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Restrictive Cardiomyopathy, Atrioventricular Block and Mild to Subclinical Myopathy in Patients With Desmin-Immunoreactive Material Deposits

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Objectives. **We present clinical data and heart and skeletal muscle biopsy findings from a series of patients with ultrastructural accumulations of granulofilamentous material identified as desmin.**

Background. **Desmin cardiomyopathy is a poorly understood disease characterized by abnormal desmin deposits in cardiac and skeletal muscle.**

Methods. **Clinical evaluation, endomyocardial and skeletal muscle biopsy, light and electron microscopy and immunohistochemistry were used to establish the presence of desmin cardiomyopathy.**

Results. **Six hundred thirty-one patients with primary cardiomyopathy underwent endomyocardial biopsy (EMB). Ultrastructural accumulations of granulofilamentous material were found in 5 of 12 biopsy samples from patients with idiopathic restrictive cardiomyopathy and demonstrated specific immunoreactivity with anti-desmin antibodies by immunoelectron microscopy. Immunohistochemical findings on light microscopy were nonspecific because of a diffuse intracellular distribution of desmin. All five patients had atrioventricular (AV) block and mild or subclinical myopathy. Granulofilamentous material was present in skeletal muscle biopsy samples in all five patients, and unlike the heart biopsy samples, light microscopic immunohistochemical analysis demonstrated characteristic subsarcolemmal desmin deposits. Two patients were first-degree relatives (mother and son); another son with first-degree AV block but without myopathy or cardiomyopathy demonstrated similar light and ultrastructural findings in skeletal muscle. Electrophoretic studies demonstrated two isoforms of desmin—one of normal and another of lower molecular weight—in cardiac and skeletal muscle of the familial cases.**

Conclusions. **Desmin cardiomyopathy must be considered in the differential diagnosis of restrictive cardiomyopathy, especially in patients with AV block and myopathy. Diagnosis depends on ultrastructural examination of EMB samples or light microscopic immunohistochemical studies of skeletal muscle biopsy samples. Familial desminopathy may manifest as subclinical disease and may be associated with abnormal isoforms of desmin.**

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Disorders of cardiac and skeletal muscle may be associated with the deposition of excessive amounts of desmin (1), a polypeptide that is normally aggregated to form intermediate filaments (2). These filaments derive their name from their diameter (8 to 10 nm), which is intermediate between that of the myosin filaments (14 nm) and that of the actin filaments (5 to 7 nm). The intermediate filaments normally form transversely oriented connections linking the Z disks of myofibrils to those of adjacent myofibrils, the sarcolemma and the nuclear membranes (1,3–5). Recent studies in transgenic mice (6) suggest that desmin plays a critical role in cardiomyocyte structure and function, indicating that desmin filaments constitute an important component of the cytoskeleton of cardiac myocytes. Pathologic studies of desmin in human cardiac and neuromuscular disorders have reported markedly increased amounts of this intermediate filament protein (7). In a recent review, Goebel (7) classified desmin-related disorders into two major groups—the granulofilamentous type and the cytoplasmic inclusion type—according to the ultrastructural characteristics.

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Abbreviations and Acronyms

AV = atrioventricular
CK = creatine kinase
EMB = endomyocardial biopsy
LDH = lactate dehydrogenase
NADH = nicotinamide adenine dinucleotide, reduced form

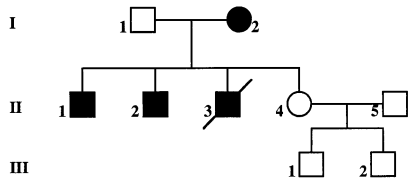
In the present report we present clinical, ultrastructural, immunohistochemical and biochemical findings in six Italian patients with deposits of desmin-immunoreactive material in cardiac and skeletal myocytes and a disorder manifested by restrictive cardiomyopathy (n = 5), atrioventricular (AV) block (n = 6) and variable degrees of skeletal myopathy (n = 6). We also analyzed previous reports of clinical and pathologic findings in patients with similar desmin deposits in the heart.

Methods

Patients. From January 1985 to December 1995, 631 patients affected by primary cardiomyopathies (dilated in 601, hypertrophic with late dilative-congestive evolution in 18, restrictive in 12) underwent EMB. Patients with secondary cardiomyopathy (e.g., amyloidosis, hemochromatosis) were not included in the series. Five (42%) of the 12 patients with restrictive cardiomyopathy who underwent EMB, and 1 other patient who underwent skeletal muscle biopsy only, were found to be affected by desmin storage cardiomyopathy. In three of these patients (Patients 1, 2 and 3) the disease was considered to be sporadic; the other three (Patients 4, 5 and 6) were from the same family (Fig. 1).

Right ventricular EMB (Caves-Shultz bioptome; three to four samples for light microscopy and one for electron microscopy) and quadriceps femoris biopsies (1 × 1 × 0.5 cm) from each of the patients were used for histopathologic, immunohistochemical and electron microscopic studies. Unfixed, frozen portions of the skeletal muscle biopsy specimens from patients 2 to 6 were used for enzyme histochemical studies; frozen samples (heart tissue from Patients 2, 4 and 5 and skeletal muscle from Patients 2 and 4 to 6) were used for biochemical studies. Cardiac and skeletal muscle tissue from 10 patients who had undergone heart transplantation for dilated (n = 6) and hypertrophic cardiomyopathy (n = 4) were used as control samples for immunohistochemical and Western blot studies; our entire series was used as the control for the ultrastructural study.

