A Novel Mutation in the Desmin Gene with an Infantile Autosomal Recessive Myopathy

Mohamed Mohamed Saad Serria[1, 2], Arbogast S[1], and Ferreiro A[1]
[1]Inserm, Institute de Myologie, Hopital Pitie Salpetriere, Paris, France,
[2]Department of Medical Biochemistry, Faculty of Medicine, Mansoura University, Egypt

ABSTRACT

Recent studies in desmin knockout (_/_) mice have shown that the targeted ablation of desmin leads to pathological changes of the extrasarcomeric intermediate filament cytoskeleton, as well as structural and functional abnormalities of mitochondria in striated muscle. Here, we report on a novel homozygous deletion in exon 6 of desmin gene in patients from two families with recurrent episodes of syncope from infancy and an aggressive course of muscles wasting and death from cardiac failure at age 20 years. Molecular analysis identified a homozygous deletion resulting in loss of 22 bp (i.e. loss of seven amino acids, in addition to a frame shift and a truncated desmin protein). The homozygous deletion changed protein structure in a highly conserved region and compromised the ability of desmin to form an intermediate filament network in transiently transfected cells, providing a confirmation of high pathogenic potential. Muscle biopsy demonstrated signs of myofibrillar myopathy with prominent subsarcolemmal desmin-reactive aggregates. From results of the present study, It could be concluded that, this deletion mutation is the molecular event leading to the development of the infantile autosomal recessive desmin myopathy. Elucidation of the genetic basis of heritable desminopathy might provide insights into the pathogenetic mechanisms of the disease. The focus of the study was to analyze the desmin gene sequences for mutation and to study the impact of mutation, if any, on the intermediate filament networks formation.

INTRODUCTION

The desmin (DES) gene provides instructions for making a protein called desmin. Desmin is found in the muscles. Within muscle fibers, desmin proteins are important to maintain the structure of sarcomeres, which are necessary for muscles to contract.[1] The desmin proteins surround rod-like structures called Z-discs that are located within the sarcomere. [2,3] Desmin connects the Z-discs to one another, linking neighboring sarcomeres and forming myofibrils, the basic unit of muscle fibers. The connection of sarcomeres to each other to form myofibrils is essential for maintaining muscle fiber strength during repeated cycles of contraction and relaxation.[4] Desmin (the main intermediate filament (IF) protein) is encoded by a single gene assigned to human chromosome 2q35 and is synthesized only in cardiac, skeletal, and smooth muscles.[5] There is only one copy of the desmin gene, which consists of nine exons and eight introns within an 8.4 kb region and encodes 470 amino acids.[6] Only one 2.2 kb desmin messenger RNA is found in human striated and smooth muscle and it is translated into a single 53 kDa protein monomer, which, in turn, assembles to form filaments of 10 nm diameter.[1] Mutations in that gene are associated with desminopathy.[7] Desmin encodes a muscle-specific class III intermediate filament. Homopolymers

of that protein form a stable intracytoplasmic filamentous network connecting myofibrils to each other and to the plasma membrane.[8] Desmin links the contractile apparatus to the sarcolemma,[3] the mitochondria[9] and the nucleus.[10] Desmin is also found in cell-cell adhesion complexes as desmosomes in cardiac muscle[11] and costamers in skeletal muscles.[4,12]

In accordance with its function, desmin is organized into three domains, a highly conserved α-helical core flanked by globular N- and C-terminal structures (known as the "head" and "tail" domains, respectively) (Figure 1). The α-helical core maintains a seven residue (heptad) repeat pattern that guides two polypeptides to form a homopolymeric coiled-coil dimer, the elementary unit of the desmin intermediate filaments.[13] The α helical rod has interruptions at three sites, resulting in four consecutive helical segments (known as 1A, 1B, 2A, and 2B) connected by short, non helical linkers.[14,15] Almost all pathogenic desmin mutations were found in the evolutionary, highly conserved α-helical coiled-coil rod domain.[16,17] The nonhelical amino-terminal (head) and carboxy-terminal (tail) domains of intermediate filament subunit proteins are highly variable in sequence and size.[18] Although their specific role is largely unknown, the head and tail domains are sites of post-translational modifications by phosphorylation and glycosylation, regulating dynamic aspects of intermediate filament organization and structure during cell cycle and developmental programs. An interplay between desmin tail and head domains is taking place, which potentially protects the amino terminus of desmin from specific proteases.[19]

