two *CRYAB* mutations.

Disease Severity in Patients with Autosomal Recessive Inheritance

Three autosomal recessive (AR) *DES* mutations are currently known. These patients had the earliest age of onset and the fastest progression of illness. A patient homozygous for deletion of seven amino acids in the 1B helix (p.Arg173_Glu179del),¹⁰ developed generalized muscular weakness and atrophy, predominantly in distal muscles of the upper extremities, atrioventricular (AV) block requiring implantation of a permanent pacemaker and intestinal malabsorption.¹⁰² EchoCG showed dilatation of the right cardiac chambers. Disease progression led to cardiac and respiratory failure and intestinal pseudo-obstruction. The patient died suddenly at age 28 years. Abundant subsarcolemmal crescent-shaped strongly eosinophilic masses in skeletal myofibers and centrally located eosinophilic bodies in the cardiomyocytes were immunoreactive for desmin and ubiquitin. Ultrastructural studies revealed electron-dense coarse granular and filamentous aggregates continuous with the Z lines.

In another AR family, three siblings were compound heterozygous for the p.Ala360Pro and p.Asn393Ile *DES* mutations.⁹ They presented with syncopal episodes and complete heart block requiring insertion of a permanent pacemaker at the age of 2, 9 and 10 years. EchoCG showed moderate to severe biatrial dilatation but normal ventricle size. Cardiac catheterization revealed left ventricle diastolic dysfunction.¹¹ Between ages 20 and 24 years, all three developed progressive muscle weakness and wasting in the extremities and trunk, weakness in the neck and facial muscles, swallowing and breathing difficulties. All three died from congestive heart failure at 28, 30 and 32 years of age. Histopathologic findings consisted of intramyofibre accumulation of amorphous desmin-immunoreactive material with a characteristic subsarcolemmal distribution. Several older family members carrying either the p.Ala360Pro or p.Asn 393Ile heterozygous mutation had no signs of muscle or heart disease.

De Novo Mutations

Desminopathy patients associated with de novo *DES* mutations represent a complex group with even wider margins of phenotypic variability. In several so-called sporadic cases with no family history, microsatellite markers and *DES* polymorphisms were used in patients and both parents to exclude false paternity and trace the mutant allele. If neither of the parents was affected and none showed *DES* mutations, but shared a haplotype with the affected offspring, this mutation was considered to be generated de novo.³

Four West European patients with de novo p.Arg406Trp mutation presented at ages 15, 18, 23 and 24 years with cardiac arrhythmia and conduction block followed in quick succession by muscle weakness.⁵⁴ The causative mutation was not present in the parents; the mutation has occurred de novo on a paternal allele transmitted to the affected offspring. All four became severely incapacitated in their twenties-early thirties and one of the patients died from decompensated congestive heart failure at the age of 28 years. Sections of skeletal muscle showed a significant accumulation of aggregates strongly positive for desmin and EM evaluation showed abnormal granulofilamentous aggregates among the myofibrils and beneath the sarcolemma.

p.Asn342Asp *DES* mutation was identified in affected mother and son, but not in the unaffected maternal grandparents.⁵⁸ The results of the haplotype analysis demonstrated that the causative mutation has occurred de novo on an allele the affected mother inherited from the grandmother and then transmitted to her son, suggesting that the grandmother was germ line mosaic.

Phenotypes Associated with CRYAB Mutations

The p.Arg120Gly *CRYAB* mutation was identified in a large family with autosomal dominant myopathy involving proximal and distal limb muscles often associated with neck, trunk and velopharynx muscle weakness, hypertrophic cardiomyopathy, respiratory disturbances and discrete lens opacities.^{8,95,103} The age of disease onset was in the mid-30s and rate of progression was moderate. Muscle biopsy results were characteristic of desminopathy.^{8,103}

Two further patients carrying c.464_465CTdel and c.451C>T (p.Q151X) *CRYAB* mutations had adult-onset cervical, limb girdle and respiratory muscle weakness in patient 1 and proximal and distal leg muscle weakness in patient 2.⁷⁵ Both had myopathic electromyogram with abnormal electrical irritability. Muscle biopsy findings were characteristic of myofibrillar myopathy and mild denervation. Disintegration begins at the Z-disk and results in abnormal local expression of desmin, alphaB-crystallin, dystrophin, neural cell adhesion molecule (NCAM) and CDC2 kinase. Neither patient had cardiomyopathy or cataracts.

The missense p.Arg157His *CRYAB* mutation was associated with dilated cardiomyopathy presenting after the fourth decade of life; no skeletal muscle involvement was observed.²⁶

Myopathology

Desmin-reactive deposits in the cardiac and skeletal muscles and granulofilamentous material at the ultrastructural level are considered morphological hallmarks of desminopathy.¹⁰⁴

Although many of the myopathological features are not specific, the overall pattern is recognizable and is being used for diagnostic purposes.