Figure 1. Family tree. The disorder appears as inherited, with an autosomal dominant pattern. I2 corresponds to Patient 4, II1 to Patient 5 and II2 to Patient 6; II3 died soon after birth; II4 was unaffected on skeletal muscle biopsy.



Histopathologic study. For histopathologic and immunohistochemical studies, the tissues were fixed in buffered 10% formalin, dehydrated rapidly (8) and embedded in paraffin. The samples were serially cut to obtain 45 sections, each 5 μm thick, that were mounted on 15 slides. Sections taken at every 75-μm cutting depth were stained with hematoxylin-eosin and with the Movat pentachrome method. Single sections were stained with Mallory trichrome, Congo red and the periodic acid-Schiff, with and without predigestion by diastase. The remaining sections were used for the immunohistochemical studies. Frozen sections were cut at a thickness of 10 μm and collected on silanized slides. These sections were stained by a modified Gomori trichrome method, standard methods for the demonstration of the activity of cytochrome oxidase, reduced form of nicotinamide-adenine dinucleotide (NADH) dehydrogenase and acid phosphatase (Bio-Optica staining kits, Bio-Optica, Milan, Italy) and were also used for some of the immunohistochemical stains (see later).

Electron microscopic study. For electron microscopy, the tissues were fixed with Karnovsky's fixative, postfixed with 1.5% OsO_4 in 0.2 mol/liter cacodylate buffer, pH 7.3, dehydrated and embedded in Epon-Araldite resin. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Zeiss 902 electron microscope.

Light microscopic immunohistochemical study. For light microscopic immunohistochemical studies, sections of formalin-fixed, paraffin-embedded tissues were reacted with antibodies against desmin (ZC18, Zymed, prediluted; D8281, Sigma, 1:20), vimentin (V9, Dako, Denmark, 1:10) and isotypes of skeletal, cardiac and smooth muscle actin (HHF35, Enzo Diagnostic, 1:10), using the avidin-biotin complex/peroxidase method and diaminobenzidine tetrahydrochloride as chromogen. Frozen sections postfixed with acetone/chloroform were used to demonstrate dystrophin (NCL-DYS1, NCL-DYS2 and NCL-DYS3, respectively, directed against the rod domain, the carboxy terminal and the amino terminal domain of dystrophin [Novocastra, Newcastle, UK; 1:20]). Appropriate positive (from 10 heart transplant donors) and negative (sections incubated without the primary antibody or with an irrelevant primary antibody) control procedures were performed in conjunction with all reactions. In addition, comparisons were made with the staining patterns observed in similarly processed sections of myocardial biopsy specimens from 10 patients with dilated cardiomyopathy.

Electron microscopic immunocytochemical study. Electron microscopic immunocytochemical demonstration of desmin on ultrathin sections was performed according to previously standardized methods (9). Immunohistochemical control procedures for this method were similar to those described for the light microscopic immunohistochemical reactions.

Western blots. One-dimensional electrophoretic studies were made of samples of cardiac muscle from Patients 2, 4 and 5 and skeletal muscle samples from Patients 2 and 4 to 6, using a 5% to 15% polyacrylamide linear gradient gel (10). The tissues were homogenized with 2 volumes of a solution containing 2.3% sodium dodecyl sulfate, 10% glycerol, 5% mercaptoethanol and 6.25 mmol/liter Tris/HCl buffer, pH 6.8, and incubated for 5 min at 95°C. Ten microliters of the extract from each sample were run on polyacrylamide gels; the protein-loading concentration, determined in accordance with Bradford's procedure (11), was 30 μg for each lane. After electrophoresis, the gels were transferred to nitrocellulose sheets (12) that were immunostained with mouse monoclonal primary antibody against desmin and a secondary antibody (goat anti-mouse) conjugated with alkaline phosphatase.

Statistical analysis. Comparison of frequencies of desmin accumulation in the groups of patients with restrictive, dilated and hypertrophic cardiomyopathy was performed by the chi-square test.

Results

Case reports. All patients in this series were born of normal pregnancies and deliveries to healthy, nonconsanguineous parents. In all patients, motor and intellectual development was normal.

Patient 1. A 17-year old boy was referred in 1989 for evaluation of restrictive cardiomyopathy (New York Heart Association functional class II) associated with second-degree (Mobitz type 2) AV block that required implantation of a pacemaker. There was no family history of cardiac or neuromuscular disease. Chest radiography showed cardiac enlargement. Echocardiographic study and cardiac catheterization revealed a restrictive pattern of function. A right ventricular EMB was performed. Serum levels of lactate dehydrogenase (LDH) and creatine kinase (CK) were normal. Neurologic evaluation was normal; however, electromyographic study revealed low amplitude motor unit potentials of short duration with an early recruitment pattern in the quadriceps femoris and deltoid muscles. Biopsy of the right quadriceps muscle was performed. In 1992 he developed complete AV block. The gluteus medius, tibialis anterior and peroneal muscles were weak, but the strength of other muscles was normal. The patient died in June 1993 while awaiting heart transplantation. A postmortem examination was not performed.