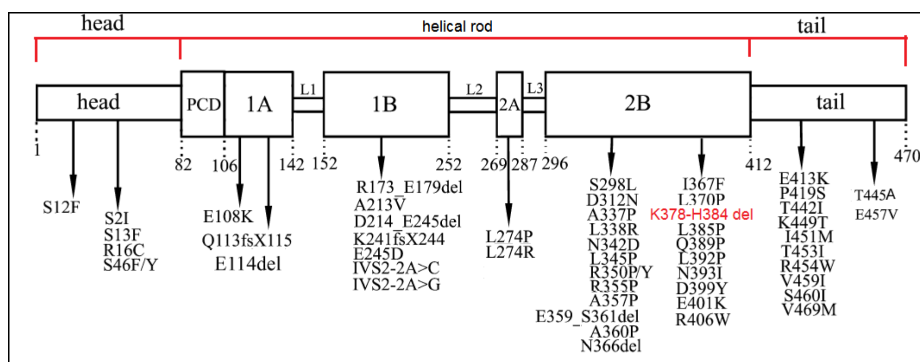

Figure 1: Schematic structure of the human desmin protein and updated chart of desmin mutations. Desmin protein represented with precoil domain (PCD) and a highly conserved α-helical rod of 303 amino acid residues. The α-helical rod is flanked by globular N- and C-terminal (`head' and `tail') structures[15] and is interrupted by three non-helical linkers in several places resulting in four consecutive α-helical segments, 1A, 1B, 2A and 2B, connected by short non-helical linkers. Mutations reported are identified by wild-type amino acid code/position/mutant amino acid; (mutation identified in current study is in RED). The deletion mutation identified in this study causes infantile onset of skeletal and cardiac myopathy. The known mutations associated with desminopathy are from the human mutation database (http://www.hgmd.cf.ac.uk/).

Desminopathy (DRMs; OMIM 601419) is a genetically heterogeneous disorder with autosomal dominant pattern of inheritance in most affected families (94%) but autosomal recessive and de novo mutations are also observed[16,20-22]. The age of disease onset is on average 30 years. Accumulated evidence suggests that desminopathy phenotype is extremely variable.[23] Desmin-related myopathy is characterized by skeletal muscle weakness and cardiomyopathy as well as the presence of intracytoplasmic aggregates of desmin-reactive material in the muscle cells[24,25]. DRMs have been associated to missense or deletion mutations of the desmin gene. Interestingly, most of desmin mutations resides in the coil 2B[26-28] and are to proline, a residue considered as a “helix-breaker”.[29] Proline is not normally present in the desmin helical rod and is known to be a potent helix breaker; its dihedral angle is fixed at –65°, creating a kink in the protein structure. In addition, proline cannot form a hydrogen bond with the residue -4 of the helix.[30] The mutant desmin gene induces numerous cytoskeletal proteins (e.g. dystrophin, vimentin, β-spectrin, and gelsolin) to form insoluble toxic aggregates that resist turnover by the cellular enzymatic machinery and trigger oxidative stress and abnormalities in the protein degradation system[31-33]. The mutant desmin interferes with the normal assembly of intermediate filaments, resulting in fragility of the myofibrils and severe dysfunction of skeletal and cardiac[34,35]. More than 50 mutations (Figure 1) have been identified in the desmin gene and most of them are missence mutations.[36] Desmin mutations are found in the human mutation database (http://www.hgmd.cf.ac.uk/). Only two deletions are previously reported[37,38] in the literature and are closely related to our observation regarding the infantile onset of the disease.

PATIENTS & METHODS

Case Selection:

Five patients from two families with healthy and consanguineous parental couples were selected for the present study. Patients were retrieved from pathological and clinical records at the Institute de Myologie (Hôpital Universitaire, Pitié-Salpêtrière, Paris). Patients included show recurrent episodes of syncope from infancy and an aggressive course of muscle wasting. The five cases were subjected to mutation screening. Blood and muscle samples were obtained after written consent in accordance to the protocols approved by the local ethic committees.

RNA Extraction and RT-PCR:

Total RNA was extracted from muscle biopsy tissue (20–25 mg) by use of RNA-PLUS extraction solution (Q Biogen, Illkirch, France). We performed reverse transcription with random hexamer primers for muscle RNA, using the Superscript kit (Life Technologies). The resulting cDNA was used as a template for PCR. The entire desmin open reading frame was PCR-amplified using primers DesFor (5-

ATGCTAGCACCATGAGCCAGGC CTA-3) and DesRev (5-ATGCGGCCTCTGTCTTTAGAGCA CTTCA -3) (GenBank TM accession number L22550). The PCR products were analyzed by electrophoresis on 0.75% agarose gel. After agarose gel electrophoresis, DNA bands (1446 bp or 1424 bp) were excised, eluted, purified by QiaEx II (Q Biogen, Illkirch, France), cloned into the TA cloning vector (Invitrogen) and cycle sequenced in at least nine clones using Dye Terminator Sequencing Protocol (Applied Biosystems).

