

**BRIEF REPORT: DEFICIENCY OF A DYSTROPHIN-ASSOCIATED GLYCOPROTEIN (ADHALIN) IN A PATIENT WITH MUSCULAR DYSTROPHY AND CARDIOMYOPATHY**

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**C**ARDIAC muscle is commonly affected in muscular dystrophies.<sup>1-4</sup> X-linked Duchenne's muscular dystrophy and Becker's muscular dystrophy are caused by mutations in the gene encoding dystrophin,<sup>5,6</sup> a membrane cytoskeletal protein.<sup>7</sup> In skeletal and cardiac muscle, dystrophin is associated with a large oligomeric complex of sarcolemmal glycoproteins.<sup>8,9</sup> This dystrophin-glycoprotein complex is composed of five glycoproteins (35, 43 doublet, 50, and 156 kd) and two proteins (25 and 59 kd). It spans the sarcolemma to provide linkage between the subsarcolemmal cytoskeleton and laminin, a major component of the extracellular matrix.<sup>9,10</sup>

In Duchenne's and Becker's muscular dystrophies, the primary deficiency in dystrophin is associated with a commensurate secondary reduction in the other dystrophin-associated proteins.<sup>11,12</sup> Disruption of the linkage between the cytoskeleton of the muscle fiber and the extracellular matrix has also been proposed as a factor in three autosomal recessive inherited diseases. In Fukuyama-type congenital muscular dystrophy, recently mapped to chromosome 9q31-33,<sup>13</sup> all the dystrophin-associated proteins, especially the 43-kd dystrophin-associated glycoprotein, have low expression.<sup>14</sup> This disorder is associated with structural abnormalities in the brain.<sup>15</sup>

The second type is merosin-negative congenital muscular dystrophy, which has been linked to chromosome 6q2.<sup>16,17</sup> Finally, severe childhood autosomal recessive muscular dystrophy has been attributed to a deficiency of the 50-kd dystrophin-associated glycoprotein.<sup>18</sup> Severe childhood autosomal recessive muscular dystrophy is prevalent in North Africa, and its clinical presentation is similar to that of Duchenne's and Becker's muscular dystrophies except that both sexes are affected.<sup>19</sup> The 50-kd dystrophin-associated glycoprotein was named "adhalin" from the Arabic *adhal* (muscle), and it has been linked in North African populations to an unidentified gene in the pericentromeric region of chromosome 13q.<sup>20</sup> Adhalin deficiency has also been reported in Europe,<sup>21</sup> Brazil,<sup>22</sup> Japan,<sup>23</sup> and Pakistan.<sup>24</sup> Interestingly,

the adhalin gene has been mapped to chromosome 17q12-21.33,<sup>25,26</sup> and missense mutations in the adhalin gene have been demonstrated.<sup>27</sup> Cardiac abnormalities develop in patients with the disease,<sup>19</sup> but the expression of the dystrophin-associated proteins, including adhalin, in cardiac muscle has not been determined.

We report here on a patient with muscular dystrophy and dilated cardiomyopathy related to adhalin deficiency in both skeletal and cardiac muscle.

**CASE REPORT**

A 13-year-old boy who had previously been given a diagnosis of Becker's muscular dystrophy was referred to the University of Wisconsin Hospital and Clinics for evaluation of dilated cardiomyopathy and congestive heart failure. He was asymptomatic until nine years of age, when proximal muscle weakness developed. Examination at that time showed flexion contracture at the ankles, hypertrophy of the calf muscles, grade 4/5 strength (according to the criteria of the British Medical Research Council) in the hip flexors, and Gowers' sign. The serum creatine kinase level was 11,560 U per liter. There was no family history of consanguinity or neuromuscular disease. Both his sister and his mother had normal serum creatine kinase values.

The results of nerve-conduction studies were normal. Electromyographic examination revealed spontaneous activity and myopathic motor units in both arm and leg muscles. A biopsy of the biceps muscle demonstrated features of muscular dystrophy, including variation in fiber size, degenerating and regenerating fibers, and an increase in endomysial connective tissue. Analysis of dystrophin by Western blotting produced normal results. Clinically, the patient's muscle power continued to deteriorate slowly. A chest radiograph obtained at the age of 13 to evaluate symptoms of persistent cough showed global cardiomegaly. An electrocardiogram revealed sinus tachycardia with an RSR pattern in lead V<sub>1</sub> and normal Q waves in leads I, aVL, V<sub>3</sub>, and V<sub>6</sub>. There were no tall right precordial R waves. Echocardiography demonstrated enlargement of all cardiac chambers and a decreased left ventricular ejection fraction (22 percent).