Patient 2. A 21-year old woman was referred in 1992 for evaluation and management of symptomatic second-degree (Mobitz type 2) AV block, right bundle branch block and left anterior hemiblock. The parents, one stepsister and one sister were clinically normal. A 30-year old paternal uncle had died suddenly of unknown cause. Chest radiography showed borderline cardiomegaly. Echocardiographic study showed very mild restrictive ventricular dysfunction and left atrial dilation. Neurologic examination showed bilateral ptosis of the eyelids and mild bilateral weakness of the extensor digitorum longus. Electromyographic study, hearing tests, measurements of serum levels of LDH and CK and evaluation of endocrine functions gave normal results. Right ventricular EMB and a quadriceps femoris muscle biopsy were performed. The diagnosis of Kearns-Sayre syndrome was excluded by the morphologic findings and by the lack of large-scale deletions or insertions in the mitochondrial DNA from the muscle biopsy. A pacemaker was implanted. The patient's clinical status has remained unchanged during a 36-month period of follow-up.

Patient 3. A 33-year old woman was referred for evaluation of restrictive cardiomyopathy (function class III). There was no family history of heart or neuromuscular disease. At the age of 14 years, in 1975, the patient was found to have elevated ventricular filling pressures, complete AV block and mild mitral regurgitation. She was diagnosed as having postmyocarditic cardiomyopathy, and she underwent implantation of a pacemaker. In 1990, the patient began to develop episodes of congestive heart failure. Conventional light microscopic study of a myocardial biopsy, performed at another hospital in 1992, described nonspecific changes of myocardial hypertrophy. On referral to our hospital in January 1995, she had dilated atria, a normal-sized hypokinetic left ventricle, severe pulmonary hypertension (65 mm Hg) and a small pericardial effusion. Serum levels of LDH and CK were at the upper limits of normal. Neurologic examination showed generalized muscle wasting, with more selected weakness of the distal muscle. The patient underwent quadriceps femoris muscles biopsy and a second EMB at our institution. She died 3 months later, while awaiting heart transplantation. Postmortem examination was not performed.

Patient 4. A 27-year old man was referred for heart transplantation because of end-stage restrictive cardiomyopathy and cardiac failure refractory to medical therapy. His mother (Patient 5) and a 28-year old brother (Patient 6) were also referred for evaluation. A 24-year old sister was also evaluated clinically and underwent skeletal muscle biopsy. Another brother had died suddenly 10 h after birth. At age 8 the patient had a syncopal episode and was found to have right bundle branch block. At age 22, he underwent implantation of a pacemaker for complete AV block. At age 24 he developed cardiac failure with a low output syndrome. Cardiac catheterization and echocardiographic study showed a normal-sized left ventricle with a restrictive pattern of dysfunction. Neurologic examination, including electromyography and repetitive nerve stimulation tests, disclosed no abnormalities. Serum levels of LDH and CK were normal. The patient had EMB and biopsy of the quadriceps femoris muscle. He underwent heart transplantation.

Patient 5. The 55-year old mother of Patients 4 and 6 developed palpitations and cardiac enlargement at age 23. At age 35, she had a pacemaker implanted because of complete AV block and left posterior hemiblock. Cardiac failure first developed at age 48. At age 54 she was referred for evaluation of continuing episodes of cardiac failure. An echocardiogram showed right ventricular dilation and normal left ventricular motion. Cardiac catheterization disclosed elevated filling pressures and a pattern of restrictive dysfunction. Neurologic