Isolation of Genomic DNAs and Desmin Exon Amplification and Sequencing:

Sequence changes identified at cDNA level were confirmed on genomic DNA by amplification and direct sequencing of the specific exons. Genomic DNAs were isolated from a skeletal muscle biopsy of the patient and from peripheral blood samples of family members and normal individuals. These DNAs served as templates for PCR amplification of exons and flanking intron segments of the desmin gene. The primer sets used were previously described by **Vicart et al.**[39] . The primers used for amplification of exon 6 are 6For (5-GCCTGCCAGCCCCAAAGCTTT-3) and 6Rev (5-ACAGAAATGGACCACC CAGCA-3). PCR products (361or 339 bp) were purified by Qiagen columns, sequenced and sub cloned (TA Cloning Kit, Invitrogen). Sequencing of genomic DNA and cDNA was performed according to the manufacturer's instructions (Dye Primer Sequencing, Perkin–Elmer Cetus) on an automated DNA sequencer (model 373A, Applied Biosystems).

Cloning and Mutagenesis:

Human desmin cDNA was subcloned into TA cloning vector. A deletion mutation at codon 1132–1153 of wild TA DES vector was introduced into the full-length human desmin cDNA by site-directed mutagenesis. The resulting desmin TA plasmid containing either mutant or wild-type cDNA were sub cloned into eukaryotic expression vector pcDNA3.1 (Invitrogen, Carlsbad, CA, USA) to generate the mutant or wild type corresponding desmin expression vectors.

Cell transfection studies:

The effects caused by desmin mutations have been evaluated in three types of cells. The eukaryotic expression vectors (mutant or wild-type desmin pcDNA3.1) were transfected into SW13 cell, MCF7cells and C2C12 cells. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum and penicillin/streptomycin (Gibco). Cells were grown to 60% confluence, the wild-type and mutant desmin vectors were transfected into cell lines using Fugene 6 according to the manufacturer's protocol (Roche, Basel, Switzerland). At 48 h after transfection, the cells were washed three times with phosphate-buffered saline and then fixed with paraformaldehyde for 15 min at room temperature. The cells were subsequently incubated with monoclonal antibody against human desmin (D33, Dako) for 1 h at 37°C and treated with a secondary antibody

conjugated with Rhodamine (Santa Cruz, Santa Cruz, CA, USA). After washing with phosphate-buffered saline, the transfected cells were analyzed by confocal immunofluorescence microscopy.

Preparation of proteins and western blot analysis:

Frozen muscles were weighed and suspended in a 0.1 M Na/KH_2PO_4 buffer containing 2 mM EDTA (1/20, w/v). Muscles were then minced with scissors, crushed in a frosted mortar, stirred for 15 min, and sonicated on ice. Homogenates were then centrifuged at 15000 g for 10 min at 4 °C. The supernatants were removed and the pellet was resuspended in gel filtration buffer at a 1/20 dilution (w/v) (PBS, 6Murea, 1mM EDTA, 2mMDTTwith protease inhibitors). Both the supernatant and the resuspended pellet were then aliquoted and stored at - 80 °C. Protein was also prepared from cells (MCF7) after transfection with DES expression vectors. When cells reach visual confluence, the monolayer was washed twice with ice-cold PBS and cell lysis was performed by adding 100 u of Laemmli sample buffer and scraping with a rubber policeman. Cell lysates were mixed several times to shear DNA and 25 u aliquots were heated for 5 minutes at 95°C prior to SDS-PAGE electrophoresis. The protein load was adjusted according to the method of Bradford[40]. Aliquots of protein (20 µg /lane for desmin, or 200 µg / lane of total cell lysate) were mixed with a loading buffer (62.5 mM Tris pH = 6.8, 5 % glycerol (v/v), 1% SDS (v/v), 2.5 % b-mercaptoethanol (v/v)), boiled for 3 min, run on 10% SDS-PAGE, electrophoresed at 115 V for 4 h at 4 °C and transferred to nitrocellulose membranes (Schleicher and Schuell). The membrane is blocked in Tris phosphate saline buffer (TBS) – 5 % milk at room temperature for 1h and probed with primary antibody (rabbit polyclonal antidesmin antibody (ICN)). The membrane and the primary antibody were incubated for 2 h at room temperature. Secondary antibodies were a Rabbit anti-mouse IgG (1/4000 (v/v) TBS–1 % milk; P0161, DAKO) conjugated to horseradish peroxidise. Secondary antibodies were used for chemiluminescent detection of proteins (ECL, Amersham). Blots were visualized by chemiluminescence (Amersham).

