

# Desmin-Related Myopathy with Mallory Body–like Inclusions Is Caused by Mutations of the Selenoprotein N Gene

Ana Ferreiro, MD, PhD,<sup>1</sup> Chantal Ceuterick-de Groote, PhD,<sup>2</sup> Jared J. Marks, BA,<sup>3</sup> Nathalie Goemans, MD,<sup>4</sup> Gudrun Schreiber, MD,<sup>5</sup> Folker Hanefeld, MD,<sup>5</sup> Michel Fardeau, MD,<sup>1</sup> Jean-Jacques Martin, MD, PhD,<sup>2</sup> Hans H. Goebel, MD,<sup>6</sup> Pascale Richard, PhD,<sup>7</sup> Pascale Guicheney, PhD,<sup>1</sup> and Carsten G. Bönnemann, MD<sup>3</sup>

**Desmin-related myopathies (DRMs) are a heterogeneous group of muscle disorders, morphologically defined by intrasarcoplasmic aggregates of desmin. Mutations in the desmin and the  $\alpha$ -B crystallin genes account for approximately one third of the DRM cases. The genetic basis of the other forms remain unknown, including the early-onset, recessive form with Mallory body–like inclusions (MB-DRMs), first described in five related German patients. Recently, we identified the selenoprotein N gene (*SEPN1*) as responsible for SEPN-related myopathy (SEPN-RM), a unique early-onset myopathy formerly divided in two different nosological categories: rigid spine muscular dystrophy and the severe form of classical multimincore disease. The finding of Mallory body–like inclusions in two cases of genetically documented SEPN-RM led us to suspect a relationship between MB-DRM and *SEPN1*. In the original MB-DRM German family, we demonstrated a linkage of the disease to the *SEPN1* locus (1p36), and subsequently a homozygous *SEPN1* deletion (del 92 nucleotide –19/+73) in the affected patients. A comparative reevaluation showed that MB-DRM and SEPN-RM share identical clinical features. Therefore, we propose that MB-DRM should be categorized as SEPN-RM. These findings substantiate the molecular heterogeneity of DRM, expand the morphological spectrum of SEPN-RM, and implicate a necessary reassessment of the nosological boundaries in early-onset myopathies.**

Ann Neurol 2004;55:676–686

Desmin-related myopathies (DRMs, [MIM 601419]) are a group of familial or sporadic muscle disorders, morphologically defined by intrasarcoplasmic aggregates of desmin, the intermediate filament of muscle cells. Although demonstration of desmin-reactive material in the muscle fibers constitute the hallmark of DRM, several other proteins accumulate in these disorders, including dystrophin, ubiquitin, nestin, vimentin,  $\alpha$ -B crystallin, amyloid precursor protein,  $\beta$ -amyloid epitopes, gelsolin,  $\alpha_1$ -antichymotrypsin, lamin-B or neural cell adhesion molecule.<sup>1,2</sup> This abnormal accumulation of proteins is constantly associated with a variable degree of myofibrillar degradation; therefore, DRMs are also known as myofibrillar myopathies.<sup>3,4</sup>

The nosological boundaries of this heterogeneous group of myopathies, most of which are autosomal

dominant and include cardiac involvement, are poorly established.<sup>5</sup> Approximately one third of the cases of DRM are caused by heterozygous or compound heterozygous mutations of the desmin gene (*DES*, [MIM 125660]) and thus are termed *primary desminopathies*.<sup>6,7</sup> A mutation in the  $\alpha$ -B crystallin gene (*CRYAB*, [MIM 123590]) was identified in a large family affected by a particular form of DRM with late-onset and autosomal dominant inheritance,<sup>8,9</sup> now called  $\alpha$ -B crystallinopathy.<sup>10</sup> The genetic basis of the remaining forms of DRM were unknown, including the early-onset, autosomal recessive form with Mallory body–like inclusion bodies or hyaline/desmin plaques.<sup>11</sup>

The “Mallory body–like” form of DRM (MB-DRM) was first described as “a form of congenital muscular dystrophy” (CMD) in five patients originat-

From <sup>1</sup>Institut National de la Santé et de la Recherche Médicale U582, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France; <sup>2</sup>Department of Neuropathology, Born-Bunge Foundation and University of Antwerp, Antwerpen, Belgium; <sup>3</sup>Children’s Hospital of Philadelphia, Philadelphia, PA; <sup>4</sup>Department of Paediatrics, University Hospital Gasthuisberg, Leuven, Belgium; <sup>5</sup>Abteilung Neuropädiatrie, Kinderklinik and Poliklinik, Georg-August Universität Göttingen, Göttingen, Germany; <sup>6</sup>Department of Neuropathology, Johannes Gutenberg University, Mainz, Germany; and <sup>7</sup>Service de Biochimie B, Groupe Hospitalier Pitié-Salpêtrière, Paris, France.

Received Sep 18, 2003, and in revised form Jan 20, 2004. Accepted for publication Jan 21, 2004.

Published online Mar 25, 2004, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20077

Accession numbers are listed in the Appendix on page 685.

Address correspondence to Dr. Ana Ferreiro, INSERM U582, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, 47 Bd. de l’Hôpital, 75651 Paris, France.  
E-mail: a.ferreiro@myologie.chups.jussieu.fr

ing from a genetic isolate in Northern Germany,<sup>12,13</sup> four of which were related by remote family links [MIM 253850]. Since then, approximately 14 additional cases have been reported.<sup>7,14</sup> All patients presented with neonatal hypotonia, axial and proximal muscle weakness, scoliosis, and normal or mildly elevated creatine kinase levels; 11 subjects died of respiratory insufficiency before adulthood.<sup>7</sup> Further morphological studies established that MB-DRM was defined by the presence, in approximately 10% of muscle fibers, of hyaline plaques devoid of any enzyme activity such as NADH, SDH, or ATPase, that corresponded at the ultrastructural level to peculiar intramyofibrillar inclusions.<sup>13,14</sup> These inclusions were composed of helical filaments, 10 to 12nm in diameter, arranged in bundles, and surrounded by irregular masses of electron-dense amorphous material. Finer filaments of 8 to 10nm in diameter frequently created a lighter perilesional halo.<sup>14</sup> Rimmed vacuoles and focal areas of Z-line streaming also were observed in the original cases.<sup>13</sup> Because the particular inclusions resembled the Mallory bodies of alcoholic liver disease,<sup>15,16</sup> they were termed MB-like inclusions. The finding that these deposits were immunoreactive for desmin, dystrophin, and ubiquitin<sup>14</sup> led eventually to the inclusion of this entity in the group of desmin-related myopathies.<sup>11,17</sup>

We recently have shown that mutations of the selenoprotein N gene (*SEPN1* [MIM 606210]), encoding a novel selenoprotein of unknown function,<sup>18</sup> are responsible for two autosomal recessive, early-onset myopathies.<sup>19,20</sup> The first one, originally referred to as rigid spine muscular dystrophy (RSMD1), is associated with early and prominent spinal rigidity and was considered as a form of congenital muscular dystrophy.<sup>19</sup> The second one includes the most severe cases of the "classical" form of multimincore disease (MmD), a congenital myopathy characterized by the presence of multiple, short-length areas of sarcomere disorganization and mitochondria depletion ("minicores") in muscle fibers.<sup>20,21</sup> Phenotypic reevaluation demonstrated that both disorders show so much clinical and morphological overlap that they are best understood as a single nosological entity (MIM 602771) for which we propose the term *SEPN-related myopathy* (SEPN-RM).<sup>20</sup> This condition is characterized by predominantly axial weakness, early scoliosis and respiratory failure, and a variable degree of spinal rigidity. The myopathological spectrum of SEPN-RM is broad and may or may not include dystrophic features such as regenerating fibers or endomysial fibrosis, whereas minicore-type lesions are a fairly constant finding.<sup>20,22</sup>

Recently, we evaluated second muscle biopsies from two brothers with SEPN-RM caused by a missense *SEPN1* mutation. The unexpected finding of Mallory body-like inclusions in these samples, together with the

previously described accumulation of desmin<sup>21</sup> and  $\alpha$ -B crystallin<sup>23</sup> within the core lesions, led us to suspect a relationship between *SEPN1* and MB-DRM. In the original MB-DRM German family, we demonstrated a linkage of the disease to the *SEPN1* locus in 1p36 and subsequently a homozygous *SEPN1* deletion (del 92 nucleotide -19/+73) in the affected patients. Clinical reassessment showed that the clinical features of MB-DRM are indistinguishable from those recognized in SEPN-RM, suggesting that MB-DRM is in fact part of the SEPN-RM spectrum.