Skeletal Muscle Pathology

In normal myofibres, desmin is located beneath the sarcolemma and at the Z-disk, whereas the intracellular desmin intermediate filament network is less clearly visualized.^{105,106} At the electron microscopic (EM) level, desmin intermediate filaments are visible as straight 8–10 nm nonhollow filaments. Desmin and nestin are normally present at the sub-neural apparatus of the neuromuscular junctions and at myotendinous junctions.^{107,108}

In patients with desminopathy, examination of skeletal muscles by light microscopy typically show irregularly shaped abnormal regions containing amorphous eosinophilic inclusions best identified with modified trichrome stain as dark green or bluish material. They are located in the subsarcolemma or within the cytoplasm. The size and shape of the inclusions varies: they may consist of “plaque-like” patches or appear as small rounded structures.^{42,47,55} Frequently, both types of inclusions coexist in the same specimen (Fig. 5). In addition, hyaline structures, cytoplasmic bodies, rods and spheroid-like bodies are reported in other cases.^{3,11,47}

Oxidative enzyme and ATPase activity is typically absent in the inclusions leading to rubbed-out lesions. Impairment of the mitochondrial respiratory chain complex has been reported,⁴² but ragged-red fibers have not been observed. With only a few exceptions, congophilia is not reported in desminopathies.

Although not specific of desminopathy, rimmed vacuoles have been observed in a majority of cases. Other nonspecific myopathic features such as variation in fibre size, scattered atrophic fibres and an increased number of internal nuclei are also frequently observed. Muscle fibre

necrosis, inflammation or regeneration have been reported in a few cases.^{40,42,46} Fibrosis and fatty replacement have been found occasionally.^{1,3,43,46,85} Under electron microscopy, all desminopathy muscle specimens showed granulofilamentous material representing a unifying feature.¹⁰⁴ The granular component of the granulofilamentous material is often more prominent than the filamentous, accumulating beneath the sarcolemma and between the myofibrils. In addition, Z-band streaming and more compact bodies of spheroid or cytoplasmic type were noted in some cases (Table 6). Autophagic vacuoles containing myelin-like lamellae and debris⁴³ and focal groupings of mitochondria^{42,47,55,56} have been observed in some specimens.

Immunohistochemical studies have consistently demonstrated the presence of inclusions or deposits reacting with antibodies against desmin and other proteins such as alphaB-crystallin and, although rarely investigated, synemin,^{42,85,97} syncoilin,¹⁰⁹ plectin,^{42,43} nestin,^{6,85} dystrophin,^{2,43,50} merosin, alpha- and beta-dystroglycan, alpha-, beta-, gamma- and delta-sarcoglycans, utrophin, collagen VI, NOS, caveolin, dysferlin, beta- and gamma-laminin, actin, actinin, N-CAM, heat shock protein 72/73,⁴³ myotilin,^{2,43,50} gamma-filamin,⁵⁰ vimentin, beta-spectrin¹¹ and ubiquitin.^{43,50,102} The size, shape and localization of protein aggregates differ from one case to another. These may be restricted to the subsarcolemmal regions or within the cytoplasm; they may be diffuse or well demarcated, and in some cases both diffuse and well demarcated small deposits are found in the same specimen (Fig. 6).

Of the large number of mutations already documented in the *DES* gene, most have been published as case reports and only a few mutations described comparatively.^{11,40,56} Thus, it has been difficult to establish genotype-morphotype correlations of individual mutations. Distinctive muscle pathology was reported in a patient carrying a homozygous p.Arg173_Glu179del mutation: prominent eosinophilic crescent-shape masses located under the sarcolemma in virtually each muscle fiber. These lesions stained dark green with modified trichrome, displayed autofluorescence and showed strong desmin immunoreactivity (Table 6).^{10,102} Identical abnormalities have been observed in an unpublished patient carrying the same *DES* deletion (Fig. 7).

In the well studied family with *CRYAB* p.Arg120Gly mutation, muscle biopsy showed disorganization of filamentous network and characteristic regions in which the intermyofibrillar network completely disappeared (rubbed-out fibers). Affected areas contained instead abnormal aggregates immuno-positive for desmin, alphaB-crystallin, dystrophin and ubiquitin. A subsarcolemmal and intermyofibrillar accumulation of dense granulofilamentous material with various degenerative changes was observed on EM.^{8,103}

Cardiac and Smooth Muscle Pathology

While cardiomyopathies are a frequent component of desminopathies, cardiac pathology has not been fully characterized. Postmortem examination of the heart from a patient carrying an p.Arg173_Glu179del mutation showed dilatation of the right chambers; cardiac cells displayed abundant centrally located globular eosinophilic bodies strongly reacting against desmin and ubiquitin.^{10,102} The right ventricle was the more severely involved area. Additional cardiopathological findings have been reported based on examination of cardiac biopsies or explanted hearts.^{37,43,52,56} In all described cases, microscopic examination revealed protein aggregates in the center of cardiac muscle fibre, rather than subsarcolemmally, hypertrophy of cardiomyocytes, prominent nuclei and considerable interstitial fibrosis (Fig. 8). Cardiac myocytes contain aggregates composed of desmin (Fig. 8) and other proteins such as alpha-B crystallin, ubiquitin, heat shock protein 72/73 and others.⁴³ At the ultrastructural level the deposits consist of granulofilamentous material, often clustered at the intercalated disks. Overall, the cardiac pathology as described is very similar to skeletal muscle pathology. Desmin-positive aggregates and other phenomena characteristic of skeletal muscle pathology were also encountered in intestinal smooth muscle cells of a desminopathy patient.¹⁰²

Diagnosis

Recognition of desminopathy can be difficult because of the heterogeneity of clinical features and nonspecificity of the histopathology. The pattern of inheritance is also variable; most of the known mutations are dominant, but others are recessive and a significant number of mutations are generated de novo.