Immunocytochemistry:

At least one skeletal muscle biopsy was obtained from each patient. Standard histological and histochemical staining was performed on cryostat sections according to previously described protocols[41]. Serial sections that were 5µm thick were stained with Hematoxylin and eosin or with antibodies against desmin at a dilution of 1:100 (clone D33, Dako).[38]. For secondary antibodies, fluorescein-conjugated goat antimouse IgG or rhodamine-conjugated goat antimouse IgG was used. Sections were examined with Axiophot 2 fluorescence microscope.

RESULTS

Analysis of the Desmin Gene Sequences:

Mutation analysis using direct nucleotide sequencing of PCR products amplified from either Genomic DNA or cDNA revealed a

novel homozygous deletion mutation (amino acids, 378-384 - Nucleotides,1132-1153) in the middle of exon 6 (GenBank ID 181539, accession M63391) of homozygous patients in contrast to normal controls. The deletion causes loss of seven amino acids and a frame shift and a premature termination signal located nine codons downstream of the deletion (figure 2), thereby leading to a truncated desmin molecule which is apparent in immunoblot analysis (Figure 7).

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 D   S   L   M   R   Q   M   R   E   L   E   D   R   F   A   S   E   A    360
GAT TCC CTG ATG AGG CAG ATG CGG GAA TTG GAG GAC CGA TTT GCC AGT GAG GCC  1080

 S   G   Y   Q   D   N   I   A   R   L   E   E   E   I   R   H   L   K    378
AGT GGC TAC CAG GAC AAC ATT GCG CGC CTG GAG GAG GAA ATC CGG CAC CTC AAG  1134

 S   G   Y   Q   D   N   I   A   R   L   E   E   E   I   R   H   L   C    378
AGT GGC TAC CAG GAC AAC ATT GCG CGC CTG GAG GAG GAA ATC CGG CAC CTC TGC  1134

 A   S   T   R   T   C   S   T   *                                        396
GCG AGT ACC AGG ACC TGC TCA ACG TGA                                      1188

 D   E   M   A   R   H   L   Q   D   L   L   R   E   Y   N   V   K   M    396
GAT GAG ATG GCC CGC CAT CTG CGC GAG TAC CAG GAC CTG CTC AAC GTG AAG ATG  1188

 A   L   D   V   E   I   A   T   Y   R   K   L   L   E   G   E   E   S    414
GCC CTG GAT GTG GAG ATT GCC ACC TAC CGG AAG CTG CTG GAG GGA GAG GAG AGC  1242
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Figure 2: Nucleotide sequence of exon 6: The normal sequence (black letters) and the deleted sequence (Red letters) with the corresponding AA sequence of the protein. The blue letters denotes the mutant transcript and the star denotes a premature stop codon in exon 6.

This mutation resides in a part of the gene that encodes an evolutionary highly conserved 2B domain of the α-helical coiled-coil rod domain of the desmin protein. The site is known to be critically involved in filament assembly[42] and interactions with other cellular proteins[43]. The sequencing profile of the heterozygous healthy parents showed turbidity in contrast to the clear profile in homozygous patients and normal control persons (Figure 3). The turbidity in the sequence profile is due to the presence of the two transcripts (i.e. normal and deleted) in the amplified sequenced product of the healthy heterozygous parents.