## Subjects and Methods

### Subjects

Family I includes two brothers (Patients 1 and 2) born from healthy Belgian parents without known consanguinity. Both presented with predominantly axial muscle weakness, scoliosis, respiratory insufficiency, spinal rigidity, and some minicore lesions in their muscle biopsies. This typical SEPN-RM phenotype led to identification of a homozygous *SEPN1* mutation (943G→A) in both cases at the ages of 23 and 20 years.

Family II includes four of the five patients (two male and two female patients, Patients 3–6) with whom the first description of MB-DRM was established. They belonged to three different family cores; all the parental couples were healthy, originated from the same genetic isolate (the Eichsfeld, Northern Germany), and were related by family links that could be traced back over 250 years.<sup>12,13</sup> The early clinical findings in these cases have been previously described.<sup>12,13</sup>

### Morphological Studies

In Family I, deltoid muscle biopsies for diagnostic purposes were obtained from both patients at 6 and 9 years of age, respectively. After informed consent, second quadriceps muscle biopsies were performed 14 years later and processed for light and electron microscopy and immunohistochemistry according to standard methods.<sup>24</sup> A large battery of antibodies was used on 7 to 8 $\mu$ m cryostat sections, to examine the reactivity of  $\alpha$ -actinin,  $\alpha$ - $\beta$  crystallin, actin, A $\beta$  amyloid, desmin, dystrophin (1, 2, 3), 8-hydroxydeoxyguanosine (8-OHdG), merosin, myosin heavy chain (MHCn and MHCd), nebulin, pan-cytokeratin 5, 6, 8, and 18 intermediate filament proteins, plectin,  $\alpha$ -,  $\beta$ - and  $\gamma$ -sarcoglycans, 43kDa DAG ( $\beta$ -dystroglycan), SERCA2 (sarcoendoplasmic reticulum Ca<sup>2+</sup> ATPase), telethonin, tropomyosin, troponin, titin, vimentin, and ubiquitin. The presence of normal and phosphorylated Tau and of heavy neurofilament subunit was analyzed using the AT100 and AT120 antibodies and the SMI-31 antibody respectively. Immunoelectron microscopy by immunogold labeling was performed using anti- $\alpha$ -B crystallin, antiactin, anti-AT100, antidesmin, and antiubiquitin. Standard indirect, double immunofluorescence microscopy on frozen cryostat sections was processed to localize  $\alpha$ -B crystallin and desmin.

In addition, a paraspinal muscle sample obtained from Patient 1 at 17 years was reviewed retrospectively.

In Family II, quadriceps muscle biopsies were taken from

all the affected members between the ages of 2 and 10 years. Samples were processed for light and electron microscopy and for immunohistochemistry as described.<sup>12,13</sup>

### Genetic Analysis

Genomic DNA was extracted from blood samples or lymphoblastoid cell lines, after informed consent according to the local ethics committees, using standard procedures, except in the deceased Patients 3 and 6, whose DNA was extracted from archival muscle sections as described.<sup>25</sup> Genetic analysis of Family I has been previously described.<sup>20</sup>

Genetic analysis of Family II originally was undertaken through a whole-genome screening using 400 microsatellite markers,<sup>20</sup> initially without material from deceased Patients 3 and 6.

Because of the morphological and clinical similarities with Family I, *SEPN1* was further analyzed as a candidate gene in Family II. Intragenic marker D1S2885 was amplified as described.<sup>26</sup> All *SEPN1* exons and the SECIS element were screened by polymerase chain reaction (PCR) single-strand conformation polymorphism and sequencing using described methods.<sup>19</sup> All PCRs were done using Platinum Taq polymerase (Gibco BRL/Life Technology, Paisley, Scotland), except for exon 1 which required the GC-rich PCR system kit (Roche Diagnostics, Mannheim, Germany), a hot start and, for archival DNA samples (Patients 3 and 6), a total of 40 cycles.

## Results

### Morphological Reevaluation of Patients with Known *SEPN1* Mutations (Family I)

The first muscle biopsies (that were) taken from Patients 1 and 2 at 9 and 6 years, respectively, showed minor myopathic changes (type I fiber predominance, sparse centrally located nuclei), together with minicores in less than half of the muscle fibers (Fig 1A). Although consistent with MmD the scarcity of core lesions was not fully typical. Therefore, novel biopsies were taken from the quadriceps at 23 (Patient 1) and 20 (Patient 2) years. An *in vitro* contracture test<sup>27</sup> was performed on the second sample from Patient 2 and demonstrated the absence of malignant hyperthermia susceptibility.

These second biopsies (see Fig 1B) disclosed a different pattern, with marked fiber size variability, centrally located nuclei (see Fig 1B, a), rare rimmed vacuoles (see Fig 1B, d) and, unexpectedly, focal myofibrillar lesions forming circumscribed, hyaline plaques (see Fig 1B, b, c) with absent or reduced oxidative enzyme reactivity (see Fig 1B, e–h). Minor dystrophic features, including sparse necrotic or regenerating fibers and mild endomysial fibrosis and fatty replacement, also were present.

At the ultrastructural level, we observed myofibrillar disorganization ranging from Z-band streaming forming rare classic minicores (Fig 2A) to Z-disk derived heterogeneous material assembled in plaques (see Fig

2B), some of which were amorphous and strongly osmiophilic (see Fig 2C). These plaques were composed by small aggregates of 7 to 12nm serpentine-like or helical filaments surrounded by irregular masses of electron-dense amorphous material and by accumulations of 8 to 10nm intermediate filaments (see Fig 2D, E). In addition, 20nm-thick sarcoplasmic tubulofilaments were observed in one patient, although never within the nuclei. Altogether, these elements were highly characteristic of MB-like inclusions and identical to those previously described in Family II.<sup>12,13</sup>

Immunohistochemical examination showed an overexpression of desmin and  $\alpha$ -B crystallin within the plaques (Fig 3A); by immunofluorescence analysis, both proteins were found to colocalize (data not shown). The plaques were also immunoreactive for dystrophin, tau AT100 and tau AT120, A $\beta$  amyloid, and ubiquitin. Variable expression patterns were found for actin,  $\alpha$ -actinin and 8-OHdG. The plaques showed weak or inconsistent staining for  $\beta$ - and  $\gamma$ -sarcoglycans, 43kDa DAG, nebulin, SERCA2, and telethonin. No immunoreactivity was observed for the remaining proteins analyzed. Few vimentin, MHCn, and MHCd-stained fibers were found. Merosin staining was normal.