Diagnostic Criteria

The diagnosis of desminopathy should be consistent with the following basic criteria.^{2,11,15,78,110} History of slowly progressive muscle weakness, dyspnea, dysphonia, dysphagia and cardiac symptoms. Physical examination reveals distal and proximal weakness; trunk, neck-flexor and facial muscles are involved in some patients. Tendon reflexes are diminished or normally active. Joint retractions at ankles may be present. A restrictive ventilatory defect may result from respiratory muscle weakness. ECG shows conduction blocks in a high proportion of cases. EMG reveals abnormal electrical irritability (fibrillation potentials, positive sharp waves, complex repetitive discharges and occasional myotonic discharges) in most patients. The motor unit potentials show myopathic features or a combination of myopathic and neurogenic changes. Serum creatine kinase concentration can be normal or elevated to no greater than seven-fold above the upper normal limit.

Muscle histology reveals: (1) characteristic alterations in trichromatically stained frozen sections consisting of amorphous, or granular material in a variable proportion of the muscle fibers, (2) sharply circumscribed decreases of oxidative enzyme activity in many abnormal fiber regions, (3) small vacuoles in a variable number of fibers; (4) abnormal ectopic expression of desmin, alphaB-crystallin and dystrophin in immunocytochemical studies. Electron microscopy shows granulofilamentous material under the sarcolemma or within the myofibrils. Autophagic vacuoles are observed in some cases.

Recommended Investigations

- Electrophysiological investigations including nerve conduction studies and EMG examination to exclude neurogenic causes of weakness, motor neuron disease and peripheral neuropathy.
- ECG used routinely to identify arrhythmias and cardiac conduction defects. Holter monitoring is indicated if symptoms suggest an intermittent arrhythmia.
- EchoCG to detect and diagnose the type of cardiomyopathy, it should be performed even in patients with no cardiac symptoms.
- Respiratory function tests even if respiratory symptoms are not present.
- Muscle imaging to differentiate desminopathies from other MFMs.

Molecular Testing

Genetic testing has become essential to establish an accurate diagnosis of desminopathy. Clinical genetic testing for desminopathy patients is now available at a Clinical Laboratory of Baylor College of Medicine, Jeffrey A Towbin, MD, Director (<http://www.genetests.org/>, go to "Laboratory Directory" and type in "desminopathy"). Clinical and myopathological diagnosis must precede genetic testing. It is expected that more patients with the clinical diagnosis of desminopathy will in fact show mutations in other interacting genes, or a combination of genes. Routine genetic testing is necessary for providing appropriate genetic counseling. The true prevalence of desminopathy may be established only when most or all patients clinically and pathologically resembling desminopathy are tested genetically. The era of gene-specific and mutation-specific treatments for inherited diseases is quickly approaching.

Relationships between Desminopathy and Other Myofibrillar Myopathies

Pathologically, desminopathies belong to a genetically heterogeneous group named myofibrillar myopathies (MFM).^{78,110} The common pathological pattern is myofibrillar dissolution, accumulation of myofibrillar degradation products and ectopic expression of multiple proteins. Mutations in six genes have been identified as causing MFM: desmin, alphaB-crystallin, selenoprotein 1, myotilin, ZASP and gamma-filamin. Further genetic heterogeneity is suspected (see ref. ¹⁵ for a review).

Differences and Similarities

Although no defined canon of prescribed myopathological characteristics for individual forms of MFM exist, subtle possible differences have already emerged (Table 7). Light and EM examination of skeletal muscles in patients with *CRYAB* mutations (crystallinopathy) reveal very similar if not identical features to those described in patients with mutations in desmin.^{8,71,75,103} However, immunocytochemical studies have shown lack of ubiquitin, gelsolin, or alpha1-antichymotrypsin accumulation in abnormal fibres as a distinguishing feature in some cases of crystallinopathy,⁷⁵ but not the others.^{8,71,103}

Some pathologic features of desminopathy make it distinct from typical images seen in myotilinopathy. First, the inclusions found in desminopathy are usually smaller and less prominent than those observed in myotilinopathy; second, nonrimmed vacuoles have been repeatedly reported in myotilinopathy but not desminopathy; third, congophilia is an important feature associated with the hyaline lesions observed in myotilinopathy, but it is usually absent or faint in desminopathy; four, typically spheroid-bodies are part of the picture in myotilinopathy but not desminopathy. Finally, ubiquitin, gelsolin and particularly myotilin expression is much more intense in myotilinopathy than desminopathy patients.^{2,50,111–114}