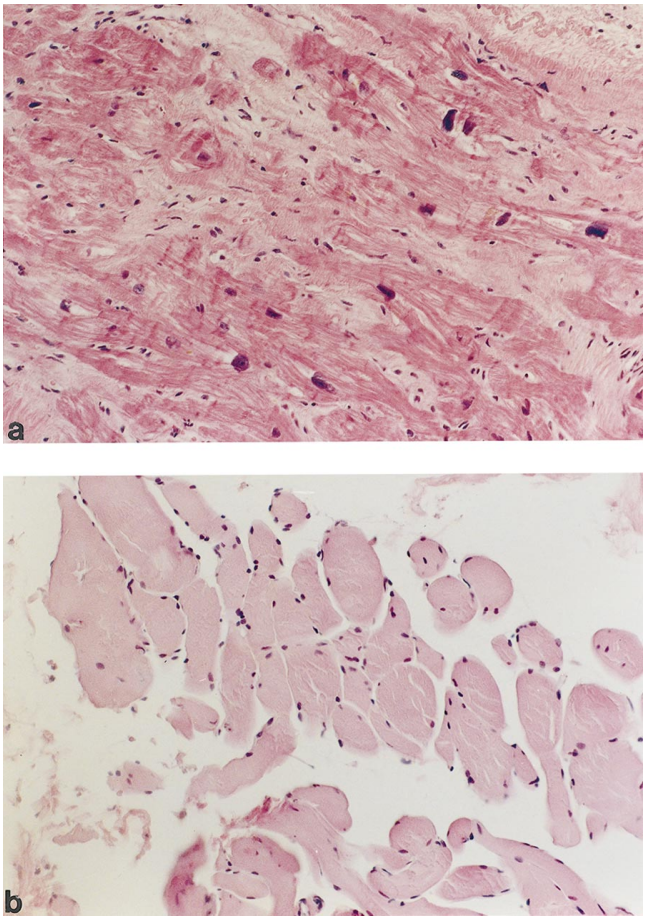


Figure 2. Histologic sections of heart and skeletal muscle. **a,** Myocardium from Patient 5, showing mild interstitial fibrosis and focal myocyte hypertrophy, with prominent nuclei and minimal disarray. **b,** Skeletal muscle from Patient 4. Variations in fiber size are evident. Hematoxylin-eosin ×250, reduced by 42%.

examination, including electromyography and repetitive nerve stimulation tests, disclosed no abnormalities. Serum levels of LDH and CK were normal. The patient had EMB and quadriceps femoris muscle biopsy. She underwent heart transplantation.

Patient 6. This 28-year old man, son of Patient 5 and brother of Patient 4, was asymptomatic and clinically normal but had first degree AV block. Neurologic examination was normal. Echocardiographic and scintigraphic studies gave normal results. Right ventricular EMB, performed at another institution, was reported as showing myocyte hypertrophy and interstitial fibrosis (no electron microscopic or immunohistochemical study was made). Needle biopsy of the quadriceps femoris muscle was performed at our institution.

Histologic observations. *Heart.* Histologic study of the EMB specimens (Fig. 2a) from five (Patients 1 to 5) of the six patients revealed only variable degrees of myocyte hypertrophy and fine, diffuse interstitial fibrosis (minimal in Patient 2, moderate in Patients 1, 3, 4 and 5). Endocardial thickening, myocardial inflammation and vascular abnormalities were not present. Minimal disarray of the myocytes was noted in all patients. Nuclei with bizarre shapes were present in some of the myocytes from Patients 4 and 5. The myofibrils were focally lysed in some cells from Patients 1, 3, 4 and 5. However, no changes suggestive of any storage disease were observed in any of the specimens. Amyloid deposits and accumulations of abnormal amounts of glycogen were not detected.

Skeletal muscle. Examination of the quadriceps femoris muscle showed mild fibrosis in Patients 1, 3, 4 and 5 (Fig. 2b). Frozen sections (Patients 2 to 6) stained by the Gomori trichrome method showed peripherally located sarcoplasmic masses that stained deep green. Ragged red fibers were not found. Areas devoid of NADH diaphorase activity, such as those found in central core disease, were not detected. Stain for acid phosphatase showed mildly increased reactivity in Patients 2 and 5, mostly in subsarcolemmal areas.

Ultrastructural observations. *Heart.* Ultrastructural examination of the five EMB specimens showed multiple deposits of granulofilamentous and electron-dense amorphous material in perinuclear, subsarcolemmal and intermyofibrillar areas (Fig. 3). These deposits were not enclosed by limiting membranes. In Patients 2 and 4 they also were distributed in register with the Z bands, with which they often appeared to be continuous. The extent to which the deposits were present varied among different cardiac myocytes and among the five patients (mild in Patient 2, moderate in Patients 4 and 5, severe in Patients 1 and 3). The mitochondria showed nonspecific abnormalities. None of the remaining control biopsies investigated at the electron microscopy level showed similar ultrastructural features.

Skeletal muscle. In all six patients the skeletal muscle cells contained deposits that were morphologically identical to those found in the cardiac muscle cells (Fig. 4). They were almost exclusively subsarcolemmal, rarely extending between the myofibrils. Endothelial cells, fibroblasts and nerves did not contain similar deposits. The deposits were most abundant in the biopsy specimens from Patients 1 and 3 and progressively less extensive in Patients 4, 5, 2 and 6. The skeletal muscle mitochondria were morphologically normal in all six patients. The skeletal muscle biopsy of the sister of Patients 4 and 6 was normal.

Light microscopic immunohistochemical observations. *Heart.* Staining for desmin (Fig. 5a–c) showed a pattern of immunoreactivity that corresponded to the distribution of the deposits observed by transmission electron microscopy. In many cells (>50%) the pattern of reactivity was irregular but was associated with the pattern of staining at the levels of the Z bands, as usually seen in idiopathic dilated cardiomyopathy (Fig. 5b).