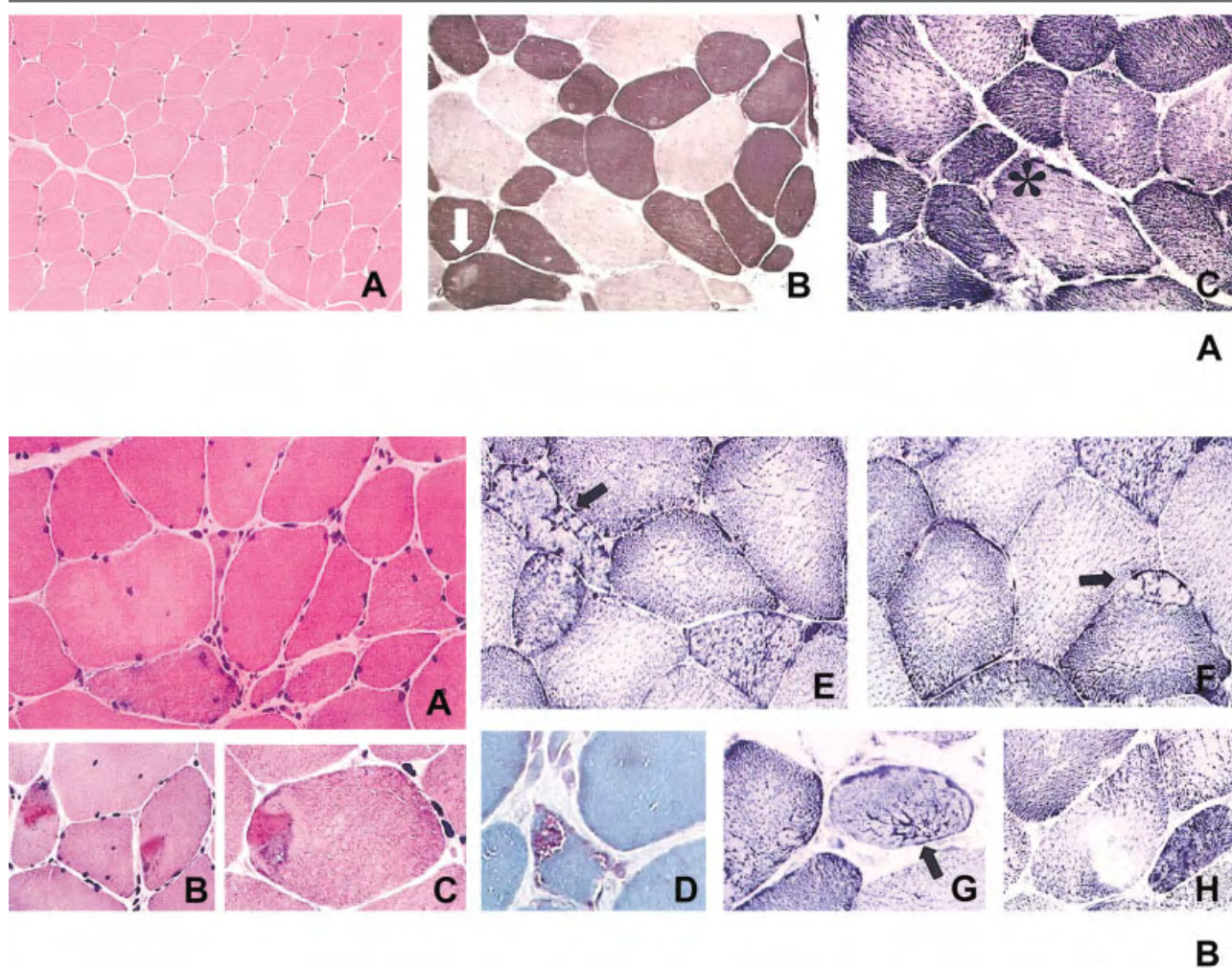
Immunoelectron microscopy (see Fig 3B) showed a strong actin immunoreactivity associated with the modified myofibrillar elements. Focal desmin immunoreactivity was associated with the Z-disk-derived material, with the peripheral 8 to 10nm intermediate filaments and with an intrasarcoplasmic, granular material. The helical filaments were desmin- and actin-negative but were immunoreactive for ubiquitin and AT100.

Review of the previous paraspinal muscle biopsy from Patient 1 showed type I fiber predominance, marked dystrophic features including endomysial fibrosis and adiposis, and numerous hyaline plaques (data not shown).

### Molecular Genetic Studies of the Original Mallory Body-like Desmin-Related Myopathy Family (Family II)

The finding of typical MB-like inclusions in patients from Family I, known to have a *SEPN1* homozygous missense mutation (943G→A) that changed a glycine codon to a serine codon (G315S), suggested *SEPN1* as a potential candidate gene for MB-DRM. To verify this hypothesis, we studied the highly informative German family in which MB-DRM was first described (Family II, Fig 4).

Initially, a genome-wide screen had been performed in this consanguineous family, including marker D1S234, located 1cM upstream *SEPN1*, that proved to be unlinked to the disease. However, further studies showed that the intragenic marker D1S2885 was homozygous by descent in all the affected individuals,



**Fig 1.** Histological and histochemical studies in Family I. Transverse cryostat sections. (A) First deltoid biopsies. Absence of dystrophic lesions (a). Type 1 fiber (darker) predominance and relative hypotrophy (b), short core areas lacking enzymatic activity, multiple (c, asterisk) or larger and unique (b and c, arrow). Patients 2 (a) and 1 (b, c) at 6 and 9 years of age. HE  $\times 40$  (a), myosin adenosine triphosphatase (ATPase), pH 4.36 (b), NADH-TR 80X (c). (B) Second quadriceps biopsies: marked fiber size variability, centrally located nuclei, rare regenerating fibers, mild endomyrial fibrosis (a), sparse hyaline plaques (b, c), rare rimmed vacuoles (d). Different patterns of abnormal oxidative activity were observed, mostly in type 1 fibers (e–h, arrows): heterogeneous oxidative activity distribution (“pseudobulbated” fibers, e); well-circumscribed, rounded, oxidative-negative core-like areas (f); presence of a dark-stained amorphous material (g) or a rubbed-out appearance (h). Patients 1 (a–f) and 2 (g, h) at 23 and 20 years. HE  $\times 80$  (a, b) and  $\times 120$  (c), Gomori’s modified trichrome  $\times 120$  (d), NADH-TR  $\times 80$  (e–h).

thus indicating a potential linkage of the disease to *SEPN1* (see Fig 4).