Histological and immunohistochemical analysis of skeletal muscles in zaspopathy¹¹⁵ reveal abnormalities very similar to those found in myotilinopathy. The hallmark of desminopathy, the granulofilamentous material, could also be present in myotilinopathy, zaspopathy and filaminopathy patients, but autophagic vacuoles, myofibrillar degeneration and accumulation of compacted and fragmented filaments and dense material seem to be more consistently found in myotilinopathy and zaspopathy.^{111–115} In addition, clusters of cytoplasmic 15–28 nm filaments have been observed in myotilinopathy.^{114,116}

In a subset of patients with mutations in *SEPN1* gene, muscle biopsies showed distinctive circumscribed hyaline plaques resembling Mallory bodies known in hepatic disorders. Immunohistochemical studies revealed accumulation of desmin, alphaB-crystallin, actin, A-beta-amyloid and many other proteins within the plaques. Z-band streaming, specific minicores, aggregates of intermediate filaments and tubulofilaments in the sarcoplasm and plaques containing Z-disc derived material were observed on EM.¹¹⁷

Frequency of Desminopathy among Other Myofibrillar Myopathies

Among 63 patients with myofibrillar myopathy studied in the Mayo clinics, only 6% show mutations in *DES*, while 3% had sequence alterations in *CRYAB*, 10% in *MYOT*, 15% in *ZASP* and 3% in *FLNC*.¹⁵ In an International sample of 52 affected families studied at the National Institutes of Health, 46% showed mutations in *DES*, 8% in *MYOT* and 2% in *ZASP*; none of the patients had mutations in *CRYAB* and no testing was done for *FLNC* mutations (Goldfarb, unpublished results). Relative numbers of myofibrillar myopathies among 23 studied Spanish families were 26% in *DES*, 60% in *MYOT*, none in *CRYAB* and 14% did not show mutations in any gene (Olivé, in preparation). Finally, in a set of 41 patients with myofibrillar myopathy from Britain, 17% had mutations in *DES*, 7% in *MYOT* and 7% in

ZASP (Bushby, in preparation). These results, although incomplete, show significant variability in the prevalence of desminopathy among other myofibrillar myopathies.

Treatment

There is no specific treatment for desminopathy, but some of the complications and premature death can be prevented. Early detection and treatment of cardiac arrhythmias and conduction defects is essential since implantation of a pacemaker can be lifesaving. Pacemaker and implantable cardioverter defibrillator (ICD) should be considered in individuals with arrhythmia and/or cardiac conduction defects. Individuals with progressive or life-threatening cardiomyopathy are candidates for cardiac transplantation. Respiratory support, consisting of continuous or bilevel positive airway pressure (CPAP and BIPAP), initially at night and later at daytime, are indicated in patients with hypercapnea and other signs of incipient ventilatory failure. Risk of chest infection should be considered in these patients. Assistive devices should be used in individuals with advanced muscle weakness. Gene and stem-cell therapy is an active area of research that promises effective treatments in the future.

Concluding Remarks

Desminopathy is associated with mutations in *DES*, *CRYAB* and perhaps other genes interacting with desmin. Disease-associated *DES* mutations affect amino-acid residues that are crucial for filament assembly; they render dominant inhibitory effect on desmin function. In humans or transgenic mice, they lead to accumulation of chimeric intracellular aggregates containing desmin and other cytoskeletal proteins. Desminopathy manifests with a variety of phenotypes depending on the type of inheritance and the location of mutations within the relatively large and structurally and functionally complex desmin molecule. Dominant mutations show a wide phenotypic variability which is probably a result of interactions between desmin, other intermediate filaments and chaperones capable of compensating for their detrimental effects. AlphaB-crystallin serves as such a chaperone for desmin but if mutated it may cause a myopathic syndrome identical to those resulting from mutations in *DES*. The current knowledge of the molecular basis of disorders resulting from mutations in *DES* and *CRYAB* genes allows the use of diagnostic genetic testing. In spite of significant progress in the studies of desminopathy there are unresolved problems. It is unclear how misfolded and aggregated desmin triggers the disease development. The muscle-specific proteolytic system involved in degradation of misfolded proteins needs further examination. Mechanisms leading to alternative disease expression in skeletal or cardiac muscle cells also require serious consideration. New technologies will help to solve these problems and facilitate novel specific therapies.

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Figure 1.
Cytoskeletal proteins. Reproduced from *The New England Journal of Medicine*, 2000; 342:778 with permission.

Figure 2.
Schematic representation of the structural organization of desmin protein domains and predicted positions of disease-causing mutations.

Figure 3.

Molecular modeling of desmin coiled-coil segment 2B. Arrows: (1) The location of the wild-type stutter; (2) the position of the abnormal stammer occurring in the p.Asn366del mutant; (3) the positions of two stutters in the p.Glu359_Ser361del mutant. Dots indicate the mutation sites. Reproduced from Human Genetics 2004; 114:311, with permission.

Figure 4.