Skeletal muscle. Study of skeletal muscle confirmed the presence of desmin-immunoreactive subsarcolemmal deposits (Fig. 5d). As in cardiac muscle, the distribution of the reactivity for desmin matched that of the material observed by transmission electron microscopy. Immunostaining for dystrophin gave normal results in all cardiac and skeletal muscle biopsy specimens. The skeletal muscle samples of our 10 control cases and

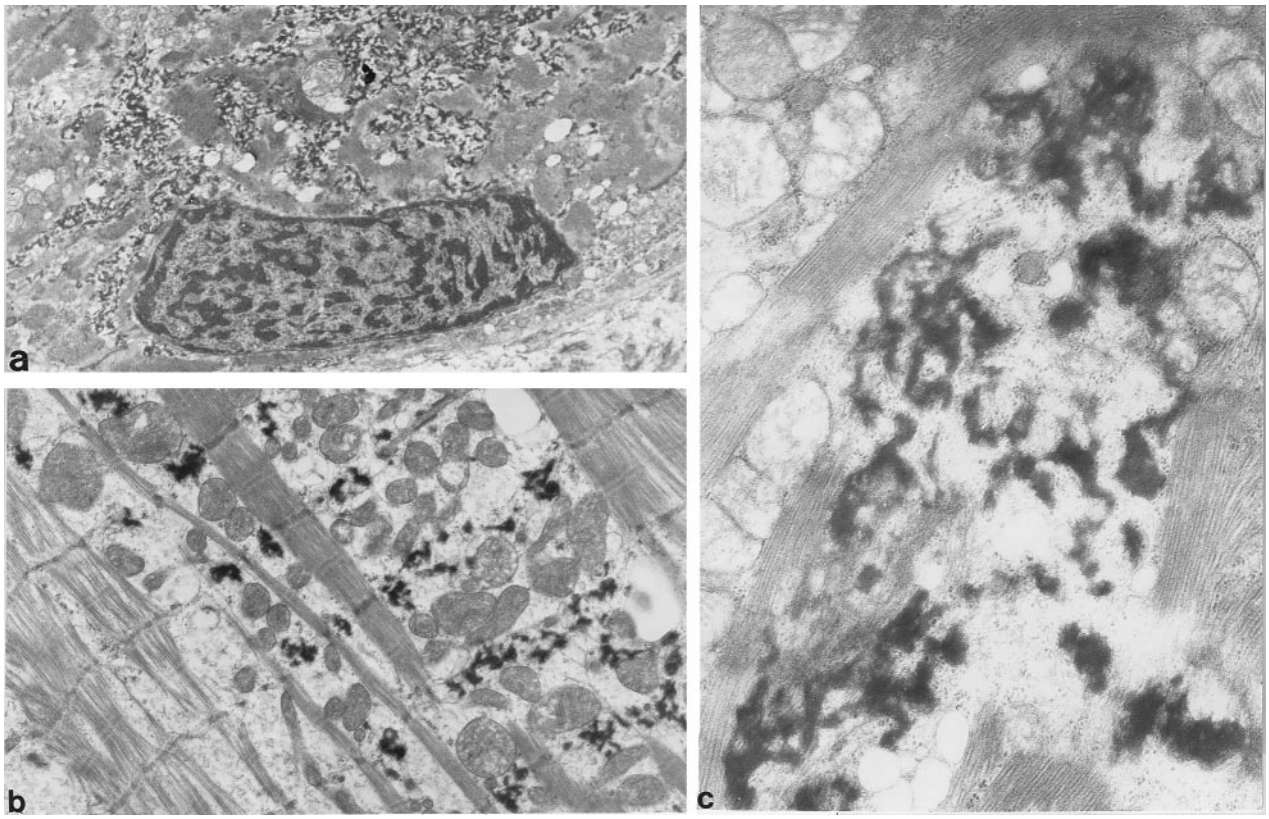


Figure 3. Transmission electron micrographs of cardiac muscle. **a,** Myocyte from Patient 4 contains multiple deposits of granulofilamentous material in intermyofibrillar areas. **b,** Longitudinal sections of myocyte from Patient 5, showing localization of deposits of granulofilamentous material at the levels of the Z bands. **c,** Higher magnification view of myocyte from Patient 5, showing aggregates of granulofilamentous material and intermyofibrillar bundles of intermediate filaments, some of which are attached to Z bands. **a** (×7,000), **b** (×12,000), **c** (×13,000), all reduced by 41%.

of the sister of Patients 4 and 6 did not show abnormal desmin immunoreactivity.

Ultrastructural immunocytochemical observations. Ultrastructural immunocytochemical study of cardiac (Fig. 6a) and skeletal (Fig. 6b) muscle sections showed a specific reactivity of anti-desmin antibody with the material described earlier.

Electrophoretic study of desmin. One-dimensional gel electrophoresis of skeletal and cardiac muscle from Patient 2 showed a single band of desmin reactivity (identified by specific immunostaining) of 55 kilodaltons (kDa). This band had the same molecular weight but was thicker than those obtained from the skeletal muscle and from the cardiac muscle of normal control subjects and patients with other types of cardiomyopathies. Two bands with desmin reactivity were found in all samples of skeletal muscle and heart from Patients 4, 5 and 6, who belong to the same family. One of the bands had a normal molecular weight (55 kDa), whereas the second band corresponded to a smaller isoform of desmin, with 53-kDa molecular weight. These observations are shown in Figure 7.

Statistical analysis. Cases of desmin accumulation were significantly higher in restrictive than in other cardiomyopathy types (chi-square 260.81, 2 degrees of freedom, $p = 0.000$).

Discussion

Characteristics of desmin cardiomyopathy. The present study describes the finding of granulofilamentous, desmin-immunoreactive material in a series of patients with restrictive cardiomyopathy, disturbances of AV conduction and skeletal myopathy of variable onset and severity. These pathologic findings are characteristic of desmin-related disorders, or desmin cardiomyopathy.