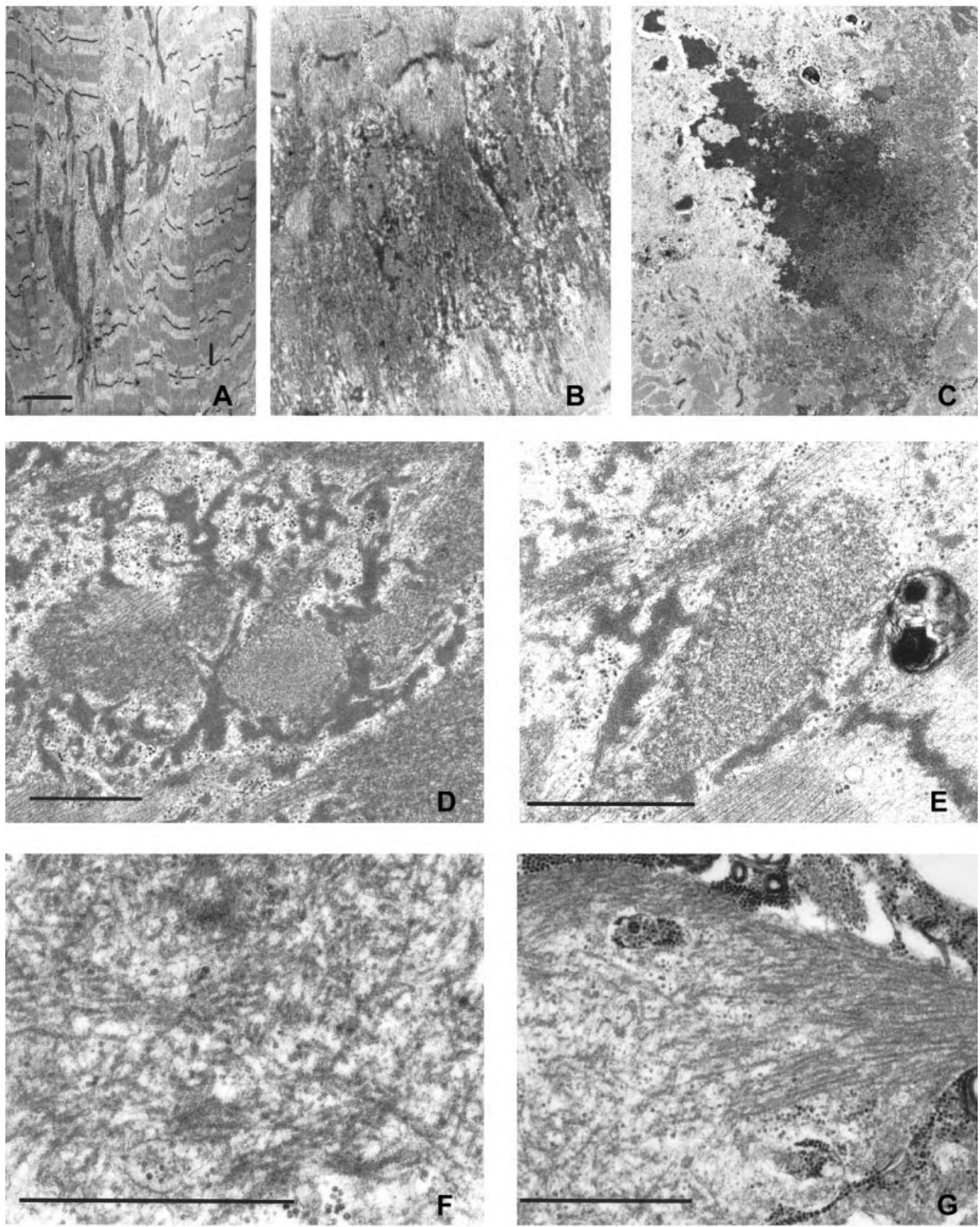
Subsequent analysis of the *SEPN1* coding sequence in Patients 3 to 6 disclosed a novel 92bp deletion, starting 17bp upstream exon 1 and including the first 73bp of this exon (del 92 nucleotide  $-19/+73$ ), thereby causing a loss of the translation starting codon ATG. This deletion was present at the homozygous state in all patients, all their parents being heterozygous carriers (Fig 5).

#### *Clinical Reevaluation: Phenotypical Homogeneity*

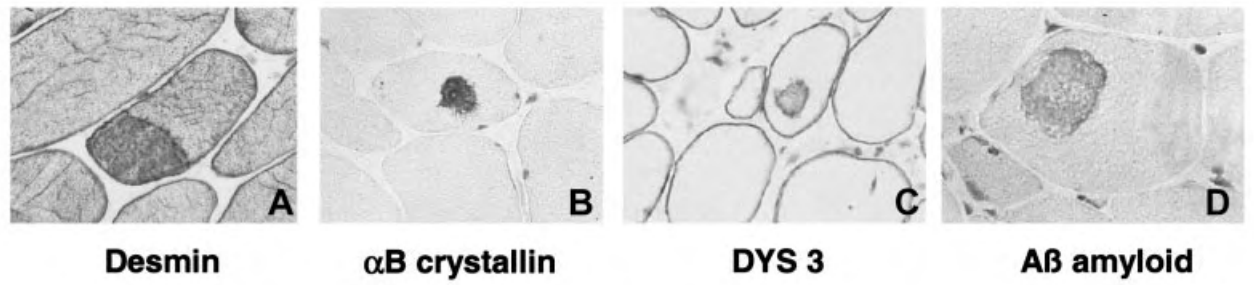
Clinical reevaluation of the surviving patients from both families (Patients 1, 2, 4, and 5, now aged 24, 21,

35, and 27 years, respectively), and a retrospective analysis of the early findings reported in Family II<sup>12</sup> demonstrated that all patients shared a highly consistent clinical picture, typical of SEP-N-RM.

Patients 1 and 2 presented with mildly delayed motor development (independent gait at 18 and 20 months), poor head control, generalized amyotrophy, difficulties climbing stairs, and frequent falls since early infancy. Most patients from Family II presented earlier with congenital muscle weakness. A flat chest, the typical scoliosis with lateral trunk deviation and the particular lower limb appearance described in SEP-N-RM patients (amyotrophic inner thighs, straight calves, and flat feet)<sup>20</sup> were evident on the pictures of the MB-



*Fig 2. Ultrastructural findings in adulthood biopsies from Family I: Mallory body-like inclusions. Longitudinal and transverse electron microscopic sections of the quadriceps biopsies from Patients 1 (A, B and D–G) and 2 (C). Myofibrillar disorganization ranged from Z-band streaming, forming rare classic minicores (A), to Z-derived material assembled in plaques (B), some of them amorphous and strongly osmiophilic (C). These plaques were composed of bundles of 12nm helical filaments surrounded by electron-dense amorphous material and by 8 to 10nm intermediate filaments (D, E). Most bundles contained randomly arranged filaments (F), but some showed parallelly arranged filaments with characteristic serrations (G). Bar = 2.5 $\mu$ m in A to C, 1.2 $\mu$ m in D to G.*