Muscle CT scans performed in three desminopathy patients at different stages of illness at mid-thigh (A, C and E) and mid-calf (B, D and F) levels. Two years after the initial symptoms muscle CT scan in a patient carrying a *DES* Ile367Phe mutation showed initial involvement of the semitendinosus, sartorius and gracilis muscles (arrows in A), whereas at the mid-calf level a decreased attenuation was seen in the peroneal group and anterior tibialis (B). More advanced changes are present in these muscles in a patient carrying a *DES* p.Arg406Trp mutation ten years after the disease onset (C and D). Almost all muscles are replaced by fatty tissue in a patient with a *DES* p.Asn366del mutation 20 years after the disease onset (E and F). A to D images are reproduced from Neuromusc Disorders 2007; 17:448, with permission.

Figure 5.

Types of lesions observed in patients with desmin mutations. A, B and C: muscle pathology in a patient with p.Leu392Pro *DES* mutation at advanced stage of illness showing variability in the size of fibers, an increased number of internal nuclei and areas of fibro-fatty tissue infiltration. Several fibers contain cytoplasmic inclusions that are eosinophilic on HE stain (A), blue-dark on the modified trichrome stain (B) and devoid of oxidative activity (C). D, E and F: muscle pathology in a patient with p. Ile367Phe *DES* mutation. The following features are observed: a large fibre containing rimmed vacuoles (thin arrow in D); a plaque-like inclusion in an adjacent fiber (thick arrow in D), an eosinophilic body in a fiber containing rimmed vacuoles (E); collections of small cytoplasmic red bodies in two muscle fibers, sometimes in association with a plaque-like inclusion (F). G, H and I: muscle pathology in patients with p.Pro419Ser, p.Asn366del and p. Arg406Trp *DES* mutations. Single small green inclusions (arrows) are seen in some fibers and plaque-like lesions in others. Cryostat sections stained with H&E (A, D and E), modified trichrome stain (B, F, G, H and I), NADH (C). Original magnification $\times 200$ before reduction, except for H which is $\times 400$.

Figure 6. Diffuse or single inclusions containing desmin (A), alphaB-crystallin (B), gamma filamin (C), dystrophin (D), myotilin (E), or ubiquitin (F) in a patient with p.Pro419Ser mutation. Cryostat sections, original magnification $\times 400$.

Figure 7.

Muscle pathology in a patient with homozygosity for the p.Arg173_Glu179del *DES* mutation. Prominent eosinophilic masses located under the sarcolemma and sometimes associated with basophilic granular material (A), displaying strong desmin immunoreactivity (B). On electronmicroscopy, the masses are composed of a matrix of dense granulo-filamentous material (C). Cryostat sections, original magnification in A and B $\times 200$; C $\times 5000$. Figure kindly provided by Dr. Ana Cabello.

Figure 8.

Formalin-fixed, paraffin embedded cardiac tissue of the explanted heart from a patient with p.Arg406Trp *DES* mutation showing severe cardiomyocyte loss and extensive fibrosis. Variability in the size of cardiac cells and prominent nuclei are observed (A). On longitudinal sections (C) masses of eosinophilic material are seen within the cardiac cells (arrows). Strong desmin immunoreactivity is present in transverse and longitudinal sections (B and D). Original magnification $\times 200$ (A and B) and $\times 400$ (C and D).

Table 1

Single nucleotide polymorphisms in DES coding region

mRNA Position (NCBI Numbering)	Nucleotide Change	Codon Position	Amino Acid Position	Amino Acid Change	Population Frequency	Reference
c.116	C > T	3	10	Arg	0.0061	NIH Lab
c.146	G > C	3	20	Gly	0.0061	NIH Lab
c.154	G > T	2	23	Gly > Val	?	NCBI
c.161	G > A	3	25	Pro	0.0246	NIH Lab
c.179	C > T	3	31	Ser	0.0246	NIH Lab
c.269	C > A	3	61	Ser	?	NCBI
c.332	C > T	3	82	Ser	0.0061	NIH Lab
c.338	C > G	3	84	Gly	0.0061	NIH Lab
c.344	C > G	3	86	Gly	0.0061	NIH Lab
c.455	C > G	3	123	Ile > Met	0.0061	NIH Lab
c.458	G > A	3	124	Glu	0.0061	NIH Lab
c.724	C > T	2	213	Ala > Val	0.0201	NIH Lab
c.806	G > A	3	240	Lys	0.0061	NIH Lab
c.914	T > C	3	276	Asp	0.3493	NIH Lab
c.1100	C > G	3	338	Leu	0.4024	NIH Lab
c.1112	C > T	3	342	Asn	0.0122	NIH Lab
c.1157	C > A	3	357	Ala	0.0061	NIH Lab
c.1190	A > G	3	368	Ala	0.3611	NIH Lab
c.1302	C > G	3	405	Tyr	0.0324	NIH Lab
c.1367	C > T	3	427	Asn	?	NCBI
c.1461	G > A	1	459	Val > Ile	* 0.01	[38]
c.1472	C > G	3	462	Ala	?	NCBI