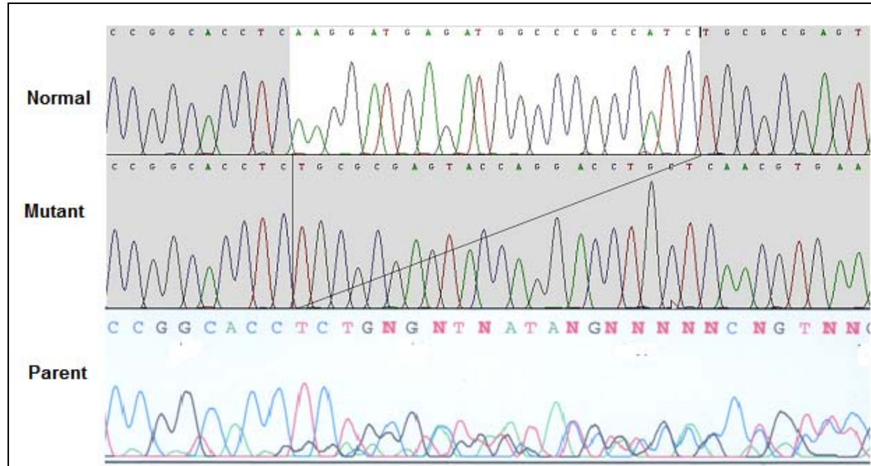

Figure 3: **Desmin mutation detection.** (A) Comparison of nucleotide Sequence of exon 6 of the desmin gene, performed by direct sequencing of PCR-amplified desmin genomic DNA in affected (Middle), control individuals (Upper) and heterozygote parent(Lower).Sequencing revealed a novel homozygous deletion mutation (1132-1153) residing in exon 6 of the desmin gene.

The length of the RT-PCR–amplified transcripts with desmin primers DesFor (5-ATGCTAGCACCATGAGCCAGGC CTA-3) and DesRev (5-ATGCGGCCTCTGTCTTT AGAGCACTTCA -3) from muscle biopsy tissue was 1446 bp in the controls and is smaller (1424bp) in the homozygous patients (Figure 4 B). This shortening of the transcript is also confirmed by PCR of exon 6 from genomic DNA (Figure 4 A). The heterozygous healthy parents have both alleles and so both fragments (Figure 4 A, B).

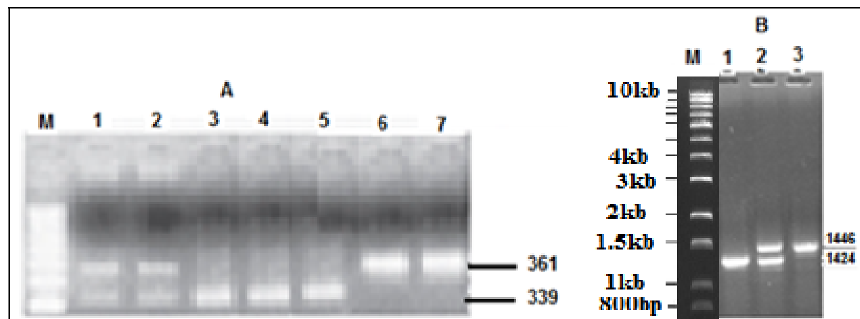

Figure 4: Mutation analysis:(A) Gel electrophoresis (1.5%) of PCR products of exon 6 from parents (1, 2), Patients (3, 4, 5) and controls (6, 7). The sequences containing the mutation showed aberrant migration and proved to be very clearly distinguishable from the wild-type band. (B) Desmin cDNA–amplified transcript run on 0.75% agarose gel, 1 Patient, 2 Parent and 3 control.

Functional Studies:

The functional consequences of the novel desmin mutation were studied by means of transfection studies. To assess the ability of the desmin mutation to assemble into a de novo filament network, wild-type and mutant desmin expression vectors were transiently transfected into SW13 and MCF-7 cells. The SW13 (human adrenocortical carcinoma cells) are completely devoid of cytoplasmic intermediate filaments and are an ideal cell culture system to investigate the potential of mutant desmin to form intermediate filaments[44]. MCF-7 epithelial cells contain an endogenous IF network composed only of keratin[45]. For further evaluation of the effects of mutant desmin on the pre-existing desmin filament network, C2C12 (mouse myoblast cells) cells were used[33]. As determined by antidesmin immunoblot analysis, both mutant and wild desmin vectors generated a single protein of expected size, with mutant desmin migrating only slightly faster than wild type (Fig. 7) Immunofluorescence microscopy was used to assess the ability of these proteins to produce IF networks in Sw13 and MCF-7. Antidesmin antibody staining was detected in '50% of all transfected cells, and not in any untransfected or mocktransfected populations.

The wild desmin eukaryotic expression vector (pcDNA3.1) produces functional desmin protein capable of building a cytoplasmic network in SW13 cells and in the MCF-7 epithelial cells (Figure 5 B). While the transfection of mutant desmin PcDNA3.1 vector produces multiple desmin-positive clumps or abnormal solid aggregates in both cell lines (Figure 5 A), and this is parallel to the abnormal cytoplasmic protein aggregation seen in the immunohistochemical and ultrastructural analysis of skeletal muscle biopsies from patients harboring pathogenic desmin mutations (Figure 6). Desmin rod domain and its C-terminal end are critical for the correct assembly of desmin intermediate filaments. Deletion or substitution of a single amino acid in this domain has been shown to impair desmin filament assembly and to disrupt the desmin-vimentin network[18,45,46]. Furthermore, the mutant desmin induces collapse of the pre-existing type III IF network in cell types that express vimentin i.e. C2C12 cell lines (Figure 5A). This is in agreement with previous reported data by **Haubold et al.**[47].