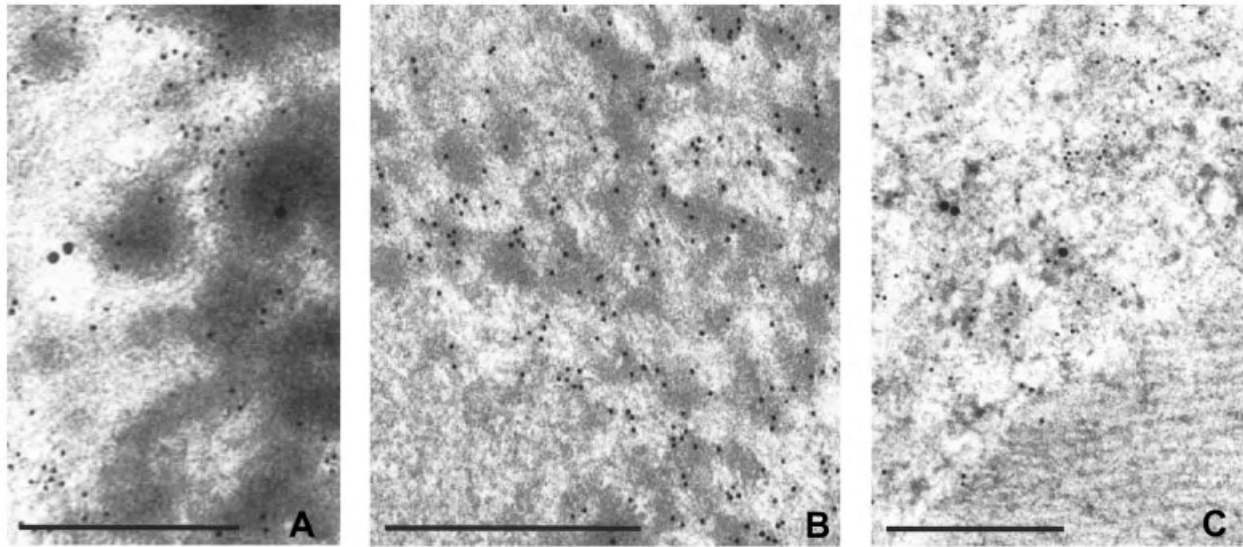
**Desmin**

**$\alpha$ B crystallin**

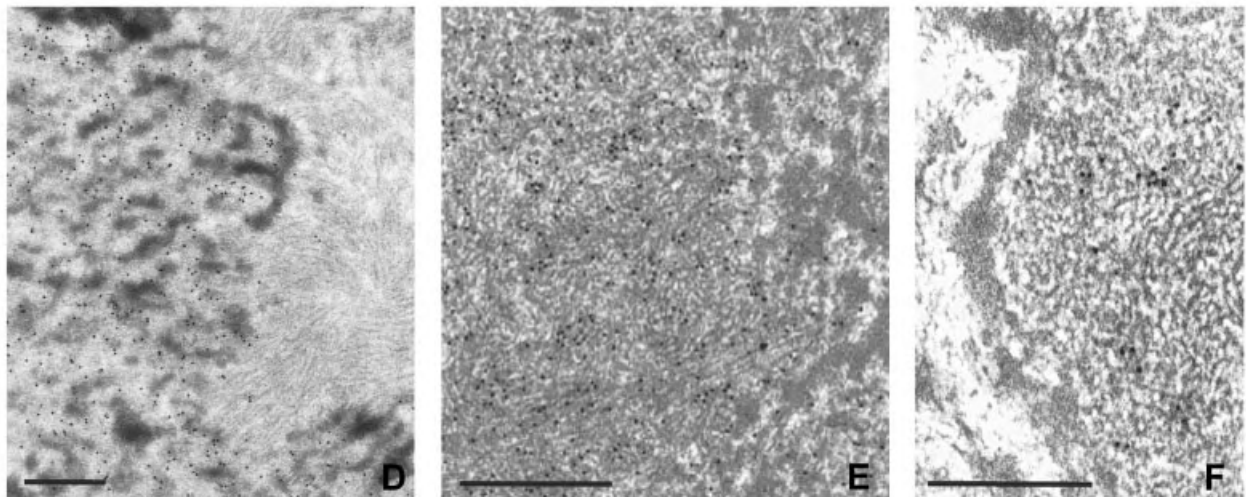
**DYS 3**

**A $\beta$  amyloid**

**A**



**Desmin**



**Actin**

**Ubiquitin**

**AT-100**

**B**

*Fig 3. Immunohistochemical studies in Family I. (A) Light microscopy.  $\times 80$  (a-c),  $\times 120$  (d). The hyaline plaques were strongly immunoreactive for desmin,  $\alpha$ -B crystallin, dystrophin (dys 1, dys 2, and dys 3) and A $\beta$  amyloid, among others. (B) Immunoelectron microscopy. Bar =  $0.5\mu\text{m}$ . Desmin immunoreactivity was associated with focal Z-derived material (a), with the outer intermediate filaments (b) and with an intrasarcoplasmic granular material. The modified myofibrillar elements were reactive for actin (d). The helical filaments were desmin- and actin-negative (b, c, d) but immunoreactive for ubiquitin (e) and AT100 (f).*

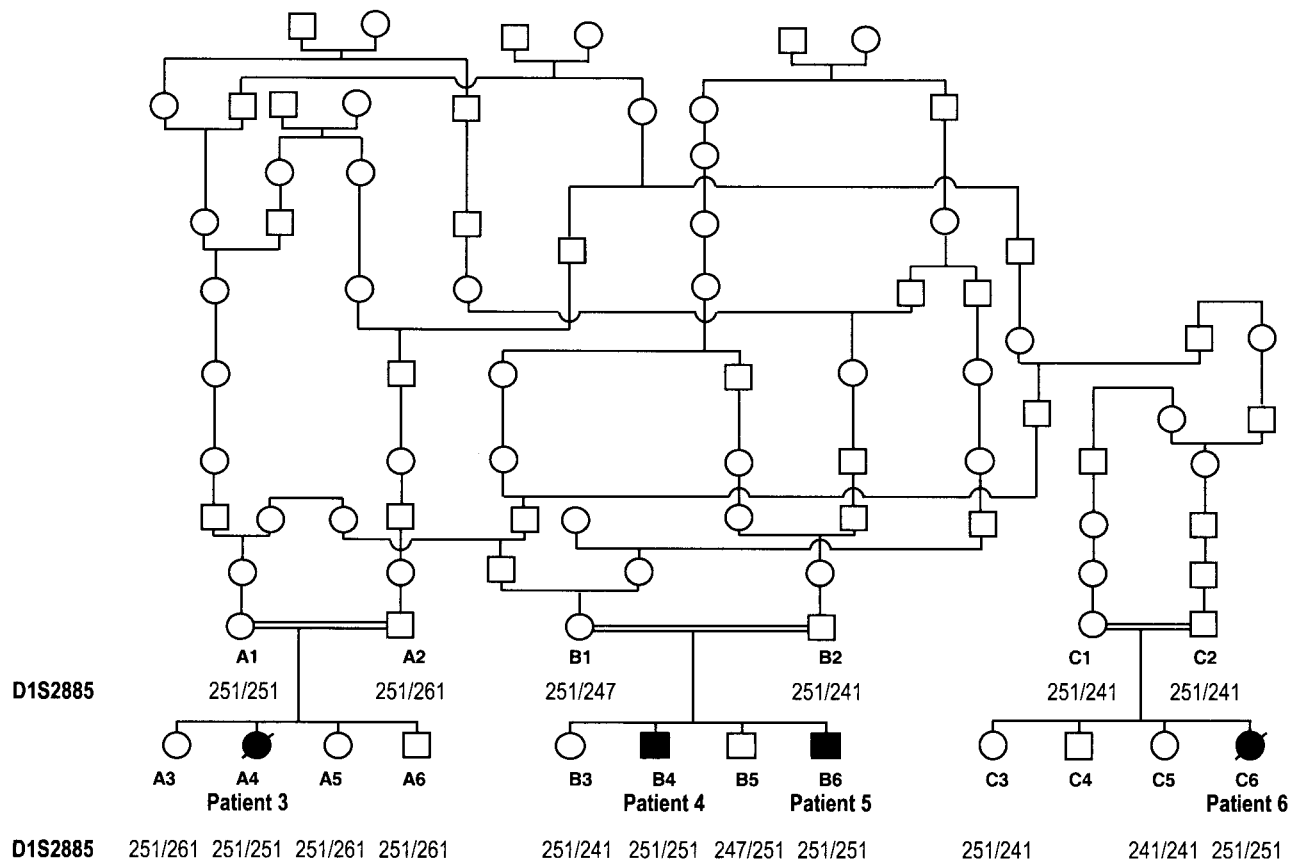


Fig 4. Simplified pedigree and D1S2885 genotypes of Family II. Affected individuals are denoted by filled symbols. For compactness, only some of the consanguinity loops and links among the three core families are represented; the common ancestors originating this genetic isolate, a Roman Catholic enclave in a Protestant Northern Germany area, are not shown. A detailed pedigree has been published previously.<sup>12,13</sup> Excepting individual A1, in whom D1S2885 was noninformative, all the parents were heterozygous carriers of the same at-risk allele (251bp) inherited by the affected individuals at the homozygous state. No DNA sample was available from individual C4.

DRM patients taken at early ages<sup>12</sup> and are still apparent in the surviving cases (Fig 6).

In all patients, axial weakness was severe and contrasted with a comparatively preserved limb muscle strength. All developed early scoliosis (from the ages of 8 to 14 years), spinal rigidity, and life-threatening respiratory insufficiency, despite preserved ambulation. Restrictive lung disease was associated with episodes of nocturnal desaturation documented in the cases who underwent polysomnographic studies (Patients 1 and 2, at 15 and 17 years, respectively) and required nighttime assisted ventilation despite a relatively preserved vital capacity (47% and 29% of the predicted values). In Family II, Patients 4 and 5 needed nocturnal nasal ventilation since early adulthood; the more severely affected Patients 3 and 6 developed pulmonary hypertension from the age of 9 years and died of secondary cardiac failure at 11 and 12 years, respectively. However, there was no evidence of primary cardiac involvement either in these patients or in the four surviving patients, who have undergone repeated cardiological

evaluations. Mild to moderate facial weakness, a high arched palate, and a nasal, high pitched voice also were frequently observed in these cases.