*
in African Americans

Phenotypic features of myopathy-causing mutations in desmin

Table 2

Predicted Amino Acid Change	Domain	Type of Inheritance	No. of Fam/ Pts Studied *	Age at Onset (Diagnosis)	Initial Symptoms	Distribution of Weakness	Respiratory Weakness	Bulbar Symptoms	Cardiac Disease	Associated Mutations	Reference
p.Ser21le	Head	?	1/1	?	?	L	no?	no?	?		2
p.Arg16Cys	Head	AR	1/1	30	Skel	L?	?	?	R,Co		37
p.Ser46Phe	Head	?	1/1	?	?	L	?	?	?		2
p.Ser46Tyr	Head	?	1/1	?	?	L	?	?	?		2
p.Glu108Lys	Head	?	1/1	60	Card	-	-	-	D,Co		38
p.Arg173_ Glu179del	1B	AR	1/1	15	Skel + Card	L,U,F	+	+	D,Co		10
p.Ala213Val	1B	?	1/1	?	Card	-	-	-	R		39
		AD	1/1	33	Skel	L,T	-	-	-	GAA**	40
		Spor	1/1	65	Card	-	-	-	D		40
		AD	1/1	31	Skel	L	+	-	-		41
p.Lys240fsX243	1B	Spor	1/1	18	Skel	L,U	-	-	Co		42
p.Glu245Asp	1B	AD?	1/1	46	Skel	L,U	-	-	R		43
		AD	/1	20	Card	-	-	-	R		43
p.Asp214_ Glu245del	1B	AD	1/2	40	Card	L,U,T,F	-	-	Co		3
		AD	1/2	29-41	Card	L,U,F	+	-	Co		44
		AD	1/3	24-48	Card	-	-	-	R,Co		37
p.Ser298Leu	2B	?	1/1	45	Card	-	-	-	D,Co		38
p.Asp312Asn	2B	?	1/1	35	Card	-	-	-	D		38
		Spor	1/1	45	Skel	L,T,F	+	-	D		41
p.Ala337Pro	2B	AD	1/2	20-38	Skel	L,U,T	-	+	Co		[11]
		AD	1/1	45	Skel	L,U	+	-	Co		45
p.Leu338Arg	2B	AD	1/2	43-46	Skel	L,U,T,F	+	-	-		40
		AD	1/1	35	Skel	L,U	-	+	Co		41
p.Asn342Asp	2B	AD	1/2	23-30	Skel	L,U,T	-	-	-		11
p.Leu345Pro	2B	AD	1/5	24-46	Skel	L,T,F	+	+	Co		6
p.Arg350Pro	2B	AD	1/3	48	Skel	L,U	+	-	D,Co		46
p.Arg350Trp	2B	?	1/1	55	Card	-	-	-	D		38
p.Arg355Pro	2B	AD	1/1	36	Skel + Card	L,U	-	-	R,Co		47
p.Ala357Pro	2B	AD	1/3	35-45	Skel	L,U,T	+	-	-		48
		AD	1/1	Teens	Skel	L	-	-	-		41
p.Glu359_ Ser361del	2B	AD	2/8	31-46	Skel	L,U	-	-	-		49
p.Ala360Pro	2B	AR	1/3	2-10	Card	L,U,T	+	+	D,Co	DES Asn393Ile	11
p.Asn366del	2B	AD	1/1	36	Skel	L,U	+	-	Co		49
p.Ile367Phe	2B	AD	1/1	25	Skel	L	-	+	H,Co		50
p.Leu370Pro	2B	AD	1/1	28	Skel	L,U	+	+	-		48
		AD	1/2	35-40	Skel	L,U,T	+	-	-		51
p.Leu385Pro	2B	denovo	1/1	21	Skel	L,U,T,F	+	-	D		52
p.Gln389Pro	2B	Spor	1/1	42	Skel	L,U,T,F	+	-	D,Co		53
p.Leu392Pro	2B	AD	1/1	25	Skel	L,U	-	-	D,Co		50
p.Asp399Tyr	2B	AD	1/1	34	Skel	L,U,T,F	+	+	H,Co		50
p.Glu401Lys	2B	AD	1/1	20	Skel	L	+	-	D,Co		40
p.Arg406Trp	2B	Spor	1/1	15-23	Skel+Card	L,U	-	+	Co		40
		denovo	3/3	24	Card	L,U,T,F	+	+	D,Co		54
		denovo	1/1	24	Skel	L,U,T	+	+	Co		54
		AD	1/2	40-50	Card+Skel	L?	?	?	R,Co		37
p.Glu413Lys	2B	AD	1/1	30	Card	L	-	-	R,Co		55
		AD	/2	30-63	Card	L	-	-	R,Co		55
p.Arg415Trp	Tail	?	1/1	30	Skel	L	-	-	-	ZASP Lys251Asp	41
p.Pro419Ser	Tail	AD	1/2	22-25	Skel	L,U	-	+	H,Co		50