Desmin cardiomyopathy, unlike other forms of cardiomyopathy, requires ultrastructural study for diagnosis. By light microscopy, the excess of desmin microfilaments is found throughout the sarcoplasm. Therefore, the immunohistochemical findings are not sufficiently different from the abnormal staining patterns seen in dilated and hypertrophic cardiomyopathy (13–15). However, ultrastructural observations in desmin cardiomyopathy are distinct from those of other forms of cardiomyopathy and consist of striking granulofilamentous deposits that we did not identify in any cases of dilated cardiomyopathy. The clinical findings of desminopathy are likewise distinct, supporting the concept that desmin cardiomyopathy is a separate clinicopathologic entity. Restrictive hemodynamic variables and AV block, characteristic of desmin cardiomyopathy, are not consistent features of hypertrophic

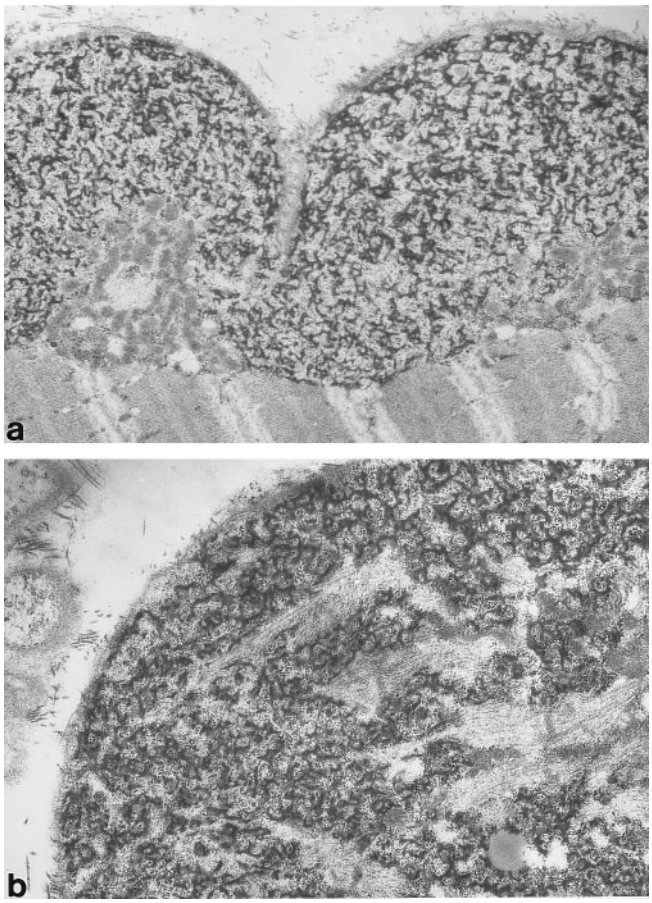


Figure 4. Transmission electron micrographs of quadriceps femoris muscle from Patient 4. Low **(a)** and high **(b)** power views of muscle fiber showing large deposits of granulofilamentous material in subsarcolemmal areas. **a**: ×7,000, **b**: ×12,000, both reduced by 36%.

cardiomyopathy and are not found in dilated cardiomyopathy. We hypothesize that the failure of desmin to aggregate into intermediate filaments, as indicated by the observed masses of granulofilamentous material, is responsible for the structural disorganization of the cytoskeleton of the myocytes, which in turn leads to impairment of both relaxation and contraction and results in the abnormal diastolic and systolic function that characterizes restrictive cardiomyopathy.

Although the light microscopic features of cardiac biopsy specimens in patients with desmin cardiomyopathy are nonspecific, our data suggest that immunohistochemical distribution of desmin filaments in skeletal muscle biopsy samples is characteristic of the disease. We demonstrated typical desmin-immunoreactive deposits localized in the subsarcolemmal areas with a crescentic, semilunar distribution. Because diagnostic findings were present in all six patients, biopsy of skeletal muscle may be helpful, even in the absence of clinical myopathy.

Review of published reports of desmin abnormalities in cardiomyopathy. In a review of published reports, we identified 26 patients with desmin cardiomyopathy on the basis of ultrastructural presence of granulofilamentous material deposits in cardiac or skeletal muscle myocytes, or both (16–40). In a few cases, this material was identified as desmin by direct immunocytochemical characterization (38–40); in the others, either desmin-immunoreactive deposits were observed on light microscopy immunohistochemical study of the same bioptic samples (21,22,24,27–37) or abnormal desmin-immunoreactive bands were demonstrated on Western blot of cardiac or skeletal muscle tissue (18,22,29,32). In a limited number of cases reported before the development of immunohistochem-