The clinical picture remained globally stable after the growth spurt. All the adult patients are able to walk independently, at least for short to moderate distances. Although the clinical pattern is highly consistent, the severity of the disease is quite variable, even among siblings. In Family II, the more severely affected of both survivors, Patient 4, shows generalized muscle weakness that is more marked in neck flexors and glutei (3/5 according to the Medical Research Council scale), spares the quadriceps and the triceps surae and remains moderate (4/5) in the other muscle groups, allowing independent gait for short distances (15–20m). As Patient 1, he (or Patient 4) required spinal fusion in early adulthood. In contrast, his brother (Patient 5) did not require arthrodesis and has an unlimited gait perimeter, although he has needed ventilatory support from the age of 22 years. Patients 1 and 2 can walk 0.5 and 2km, respectively.

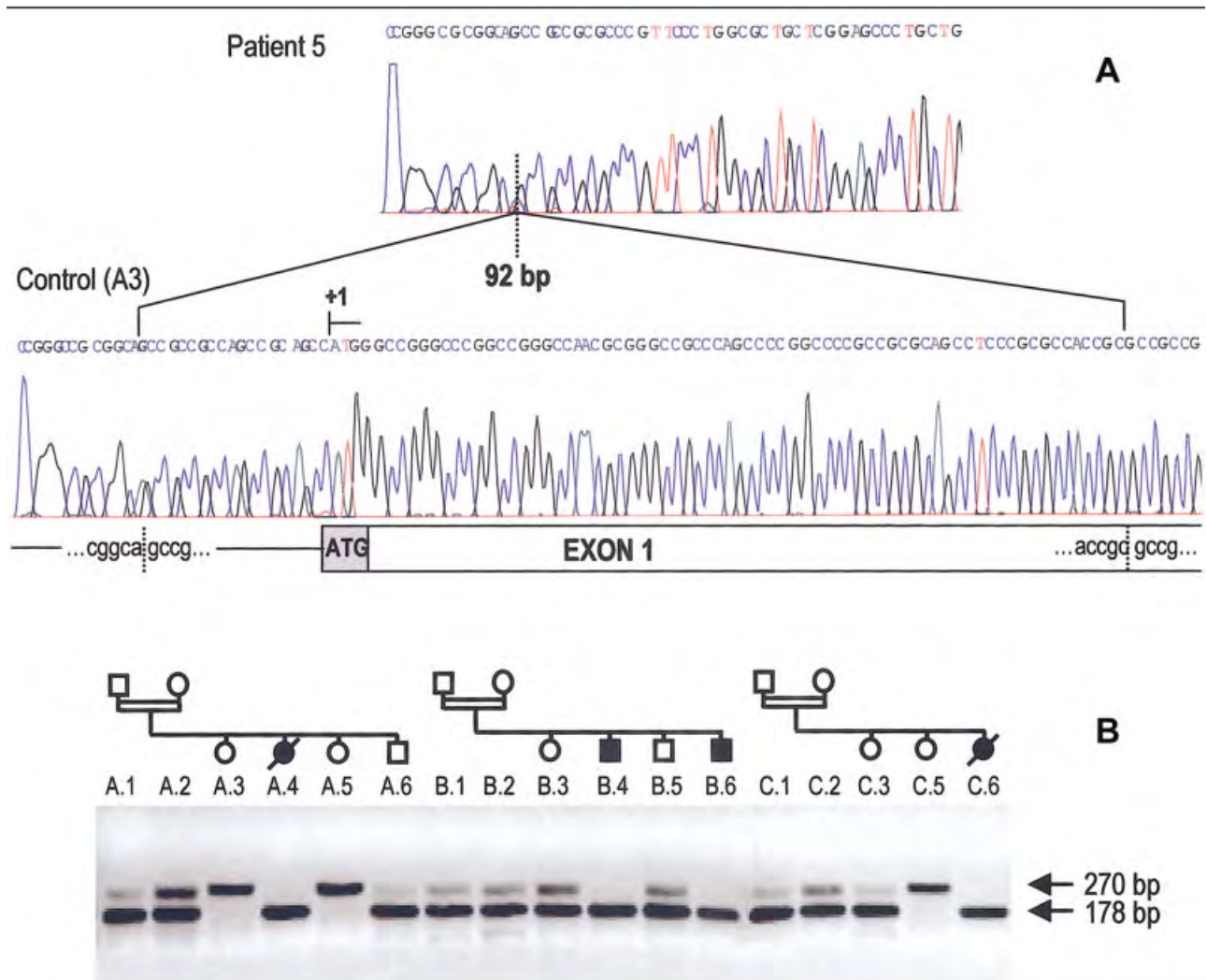


Fig 5. Segregation of the *SEPNI* (del 92 nucleotide –19/+73) in Family II. The 92bp deletion (A) reduced the size of exon 1 polymerase chain reaction product from 270 to 178bp, allowing its identification by electrophoresis on agarose gels (B). All the affected individuals were homozygous for the deletion; all parents and four unaffected siblings were heterozygous carriers.

Joint contractures correlate with the degree of muscle weakness, being more severe in Patients 1 and 4 (particularly at elbows and hip flexors, less prominently at Achilles tendon and finger flexors) and milder in Patients 2 and 5 (see Fig 6).

### Discussion

With the elucidation of the genetic basis of many neuromuscular disorders, revised classifications are now emerging; however, numerous muscular diseases are still mainly classified on purely clinical and morphological criteria. This is particularly true of congenital myopathies (including MmD) and of the desmin-related myopathies, for which there are divergent definitions, classifications, and nomenclatures<sup>28</sup> (DRM, myofibrillar myopathies,<sup>3,29</sup> protein-surplus myopathies<sup>1,7</sup>).

Recently, a subgroup of DRM was delineated in two European Neuromuscular Center-sponsored work-

shops<sup>30,31</sup> as congenital myopathies characterized by various desmin-positive inclusions,<sup>11,32</sup> such as cytoplasmic bodies,<sup>33,34</sup> or MB-like inclusions. However, most of these inclusions are not specific; for instance, cytoplasmic bodies may coexist with reducing bodies,<sup>35–38</sup> appear in other desmin-storage conditions,<sup>39,40</sup> or occur nonspecifically in other neuromuscular entities.<sup>32</sup> Although it has been proposed that most inclusions would have the same origin,<sup>3</sup> this has never been supported by genetic data.

The identification of *SEPNI* mutations in our patients contributes to clarify this intricate field by elucidating the genetic basis of MB-DRM, long recognized as a distinct clinicopathological entity. Furthermore, *SEPNI* is identified as the third gene responsible for a myopathy with desmin-positive foci, thereby expanding the molecular spectrum of DRM.

Recently, we established that mutations in *SEPNI*

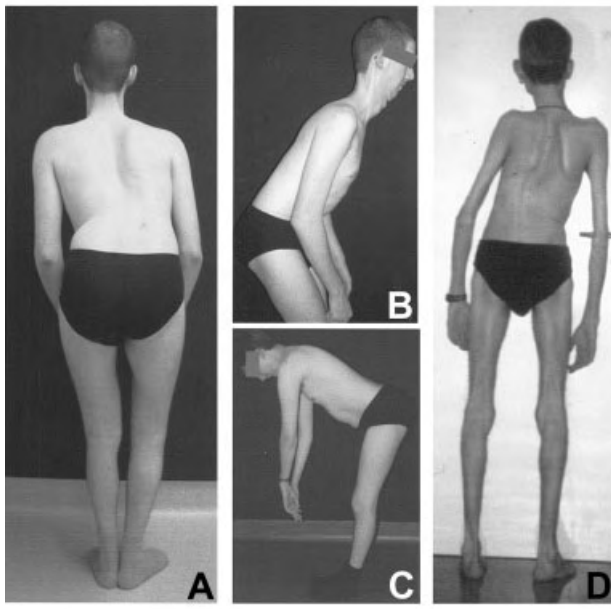


Fig 6. Clinical features. Patients 1 (A, B), 2 (C), and 4 (D). Slender neck, flat thorax, moderate deltoid atrophy, particular leg appearance (A, D), and typical scoliosis, characterized by dorsal lordosis ("hollow back"), lateral trunk deviation, and balanced hips (A, D). Retrognathia was common (B). Note a marked variability in the severity of joint contractures and spinal rigidity, which was greatest and associated with fixed neck hyperextension in Patient 1 (B, maximum neck flexion and elbow extension). Patient 1 had also marked elbow, hip flexors, knee, and heel contractures (B), much less patent in his brother (Patient 2, C).