Predicted Amino Acid Change	Domain	Type of Inheritance	No. of Fam/ Pts Studied	Age at Onset (Diagnosis)	Initial Symptoms	Distribution of Weakness	Respiratory Weakness	Bulbar Symptoms	Cardiac Disease	Associated Mutations	Reference
p.Thr442Ile	Tail	AD	1/2	27–35	Skel	L,U,T	+	–	Co		56
p.Lys449Thr	Tail	denovo	1/1	38	Skel	L,U	–	–	H,Co		56
		?	1/1	?	?	L	?	?	?		2
		Spor	1/1	14	Skel	U	–	–	?		41
p.Ile451Met	Tail	AD	1/2	15–37	Card	–	–	–	D		57
		AD	1/3	25–35	Skel	L,U,T	+	–	–		58
		Spor	3/3	38–55	Card	–	–	–	D		59
p.Thr453Ile	Tail	Spor	1/1	17	Card	–	–	–	R,Co		37
		AD	1/1	20	Skel	L,U,T	–	–	?		41
p.Arg454Trp	Tail	denovo	1/1	15	Card	L,U,T,F	–	–	H	MYOT-74	56
p.Val459Ile	Tail	?	1/1	34	Card	–	–	–	D,Co		38
		?	1/1	44	Card	–	–	–	D		38
p.Ser460Ile	Tail	AD?	1/1	29	Card	L,U	–	–	Co		56
p.Val469Met	Tail	AD	1/1	14	Card	L,T	–	–	H,Co	Lamin A p.R644C	60

AD: autosomal dominant pattern of inheritance; AR: autosomal recessive; Spor: sporadic; Skel: skeletal myopathy; Car: cardiomyopathy; Codon numbering according to updated sequence in GenBank submission # AF167579.

Muscle weakness predominant topography: L—lower limbs; U—upper limbs; T—trunk; F—face.

Type of cardiomyopathy: D—dilated; R—restricted; H—hypertrophic. Co—conduction abnormalities.

* Number of families and number of patients studied for the report.

** Compound heterozygosity for alpha-glucosidase (GAA) p.Asn91Asp and p.Ala261Thr mutations.

Table 3

Splice site mutations resulting in deletion of 32 amino acids encoded by exon 3 of DES

Sequence	Acceptor Site (End of Intron 2)	Exon Three	Donor Site (Start of Intron 3)
Wild type	... tcccag	GAC...GAG	gtatac...
IVS2-1G→A	... tccc aa	GAC...GAG	gtatac...
IVS2-2A→T	... tcc ct g	GAC...GAG	gtatac...
IVS3+1G→A	... tcccag	GAC...GAG	a tatac...
IVS3+3A→G	... tcccag	GAC...GAG	gt g tac...

Nucleotide replacements are bolded.

Table 4Single nucleotide polymorphisms in CRYAB gene⁷⁰

SNP Location	Change	Amino Acid Change	Frequency
c.-652	G > A	-	0.35
c.-650	G > C	-	0.05
c.-249	C > G	-	0.26
c.1120	C > A	p.Ser41Tyr	0.02
c.1150	C > T	p.Pro51Leu	0.02

Table 5

Desminopathy clinical phenotypes caused by mutations located in different functional domains of desmin

Phenotypes	Desmin Domain		
	1B	2B	Tail
Isolated Progressive Skeletal Myopathy	1	11	3
Skeletal Myopathy with Respiratory Insufficiency	1	6	3
Skeletal Myopathy Followed by Cardiomyopathy	3	24	5
Cardiomyopathy Followed by Skeletal Myopathy	4	10	3
Isolated Cardiomyopathy	6	4	8
Total number of patients	15	55	22