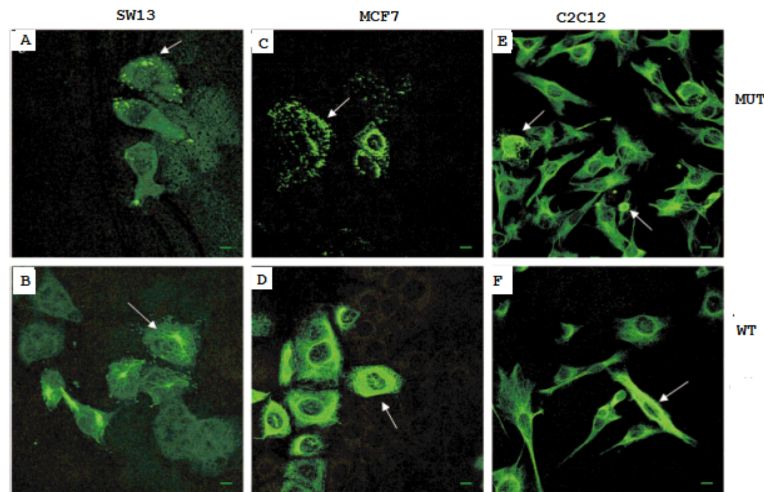

Figure 5: The functional consequences of the novel desmin mutation: SW13 (A,B), MCF7 (C, D) (desmin-negative :) and C2C12 (E, F) cell lines transfected with wild type or mutant desmin cDNA. Cells were processed for indirect immunofluorescence to detect the distribution of desmin (green fluorescence) and viewed using a confocal microscope. Aggregates (arrowheads) were detected by the desmin antibody (mab-D33) in cells transfected with mutated desmin cDNA. Such aggregates were never observed in cells transfected with wild type desmin cDNA. Wild-type desmin forms filaments in SW13 cells.

Immunohistological examination of muscle tissues revealed immunopositivity of desmin in the aggregated materials (Figure 6), leading to diagnosis of desminopathy[48,49].

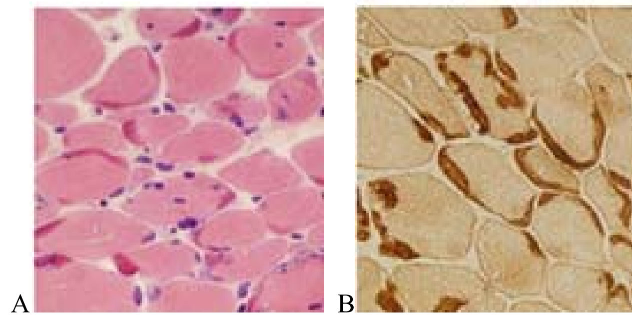

Figure 6: Section of affected skeletal muscle of a patient: Haematoxylin and eosin (A) staining revealed a Prominent crescent-shaped eosinophilic masses located under the sarcolemma, sometimes associated with basophilic granular material (B) immunocytochemical analysis for desmin displaying strong desmin immunoreactivity below the sarcolemma with atrophic fibres.

Moreover, immunoblot studies revealed abnormal band of desmin. These abnormal bands of desmin, in addition to normal desmin band, suggested mutant proteins of desmin and thus mutations in the desmin gene. Desmin immunoreactions (Fig.7A) revealed a single band corresponding in size to 53 kDa in controls and a smaller band in patients. The 3 to 4 kDa reduction in the molecular mass of mutant desmin in patients could be explained either by removal of an –28 amino acids peptide from the carboxyl terminus of the α helical rod domain (the mutation results in loss of 7 amino acids and the premature stop codon result in loss of 21 amino acids. Also, the smaller size of the fragment may be due to loss of the post-translational modification. However, the signal intensity in the insoluble fraction from patients muscle biopsy was far more intense, indicating the presence of desmin aggregates in diseased muscle (Fig.7C). Further confirmation is by immunoblot analysis of the recombinant proteins expressed in MCF7 cell lines transfected with wild or mutant desmin constructs showed a single protein of the expected size of 53KDa (Figure 7D No.1,2) or 50KDa (Figure 7D No.3,4) respectively.