were responsible for an early-onset myopathy encompassing a congenital muscular dystrophy (RSM1) and a congenital myopathy (MmD), for which we propose the joint term of SEP-N-RM.<sup>20</sup> Patients with *SEPNI* mutations previously were categorized under either of two separate entities because of the large myopathological spectrum of SEP-N-RM, that encompasses both dystrophic features and minicore lesions neither of which are obligatory. Our findings represent a further expansion of this SEP-N-RM morphological spectrum, now including also "positive" structural phenomena of the inclusion type. Indeed, the previously observed accumulation of desmin,<sup>21</sup>  $\alpha$ -B crystallin, or filamin C<sup>23</sup> within the minicore lesions pointed in this direction. Differential involvement of some muscle groups in SEP-N-RM might play a role in this remarkable morphological variability.<sup>20,22</sup> Thus, all the biopsies leading to the identification of MB-like inclusions in the MB-DRM cases reported in the literature were taken from the quadriceps femoris,<sup>13,14</sup> a muscle particularly spared in SEP-N-RM.<sup>20,22</sup> Furthermore, in Family I, only the second quadriceps biopsies disclosed the typical inclusions, not found in previous deltoid samples.

In contrast with this morphological variability, the

clinical phenotype of all the patients carrying *SEPNI* mutations is remarkably defined, highly consistent, and more reliable for diagnosis than morphological criteria. Its hallmark is a predominantly axial muscle involvement, leading to a particular scoliosis and life-threatening respiratory insufficiency that, if adequately treated, tend to remain stable after the growth spurt. This severe respiratory insufficiency, most uncommon in other ambulant neuromuscular patients, is partly caused by episodes of nocturnal desaturation; early and systematic polysomnographic studies must be performed in all these patients, even in those with a relatively preserved vital capacity and in the absence of day symptoms. A variable degree of spinal rigidity is frequent although inconstant and was lately remarked during follow-up in 14 typical MB-DRM patients.<sup>7</sup> Limb muscle strength is relatively preserved, which explains the fact that major developmental milestones are frequently considered normal, although difficulties with holding up head from the lying position due to pronounced neck flexor weakness are constant both in MB-DRM<sup>7,14</sup> and in SEP-N-RM.<sup>20,22</sup> Muscle weakness distribution is thus consistent, although its severity may be variable: among the original MB-DRM cases, deceased Patient 3 had severe respiratory involvement from birth and walked at 32 months of age, whereas Patient 6 acquired gait at 11 months and leads an autonomous life at the age of 27 years.

The primary molecular abnormality in MB-DRM is a *SEPNI* defect and not a desmin one. Furthermore, the *SEPNI* deletion observed in the original MB-DRM family has been found recently at the compound heterozygous state (1A→G/del 92 nucleotide -19/+73) in a German-French family showing a typical SEP-N-RM phenotype (unpublished data). These molecular findings, together with their common phenotypical features, suggest that SEP-N-RM and MB-DRM represent not only allelic disorders, but the same entity. In this sense, note that in their first description of MB-DRM Goebel and colleagues referred to it as "a form of congenital muscular dystrophy"<sup>12</sup>; later on, the disease was termed "a new familial congenital myopathy,"<sup>14</sup> before being explicitly classified as a DRM because of the inclusions desmin immunoreactivity.<sup>3,11,17</sup> We suggest that this nosological position should now be reconsidered, and that the morphology-based diagnostic criteria and nosological boundaries within the DRM group need to be reassessed taking into account genetic and clinical data. Actually, most patients with desmin and  $\alpha$ -B crystallin mutations show a late presentation, prominent distal weakness and primary cardiac involvement, which differentiate them on clinical grounds from the patients with *SEPNI* mutations.

*SEPNI* is a 70kDa glycoprotein localized within the endoplasmic reticulum, whose primary structure suggests a hypothetical enzymatic activity.<sup>42</sup> A defect of

this protein appears to cause not only focal sarcomere disorganization and mitochondria depletion (minicores) but also an abnormal accumulation of multiple proteins including desmin. This raises interesting questions about the unknown function of *SEPN1*. Desmin forms an intracellular network of transversal connections between Z-discs and with the sarcolemma,<sup>7</sup> suggesting a role as a mechanical integrator for myofibrillar contraction.<sup>43</sup> Besides, minicore lesions may also be caused by stretching and by repeated eccentric contractions.<sup>44</sup> They could thus be, in part, the secondary morphological manifestation of a diminished resistance of sarcomere to tension,<sup>21</sup> in which desmin abnormalities could play a role. On the other hand, the onset of desmin expression during muscle development suggests a role for desmin in muscle differentiation.<sup>4</sup> Similarly, *SEPN1* is preferentially expressed in fetal or proliferating cells.<sup>42</sup> Nevertheless, there is no evidence so far of a direct or indirect functional link between both proteins. Desmin abnormalities are probably only one of the pathophysiological consequences of *SEPN1* mutations that most likely involve several biological pathways.<sup>20</sup> Further insight into *SEPN1* function is required to determine the relevance of desmin abnormalities in the final phenotypical expression of *SEPN*-RM.

In conclusion, this work expands the spectrum of *SEPN*-RM to include MB-DRM, thereby revealing an unexpected overlap between protein-surplus myopathies/DRM, congenital muscular dystrophies, and congenital myopathies. This opens the way to a genetically based redefinition of the classic, morphology-based nosological boundaries in the field of early-onset myopathies.

## Appendix

OMIM, <http://www.ncbi.nlm.nih.gov/Omim> (for DRM [MIM 601419], RSMD1 [MIM 602771], *DES* [MIM 125660], *CRYAB*, [MIM 123590], *SEPN1* [MIM 606210], minicore myopathy [MIM 602771] [MIM 255320] [MIM 117000] [MIM 607552]). GenBank, <http://www3.ncbi.nlm.nih.gov/Genbank/> (accession numbers for *SEPN1* genomic sequence, AJ306398; for *SEPN1* cDNA sequence, AJ306399).

This work was supported by funds from the Institut National de la Santé et de la Recherche Médicale (National Institute of Health and Medical Research, ISERM) (P.G.), the Association Française contre les Myopathies (French Association against Myopathies, AFM) (A.F.), and the Florence R.C. Murray Fellowship Program at The Children's Hospital of Philadelphia (C.G.B.). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of their respective institutions. A.F. is an Avenir Program investigator. C.G.B. is a Pew Scholar in the Biomedical Sciences.

We thank the patients and their families for their participation in this study, as well as Dr A. Lemaïque and C. Bétard for technical

assistance with the linkage analysis. Dr L. Heytens performed the malignant hyperthermia in vitro contracture test, and Dr R. Sciot provided a paraspinous muscle sample from Family I. We thank Drs H. Sasaki and D. Louis for help with the archival DNA extraction and Dr L. Kunkel for additional support with this study. Finally, we are grateful to L. Dewit, G. Seeldraeyers, I. Bats, U. Lübke, and E. Kraemer for technical assistance.