Examination showed atrophy in the upper portions of the trapezius, the latissimus dorsi, the sternal and pectoral portions of the pectoralis major, and the distal vastus lateralis muscles. There was hypertrophy of the gastrocnemius, extensor digitorum brevis, and adductor hallucis muscles. The patient had 4/5 strength in the deltoids, biceps, wrist flexors, hip flexors and adductors, and foot dorsiflexors and evertors on both sides of the body. There was mild winging of the scapulae. During the next three weeks, his congestive heart failure became refractory to diuretic therapy and to inotropic therapy with dopamine, dobutamine, and milrinone. He required a left ventricular assist device for circulatory support for six weeks and then underwent successful orthotopic heart transplantation.

**METHODS**

**Muscle-Biopsy Specimens**

After informed consent had been obtained, a biopsy of the vastus lateralis muscle was performed with the patient under local anesthesia. All studies were reviewed and approved by the institutional review board. At the time of cardiac transplantation, a sample of the left ventricle was obtained. The specimens were snap-frozen first in isopentane chilled to -150°C and later in liquid nitrogen, and were stored at -80°C. Standard histologic and histochemical techniques were used to prepare 7-µm-thick frozen-muscle sections.<sup>26</sup> Samples were also processed for electron microscopy according to standard methods.<sup>26</sup> Muscles without histochemical abnormalities and muscles from patients with other muscular dystrophies were used as controls.

**Antibodies**

Monoclonal antibodies against the amino-terminus, carboxyl-terminus, and rod domain of dystrophin were obtained from Vector Laboratories (Burlingame, Calif.). A monoclonal antibody against adhalin (IVD3) had been previously characterized.<sup>28</sup> Polyclonal anti-

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Supported in part by a grant from the Muscular Dystrophy Association. Dr. Campbell is an Investigator of the Howard Hughes Institute.

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bodies against syntrophin triplet (59-kd dystrophin-associated protein), beta-dystroglycan (43-kd dystrophin-associated glycoprotein), and 35-kd dystrophin-associated glycoprotein were affinity-purified from sheep antiserum against purified dystroglycan as previously described.<sup>29</sup>

Indirect immunofluorescence microscopy of 7- $\mu$ m-thick cryosections from skeletal- and heart-muscle specimens was performed as described previously.<sup>8,18,28-30</sup> The sections were examined under a Zeiss axioplan fluorescence microscope (Berlin, Germany), and photographs were taken under identical conditions with the same exposure time.

### RESULTS

Light microscopical examination of the biopsy specimen of vastus lateralis muscle showed necrosis and regeneration of muscle fibers, some of which occurred in groups of fibers; increased endomysial fibrosis; splitting of muscle fibers; abnormal variation in muscle-fiber size; an increased number of muscle fibers containing internal nuclei; and an increased number of hypercontracted fibers. Many necrotic fibers were invaded by macrophages. The random distribution of histochemically defined fiber types was preserved. In cardiac muscle, there was an abnormal variation in the sizes of both muscle fibers and nuclei, increased endomysial fibrosis, and in many fibers, a marked increase in acid phosphatase and glycogen, especially in perinuclear vacuoles. There was no clear evidence of necrosis or regeneration of muscle fibers.

Electron microscopy showed breaks in the sarcolemma, with preserved basal laminae, in both skeletal and cardiac muscle (Fig. 1). In cardiac muscle, the most characteristic finding was the presence of autophagic

vacuoles located in perinuclear regions, many of which contained glycogen particles. In skeletal muscle, there were ultrastructural changes corresponding to necrosis and regeneration of muscle fibers, including hypercontracted fibers. In muscle fibers with focal sarcolemmal defects, focal myofibrillar contractures were present adjacent to the breaks in some fibers.