Table 6

Genotype-morphotype correlations in patients with various

<i>DES</i> mutation	Biopsied muscle	Myopathology	Electron microscopy	Reference
p.Arg173_Glu179del	deltoid	subsarcolemmal crescent-shaped eosinophilic masses, greenish autofluorescence, desmin-immunoreactive atrophic fibers, increase in internal nuclei, eosinophilic desmin-positive masses	GFM, abnormal sarcomeres	10-102
p.Ala213Val p.Lys240fsX243	not mentioned vastus lateralis	necrosis, desmin & alphaB-crystallin aggregates variation in fiber diameters, mild endomysial fibrosis, regenerating fibers, rubbed-out lesions, inclusions positive for desmin, alphaB-crystallin, synemin, plectin, ubiquitin	not mentioned GFM	40 42
p.Glu245Asp	deltoid, gastrocnemius (same patient)	fiber size variation, internal nuclei, mild fibrosis, rimmed vacuoles, patches of desmin aggregates	GFM, tubulo-filamentous aggregates	43
Ip.Asp214_Glu245del	gastrocnemius	fiber size variation, internal nuclei, rimmed vacuoles, fibrosis, cytoplasmic bodies, subsarcolemmal and cytoplasmic desmin aggregates	GFM	1-3
p.Ala337Pro	not mentioned	intracytoplasmic desmin aggregates	GFM, Z band streaming	11-40
p.Leu338Arg p.Asn342Asp	not mentioned not mentioned	desmin aggregates rimmed vacuoles, desmin deposits	not mentioned GFM, Z band streaming	40 11
p.Leu345Pro	vastus & deltoid (same patient)	variation in fiber size, fibrosis, fat cell replacement, increase in internal nuclei, subsarcolemmal and cytoplasmic desmin aggregates	not mentioned	85
p.Arg350Trp	not mentioned	fiber size variation (8-175µm), necrotic & regenerating fibers, increase in internal nuclei, increased endomysial fibrosis, cytoplasmic and subsarcolemmal desmin aggregates	GFM	46
p.Arg355Pro	biceps	variation in fiber size, round eosinophilic inclusions in type II fibers, single and diffuse desmin aggregates	GFM, vacuolar mitochondria, spheroid-like ellipsoid bodies	47
p.Ala357Pro	not mentioned	atrophic fibers, variation in fiber size, vacuoles, subsarcolemmal and cytoplasmic desmin aggregates	GFM	48
p.Glu359_Ser361del p.Ala360Pro p.Asn393Ile p.Asn366del	not mentioned not mentioned deltoid	atrophic fibers, granular desmin aggregates subsarcolemmal desmin aggregates marked variation in fibre size, many internal nuclei, rimmed vacuoles, ring fibres, subsarcolemmal aggregates of desmin and synemin	GFM GFM GFM	49 11-40 49-97
p.Ile367Phe	gastrocnemius	variation in fiber size, large numbers of rimmed vacuoles, subsarcolemmal and cytoplasmic, desmin, alphaB-crystallin, dystrophin, gamma-filamin aggregates	GFM	50
p.Leu370Pro	deltoid, gastrocnemius (two patients)	fiber size variation, internal nuclei, rimmed vacuoles, subsarcolemmal and cytoplasmic desmin aggregates	GFM	51
p.Leu385Pro p.Leu392Pro	anterior tibialis Biceps brachii	variation in fiber size, fibrosis, vacuoles, desmin aggregates, variation in fiber size, few rimmed vacuoles, fibrofatty tissue replacement, subsarcolemmal and cytoplasmic desmin, alphaB-crystallin, dystrophin, gamma-filamin aggregates	GFM GFM	52 50
p.Asp399Tyr p.Glu401Lys	not mentioned deltoid	atrophic fibers, desmin aggregates atrophic fibers, rimmed vacuoles, myophagocytosis, desmin aggregates	not mentioned not mentioned	40 40
p.Arg406Trp	deltoid	mild variation in fibre size, few internal nuclei, ring fibers, cytoplasmic and subsarcolemmal aggregates of desmin	GFM, Z band streaming	11-97
p.Pro419Ser	gastrocnemius	variation in fiber size, rimmed vacuoles, subsarcolemmal and cytoplasmic desmin, alphaB-crystallin, dystrophin, gamma-filamin aggregates	GFM	50
p.Ile451Met	not mentioned	desmin deposits	GFM, Z band streaming	11
CRYAB p.Arg120Gly	not mentioned	cytoplasmic inclusions with reduced or absent oxidative enzyme histochemical activities, desmin, ubiquitin, alphaB-crystallin and dystrophin deposits	GFM	71-103

<i>DES</i> mutation	Biopsied muscle	Myopathology	Electron microscopy	Reference
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GFM: granulofilamentous material.

Table 7

Myopathology of myofibrillar myopathy associated with mutations in MYOT, ZASP, SEPN1, FLNC and VCP genes

Mutant Gene (Protein)	Myopathology Features	Electron Microscopy	Reference
<i>MYOT</i> (myotilin)	variation in fiber size, fibrosis, increased number of internal nuclei, rimmed vacuoles, spheroid bodies, inclusions with myotilin, filamin C, α -actinin, ZASP, desmin, alpha-B crystallin, dystrophin, plectin, gelsolin, ubiquitin, prion protein, CD10 kinase, alpha1-antichymotrypsin	GFM, autophagic vacuoles, tubulofilament-like structures, 15–28 nm filaments	111·112·114
<i>ZASP</i>	hyaline, congophilic and amorphous deposits devoid of oxidative Z disk streaming, autophagic enzymes, small vacuoles, increased number of internal nuclei, fibervacuoles, remnants of sarcomeres, splitting, necrotic & regenerating fibers, aggregation of myotilin, degraded filamentous material desmin, alpha-B crystallin, dystrophin, N-CAM, prion protein, plectin, ubiquitin, gelsolin		115
<i>SEPN1</i> (selenoprotein N)	variation in fiber size, increase in internal nuclei, hyaline plaques with reduced or absent oxidative enzyme histochemical activities, necrosis and regeneration of muscle fibres, endomysial fibrosis, fat cell replacement, desmin, alpha-B crystallin, dystrophin, tau AT100 and AT120, A-beta amyloid, ubiquitin, actin, alpha-actinin, 8-OHdG, beta- and gamma-sarcoglycans, nebulin, SERCA2, telethonin	Z disk streaming, minicores, aggregates of 7–12 and 8–10 nm filaments, 20 nm filaments, 20 nm tubulofilaments in sarcoplasm, Mallory body-like inclusions	117
<i>FLNC</i> (filamin C)	desmin, filamin C, myotilin, dystrophin, sarcoglycans deposits	GFM, rods, Z disk streaming	118
<i>VCP</i> (valosin-containing protein)	marked to moderate to mild myopathic features, rimmed vacuoles, foci of desmin, α -B crystallin, ubiquitin, valosin-containing protein	GFM, destroyed sarcomeres,	119

GFM: granulofilamentous material.