## References

- Goebel HH, Warlo I. Gene-related protein surplus myopathies. *Mol Genet Metab* 2000;71:267–275.
- Nakano S, Engel AG, Akiyoshi I, et al. Myofibrillar myopathy. III. Abnormal expression of cyclin-dependent kinases and nuclear proteins. *J Neuropathol Exp Neurol* 1997;56:850–856.
- Nakano S, Engel AG, Waclawik AJ, et al. Myofibrillar myopathy with abnormal foci of desmin positivity. I. Light and electron microscopy analysis of 10 cases. *J Neuropathol Exp Neurol* 1996;55:549–562.
- De Bleecker JL, Engel AG, Ertl BB. Myofibrillar myopathy with abnormal foci of desmin positivity. II. Immunocytochemical analysis reveals accumulation of multiple other proteins. *J Neuropathol Exp Neurol* 1996;55:563–577.
- Engel AG. Myofibrillar myopathy. *Ann Neurol* 1999;46:681–683.
- Goldfarb LG, Park KY, Cervenakova L, et al. Missense mutations in desmin associated with familial cardiac and skeletal myopathy. *Nat Genet* 1998;19:402–403.
- Goebel HH, Fardeau M. Desmin-protein surplus myopathies. 96th European Neuromuscular Centre (ENMC)-sponsored International Workshop held 14–16 September 2001, Naarden, The Netherlands. *Neuromuscul Disord* 2002;12:687–692.
- Fardeau M, Godet-Guillain J, Tomé FM, et al. [A new familial muscular disorder demonstrated by the intra-sarcoplasmic accumulation of a granulo-filamentous material which is dense on electron microscopy (author's transl)]. *Rev Neurol (Paris)* 1978;134:411–425.
- Rappaport L, Contard F, Samuel JL, et al. Storage of phosphorylated desmin in a familial myopathy. *FEBS Lett* 1988;231:421–425.
- Vicat P, Caron A, Guicheney P, et al. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet* 1998;20:92–95.
- Goebel HH, Fardeau M. Desminopathies. In: Emery AEH, ed. *Diagnostic criteria for neuromuscular disorders*. 2nd ed. London: The Royal Society of Medicine Press, 1998:75–79.
- Goebel HH, Lenard HG, Langenbeck U, et al. A form of congenital muscular dystrophy. *Brain Dev* 1980;2:387–400.
- Fidzianska A, Goebel HH, Osborn M, et al. Mallory body-like inclusions in a hereditary congenital neuromuscular disease. *Muscle Nerve* 1983;6:195–200.
- Fidzianska A, Ryniewicz B, Barcikowska M, et al. A new familial congenital myopathy in children with desmin and dystrophin reacting plaques. *J Neurol Sci* 1995;131:88–95.
- Mallory F. Cirrhosis of the liver: five different lesions from which it may arise. *Bull Johns Hopkins Hosp* 1911;22:69–75.
- Bianchi L, Winckler K, Mihatsch M, et al. Mallory bodies and giant mitochondria—two different structures in liver biopsies from alcoholics. *Beitr Pathol* 1973;150:298–310.
- Goebel HH. Desmin-related myopathies. *Curr Opin Neurol* 1997;10:426–429.
- Lescure A, Gautheret D, Carbon P, et al. Novel selenoproteins identified in silico and in vivo by using a conserved RNA structural motif. *J Biol Chem* 1999;274:38147–38154.

19. Moghadaszadeh B, Petit N, Jaillard C, et al. Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. *Nat Genet* 2001;29:17–18.
20. Ferreiro A, Quijano-Roy S, Pichereau C, et al. Mutations of the selenoprotein N gene, implicated in rigid spine muscular dystrophy, cause the classical phenotype of multi-minicore disease. Reassessing the nosology of early-onset myopathies. *Am J Hum Genet* 2002;71:739–749.
21. Ferreiro A, Estournet B, Chateau D, et al. Multi-minicore disease—searching for boundaries: phenotype analysis of 38 cases. *Ann Neurol* 2000;48:745–757.
22. Flanigan KM, Kerr L, Bromberg MB, et al. Congenital muscular dystrophy with rigid spine syndrome: a clinical, pathological, radiological, and genetic study. *Ann Neurol* 2000;47:152–161.
23. Bönemann CG, Thompson TG, van der Ven PF, et al. Filamin C accumulation is a strong but nonspecific immunohistochemical marker of core formation in muscle. *J Neurol Sci* 2003;206:71–78.
24. Ceuterick C, Martin J. Sporadic early adult-onset distal myopathy with rimmed vacuoles: immunohistochemistry and electron microscopy. *J Neurol Sci* 1996;139:190–196.
25. Louis DN, von Deimling A, Seizinger BR. A (CA)<sub>n</sub> dinucleotide repeat assay for evaluating loss of allelic heterozygosity in small and archival human brain tumor specimens. *Am J Pathol* 1992;141:777–782.
26. Moghadaszadeh B, Desguerre I, Topaloglu H, et al. Identification of a new locus for a peculiar form of congenital muscular dystrophy with early rigidity of the spine on chromosome 1p35–36. *Am J Hum Genet* 1998;62:1439–1445.
27. Ellis FR. European malignant hyperpyrexia group. *Br J Anaesth* 1984;56:1181–1182.
28. Goebel HH. Desmin-related neuromuscular disorders. *Muscle Nerve* 1995;18:1306–1320.
29. Amato AA, Kagan-Hallet K, Jackson CE, et al. The wide spectrum of myofibrillar myopathy suggests a multifactorial etiology and pathogenesis. *Neurology* 1998;51:1646–1655.
30. Goebel HH, Fardeau M. Desmin in myology. 24th European Neuromuscular Center-sponsored workshop held 5–6 November 1993, Naarden, The Netherlands. *Neuromuscul Disord* 1995;5:161–166.
31. Goebel HH, Fardeau M. Familial desmin-related myopathies and cardiomyopathies—from myopathology to molecular and clinical genetics. 36th European Neuromuscular Center (ENMC)-sponsored International Workshop 20–22 October, 1995, Naarden, The Netherlands. *Neuromuscul Disord* 1996;6:383–388.
32. Goebel HH. Congenital myopathies with inclusion bodies: a brief review. *Neuromuscul Disord* 1998;8:162–168.
33. Engel WK. The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. *Neurology* 1962;12:778.
34. Goebel HH, Schloon H, Lenard HG. Congenital myopathy with cytoplasmic bodies. *Neuropediatrics* 1981;12:166–180.
35. Brooke MH, Neville HE. Reducing body myopathy. *Neurology* 1972;22:829–840.
36. Tomé FM, Fardeau M. Congenital myopathy with “reducing bodies” in muscle fibres. *Acta Neuropathol (Berl)* 1975;31:207–217.
37. Bertini E, Salviati G, Apollo F, et al. Reducing body myopathy and desmin storage in skeletal muscle: morphological and biochemical findings. *Acta Neuropathol (Berl)* 1994;87:106–112.
38. Goebel HH, Halbig LE, Goldfarb L, et al. Reducing body myopathy with cytoplasmic bodies and rigid spine syndrome: a mixed congenital myopathy. *Neuropediatrics* 2001;32:196–205.
39. Sabatelli M, Bertini E, Ricci E, et al. Peripheral neuropathy with giant axons and cardiomyopathy associated with desmin type intermediate filaments in skeletal muscle. *J Neurol Sci* 1992;109:1–10.
40. Schröder JM, Sommer C, Schmidt B. Desmin and actin associated with cytoplasmic bodies in skeletal muscle fibers: immunocytochemical and fine structural studies, with a note on unusual 18- to 20-nm filaments. *Acta Neuropathol (Berl)* 1990;80:406–414.
41. Mercuri E, Talim B, Moghadaszadeh B, et al. Clinical and imaging findings in 6 cases of congenital muscular dystrophy with rigid spine syndrome linked to chromosome 1p (RSMD1). *Neuromuscul Disord* 2002;12:631–638.
42. Petit N, Lescure A, Rederstorff M, et al. Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. *Hum Mol Genet* 2003;12:1045–1053.
43. Lazarides E. Intermediate filaments as mechanical integrators of cellular space. *Nature* 1980;283:249–256.
44. Fridén J, Sjöström M, Ekblom B. Muscle fibre type characteristics in endurance trained and untrained individuals. *Eur J Appl Physiol* 1984;52:266–271.