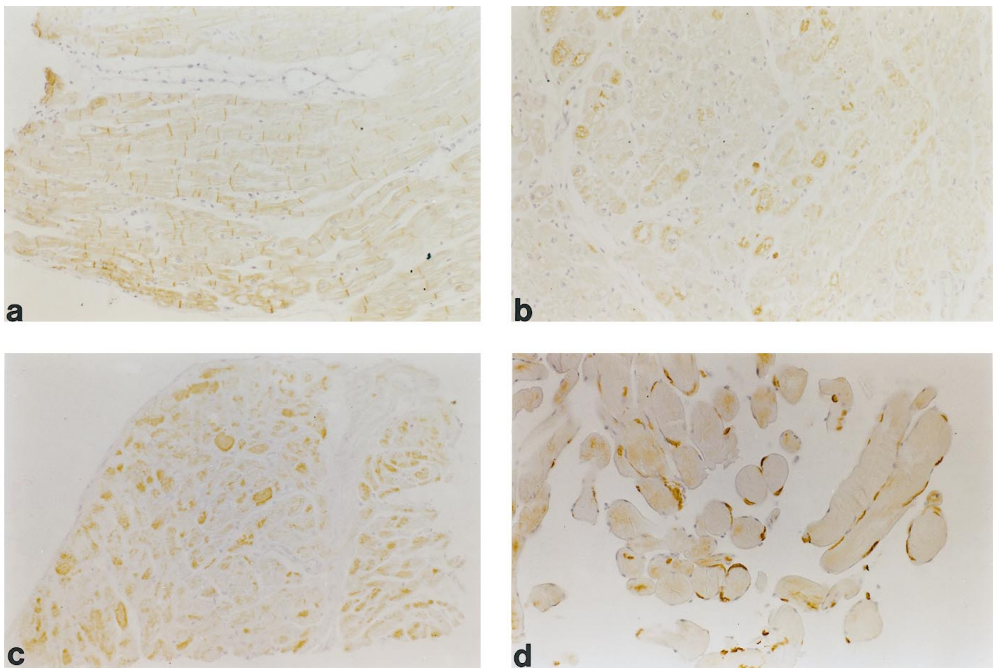


Figure 5. Light micrographs of immunohistochemically stained (avidin-biotin complex/peroxidase; nuclei were counterstained with hematoxylin) preparations; ×250, reduced by 40%. **a,** Normal pattern of distribution of desmin in pretransplant biopsy of donor heart. The reaction is localized along the Z bands and the intercalated disks. **b,** In cardiac myocytes of a patient with dilated cardiomyopathy, focal accumulations of desmin are present, but their morphology is not sufficiently distinctive to differentiate them from those shown in Figure 4c. **c,** Myocardium from Patient 2 shows marked increase in immunoreactivity for desmin, which is localized in irregular masses. **d,** Subsarcolemmal deposits of desmin-immunoreactive material are present in skeletal muscle fibers from Patient 4. Compare with Figure 4.

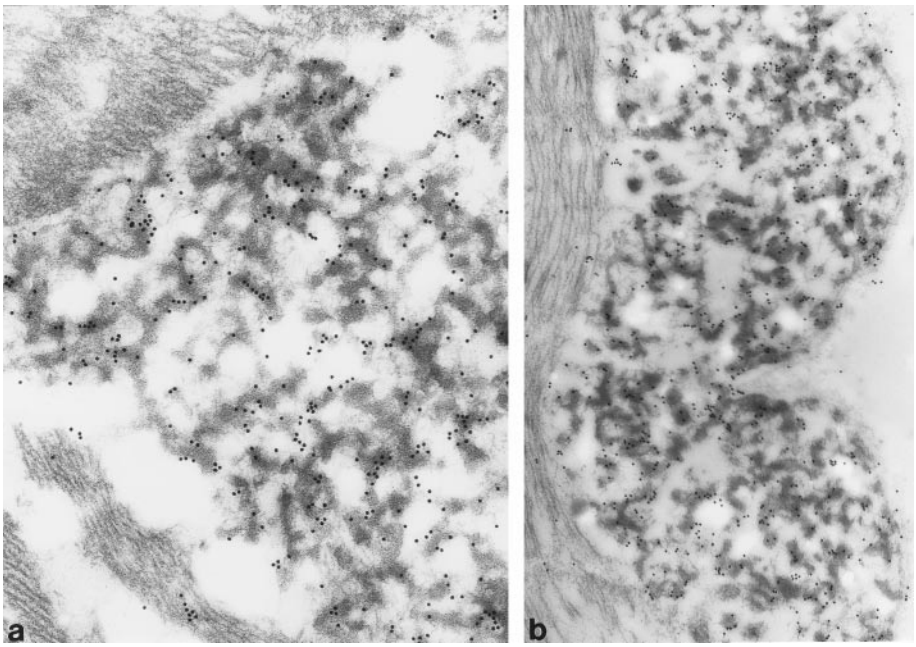
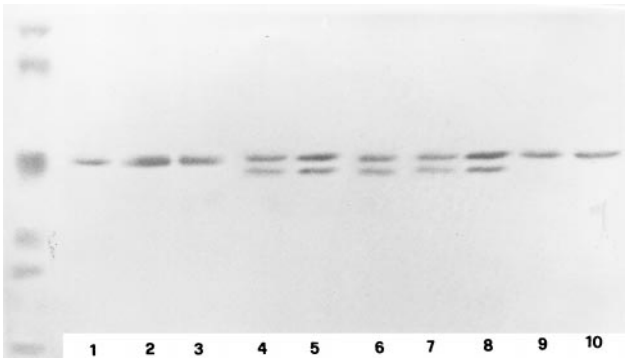