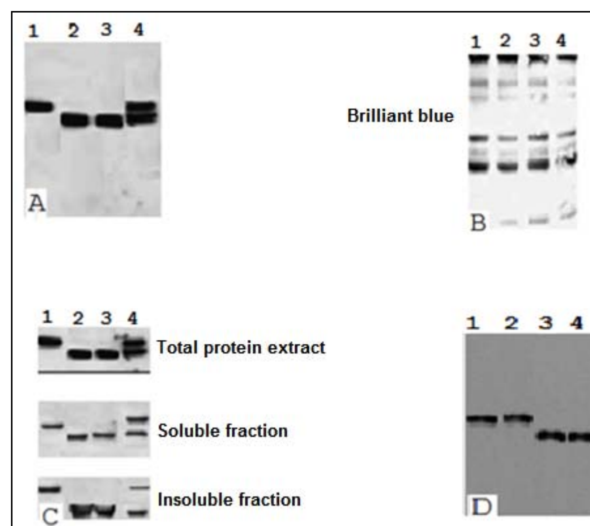

Figure 7: A mutations at the 2B domain region of the highly conserved helical rod of desmin intermediate filament has an apparent molecular mass 3–4 kDa lower than expected. A) Immunoblot analysis for desmin, performed using protein extracts from skeletal muscles of Control, (lane 1), homozygote mutant patients (lane2, 3), heterozygote normal parent (lane 4) the rabbit polyclonal antidesmin antibody (ICN)) was used. B) For loading control, same amounts of protein extracts (as calculated by Bradford) used for Western blot were analyzed in the same SDS-PAGE, blotted to PVDF membrane, and stained with Brilliant Blue R (numbering of lanes same as in A). C) Western blot analysis of the soluble fraction (supernatant of 3000 g), insoluble fraction (0.6 M KCl pellet), and total muscle protein extracts reveals that the major part of the mutant desmin is present in the insoluble fraction (numbering of lanes same as in A). D) Immunoblot analysis for recombinant desmin expressed in MCF7 cells (1, 2 wild construct, 3, 4 mutant construct).

DISCUSSION

Desmin myopathy is a genetically distinct subgroup of myofibrillar myopathies characterized by pathogenic mutations in the desmin gene and abnormal aggregates of desmin-type intermediate filaments, which affects cardiac and skeletal muscle, and rarely the intestinal smooth muscle. [24,25] Thirty years ago, morphological evidence revealed that the thin filaments and desmin intermediate filaments comprise a "collar" surrounding the Z-discs of adjacent myofibrils.[50] Biochemical support for this became available when it was shown that the giant actin filament– binding protein, nebulin, interacts with desmin at the periphery of the Z-discs.[43]. Desmin filament assembly is a complex process that 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.[30,31]

Defects in the function of desmin may therefore cause fragility of the myofibrils and impair contraction. Because intermediate filaments participate in the transmission of active force, the disruption of the filamentous network by the mutant desmin impairs the force generated within the contractile filaments and weakens the sarcomere resulting in myofibrillar damage over a period of years because of the cumulative effects of mechanical stress and muscle use[51]. Desmin-related myofibrillar myopathy (OMIM #601419) is inherited as an autosomal dominant disorder. Approximately 25% of affected individuals have an affected parent, but the proportion of *de novo* mutations is unknown[52]. In mice that lack desmin, the histologic features in muscle specimens from these mice are very similar to those of patients with desmin myopathy, including disrupted myofibrils and streaming of Z bands[53,54].

Mutation screening analysis of the desmin gene sequences reported the identification of an autosomal recessive mutation responsible for an infantile desmin myopathy. This novel recessive deletion mutation detected in desmin gene has clinical effects only in homozygous cases. The sequencing profile of heterozygous healthy parents shows turbidity in contrast to the clear profile of homozygous patients and normal control persons (Figure 3). The turbidity in the sequence profile is due to the presence of two transcripts (normal and deleted) in the amplified sequenced product of the healthy heterozygous parents. The heterozygous healthy parents have both alleles and so two bands appear in the gel of amplified PCR products of the desmin gene in contrast to a single band in healthy control and patient PCR products (Figure 4 A, B). The detected mutation resides in a part of the gene that encodes an evolutionary highly conserved 2B domain of the α-helical coiled-coil rod domain of the desmin protein. The site is known to be critically involved in filament assembly[41] and interactions with other cellular proteins[43]. The integrity of the helix 2B (carboxy-terminal part of the desmin rod) is critically important for the proper

assembly of intermediate filaments.[26-28] The potential importance of that region lies in its hydrophilic[5] nature which form the underlying basis for coiled-coil formation of intermediate filament dimer subunits and its high degree of homology among the type III intermediate filament proteins and cytokeratins[29]. Deletion or substitution of a single amino acid in this domain has been shown to impair desmin filament assembly and to disrupt the desmin-vimentin network[18,45,46]. The deletion detected here change the periodicity of the amino acids in the coiled-coil α helical domain and produced a truncated protein. It might affect the ability of dimer subunits to form functional higher order structures.