Figure 6. Transmission electron micrograph of sections stained to show the ultrastructural localization (gold-labeled antibody method) of desmin. Myocardium (**a**) and skeletal muscle (**b**) from Patient 5 show deposits of granulofilamentous material that are intensely labeled (desmin) by gold particles. ×14,000, reduced by 40%.

istry, unequivocal granulofilamentous deposits were not further characterized as desmin (16,25). Of these cases, restrictive cardiomyopathy was present in nine (definite in six, presumed from the clinical description in three) (16,19,21,25,26,29,32–34,40), hypertrophic cardiomyopathy in two (17,19,21), dilated cardiomyopathy in one (26), cardiomyopathy not further specified in eight (19,28,31,36–39), not otherwise specified congestive heart failure with pulmonary hypertension in two (22,24) and only AV conduction disturbances in four (17,35). Although most of the 26 patients described had some form of AV block, the structure of the AV conduction system was studied in only one patient who had undergone transplantation (28), in whom the desmin deposits formed well defined intermediate filaments and involved the bundle of His and its branches (especially the left) to a much greater extent than the atrial and ventricular myocardium. Because the conduction pathways were not interrupted, the AV block was attributed to the deposition of desmin in the conduction cells.

Figure 7. Nitrocellulose sheet immunostained with mouse monoclonal antibody against desmin. The protein loading concentration was 30 g for each lane. MW = molecular weight. **Lane 1,** normal control (normal donor heart); **lane 2,** heart from Patient 2; **lane 3,** skeletal muscle from Patient 2; **lane 4,** heart from Patient 4; **lane 5,** skeletal muscle from Patient 4; **lane 6,** heart from Patient 5; **lane 7,** skeletal muscle from Patient 5; **lane 8,** skeletal muscle from Patient 6; **lanes 9 and 10,** hearts from two patients with dilated cardiomyopathy.



Although most patients reported in the published reports had clinically overt myopathy, the age at onset, pattern of involvement of muscle groups and severity were all variable. In our patients, only one of six patients had early symptoms of muscular impairment, and these were mild. In three others, skeletal myopathy became evident only very late in the disease.

Diagnostic criteria. The prevalent restrictive pattern in affected hearts described in the published reports, as well as in our series, suggests that the combination of restrictive cardiomyopathy and disturbances of the AV conduction should raise the possibility of a desmin-related disorder. The diagnosis needs confirmation by immunohistochemical studies for desmin and electron microscopic studies. Ultrastructural analysis constitutes the only means for identifying this type of disorder on EMB samples. If, for clinical reasons, a skeletal muscle biopsy is available and shows features diagnostic for desminopathy in a patient who also has restrictive cardiomyopathy or AV conduction disturbance, an EMB is not necessary for diagnosis.

Biochemical considerations. In the present study, we demonstrated by immunogold labeling that the abnormal filaments seen ultrastructurally are composed of desmin myofilaments. By one-dimensional gel electrophoresis, we demonstrated an increase in desmin band thickness in one patient with sporadic disease, a finding similar to that reported by Rappaport et al. (18), Bertini et al. (30), Sabatelli et al. (32), Edstrom et al. (22) and Baeta et al. (39). To our knowledge, we demonstrated for the first time by one-dimensional gel electrophoresis, the coexistence of two isoforms of desmin—one of normal molec-

ular weight, compared with that of the control subjects and in accordance with previous biochemical characterization, and the second of 53-kDa molecular weight. This isoform was demonstrated only in the three related patients with familial desmin cardiomyopathy. Phosphorylated acidic isovariants of desmin have been described in patients with desmin cardiomyopathy (18,30,32), an interesting finding given that the ability of desmin to become organized into intermediate filaments is inversely related to the degree to which this protein is phosphorylated (41,42). However, by one-dimensional gel electrophoresis these isovariants resulted in a single band of normal molecular weight and increased thickness compared with that in control subjects (18,30,32). Although the presence of abnormal isoforms of desmin suggests a genetic defect, none has been detected by sequencing of desmin cDNA or genetic linkage studies in three families (43). It is possible that the genetic defect lies in a second protein that interacts with the transcription or translation of the desmin gene or with the protein itself (43). The significance of our finding of an abnormal isoform of desmin has yet to be clarified.

Conclusions. Desmin cardiomyopathy is characterized by restrictive cardiomyopathy, AV conduction disturbances and myopathy that is histologically manifest early but clinically only later in the course of disease. A desminopathy should be suspected in patients presenting with restrictive cardiomyopathy, especially when combined with AV block. The diagnosis is confirmed by accumulations of desmin-immunoreactive material in cardiac muscle, which must be confirmed ultrastructurally, and in skeletal muscle, which may be seen on light microscopic immunohistochemical analysis. Optimally, immunohistochemical and ultrastructural studies of heart and skeletal muscle are both necessary because they constitute the best means of distinguishing the deposits of desmin-immunoreactive material from those that occur as nonspecific cytoskeletal abnormalities secondary to cardiomyopathy associated with cardiac dilation or hypertrophy.

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