Functional studies, in vivo transfection experiments, with wild desmin expression vectors produces a functional desmin protein capable of building a cytoplasmic network in SW13 cells and in the MCF-7 epithelial cells (Figure 5 B) while mutant desmin expressed in SW13 (vimentin-negative) cells produced aggregates similar to those seen in muscle-biopsy specimens (Figure 6). Furthermore, the mutant desmin induces collapse of the pre-existing type III IF network in cell types that express vimentin i.e C2C12 cell lines (Figure 5A).This is in agreement with previous reported data by **Haubold et al.**[47].These functional studies provide compelling evidence that the mutation within helix 2B is deleterious to desmin filament assembly. The identification of a desmin gene deletion that functionally compromises desmin intermediate filament assembly in these patients underscores the significance of desmin gene alterations in the production of desmin-related muscle pathology in humans.

Moreover, immunoblot studies revealed a single band corresponding in size to 53 kDa in controls and a smaller band in patients. The 3 to 4 kDa reduction in the molecular mass of mutant desmin in patients could be explained either by removal of 28 amino acids peptide from the carboxyl terminus of the α helical rod domain (the mutation results in loss of 7 amino acids and the premature stop codon result in loss of 21 amino acids) or may be due to loss of the post-translational modification. In addition to the truncated protein, the mutation change the periodicity of the amino acids in the α helical rod domain of desmin protein and this is deleterious to desmin filament assembly and function.

From the present study, it could be concluded that, this deletion mutation has a recessive negative effect on the polymerization process of desmin intermediate filaments and is the molecular event leading to the development of the infantile autosomal recessive desmin myopathy. Elucidation of the genetic basis of heritable desminopathy might provide insights into the pathogenetic mechanisms of this disease.

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طفرة متنحية جديدة فى ديسمن جين مصاحبة لمرض العضلات (الديسمونوباسى) الطفولى

محمد سعد سرية[1,2] - أربوجاست س[1] -فيرريرو أ[1]
[1] Inserm, Institut de Myologie, hopsital pitie salpetriere, باريس -فرنسا
[2] قسم الكيمياء الحيويه- - كلية الطب – جامعة المنصوره

الدراسات الحديثة فى الفئران المنتجة عديمة الديسمن بروتين لاحظت وجود علامات مرضية فى الشعيرات الهيكلية الدقيقة خارج الوحدة البنائية للعضلات بالإضافة لتركيبات ووظائف غير طبيعية للميتوكوندريا فى العضلات. فى هذه الدراسة وجد فقدان متماثل لجزء من جين الديسمن فى حالات مرضية لعائلتان مصاحبة بإغماء متكرر من الطفولة وفقدان متطورللعضلات و موت نتيجة لفشل القلب عند سن العشرين. التحاليل البيولوجية أثبتت فقدان متماثل ل ٢٢ قاعدة من الحمض النووي الدي أوكسي ريبوزي (فقدان ٧ أحماض أمينيه وطفرة تؤدى إلى تغير فى الشفرة الوراثية مع استحداث نقطة نهاية متقدمة فى طباعة البروتين).هذه الطفرة المتماثلة غيرت تركيب البروتين فى منطقة محافظة وعاقت البروتين عن القدرة على عمل الخيوط الهيكلية فى الخلايا المحقونة بالجين المعاق و هذا دليل على التأثير المرضى لهذة الطفرة. الفحص الهوستولجى للعضلات يظهر علامات مرض العضلات الديسمونوباسى بالإضافة إلى تجمعات من الديسمن تحت وحدة بناء العضلات. هذه الدراسة أوضحت أن هذه الطفرة هي السبب فى مرض العضلات الطفولى (الديسمونوباسى). توضيح الأساس الجينى لمرض العضلات الوراثي (الديسمونوباسى) ربما يسهم فى إضاءة الآلية التي ينتج عنها المرض. هدفت الدراسة لفحص جين الديسمن من حيث وجود طفرات و ما مدى تأثير هذه الطفرات إن وجدت على تكوين الخيوط الهيكلية العنكبوتية الخلويه.