The results of immunostaining in both skeletal and cardiac muscle showed normal reactivity for antibodies directed against the three portions of the dystrophin molecule (Fig. 2). Immunostaining of adhalin was drastically reduced in skeletal muscle, but a trace amount of adhalin was still present. However, in cardiac muscle, adhalin was undetectable by immunofluorescence. Immunostaining of the 35-kd dystrophin-associated glycoprotein was also significantly reduced in skeletal muscle and undetectable in cardiac muscle. It has been shown that adhalin deficiency causes a secondary reduction of 35-kd dystrophin-associated glycoprotein.<sup>18</sup> Immunostaining of other components of the dystrophin-glycoprotein complex, including  $\beta$ -dystroglycan and syntrophin triplet, was normal in both skeletal and cardiac muscle.

### DISCUSSION

Adhalin is deficient in the skeletal muscle of patients with severe childhood autosomal recessive muscular dystrophy.<sup>18</sup> This disease is relatively frequent in countries with a high rate of consanguinity.<sup>19</sup> Cardiomegaly has been described in patients with the disease,

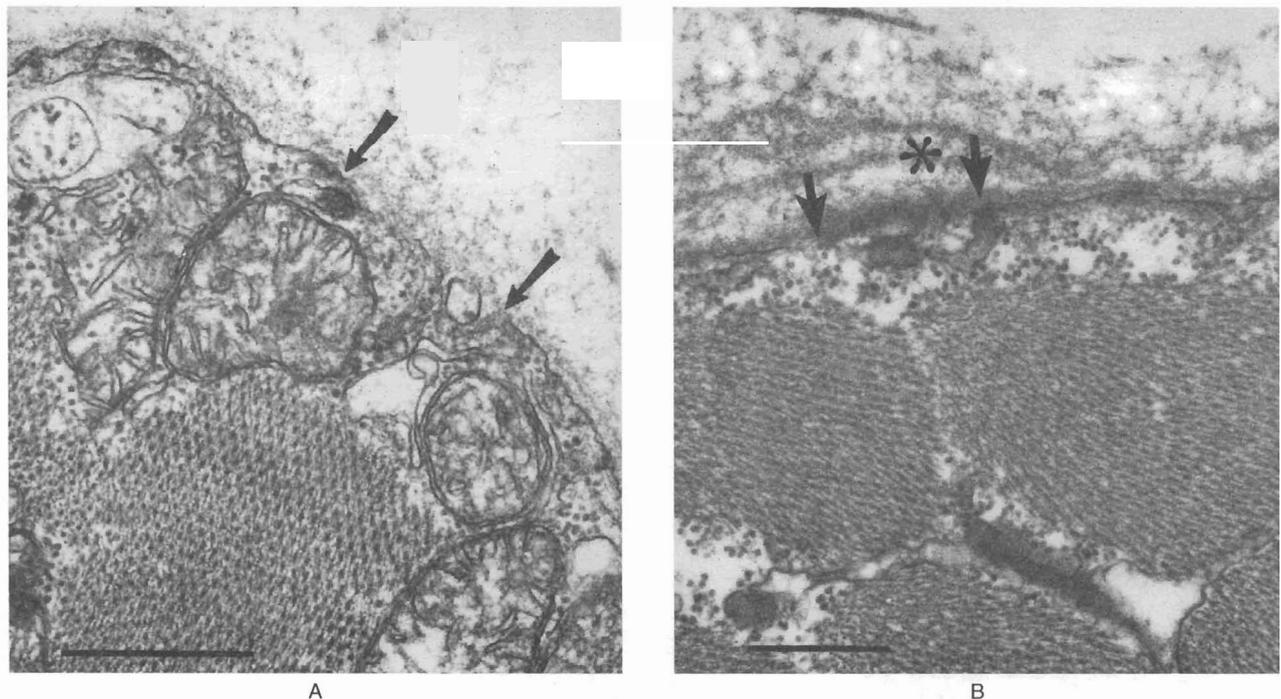


Figure 1. Electron Micrographs of Skeletal Muscle (Panel A) and Cardiac Muscle (Panel B) from the Patient. Sarcolemmal defects can be seen between the arrows. In the fiber regions under the defects, the myofibrils are not contracted. Multiple replications of the basal laminae are seen in relation to the sarcolemmal defect in Panel B (asterisk). The scale bars represent 500  $\mu$ m.

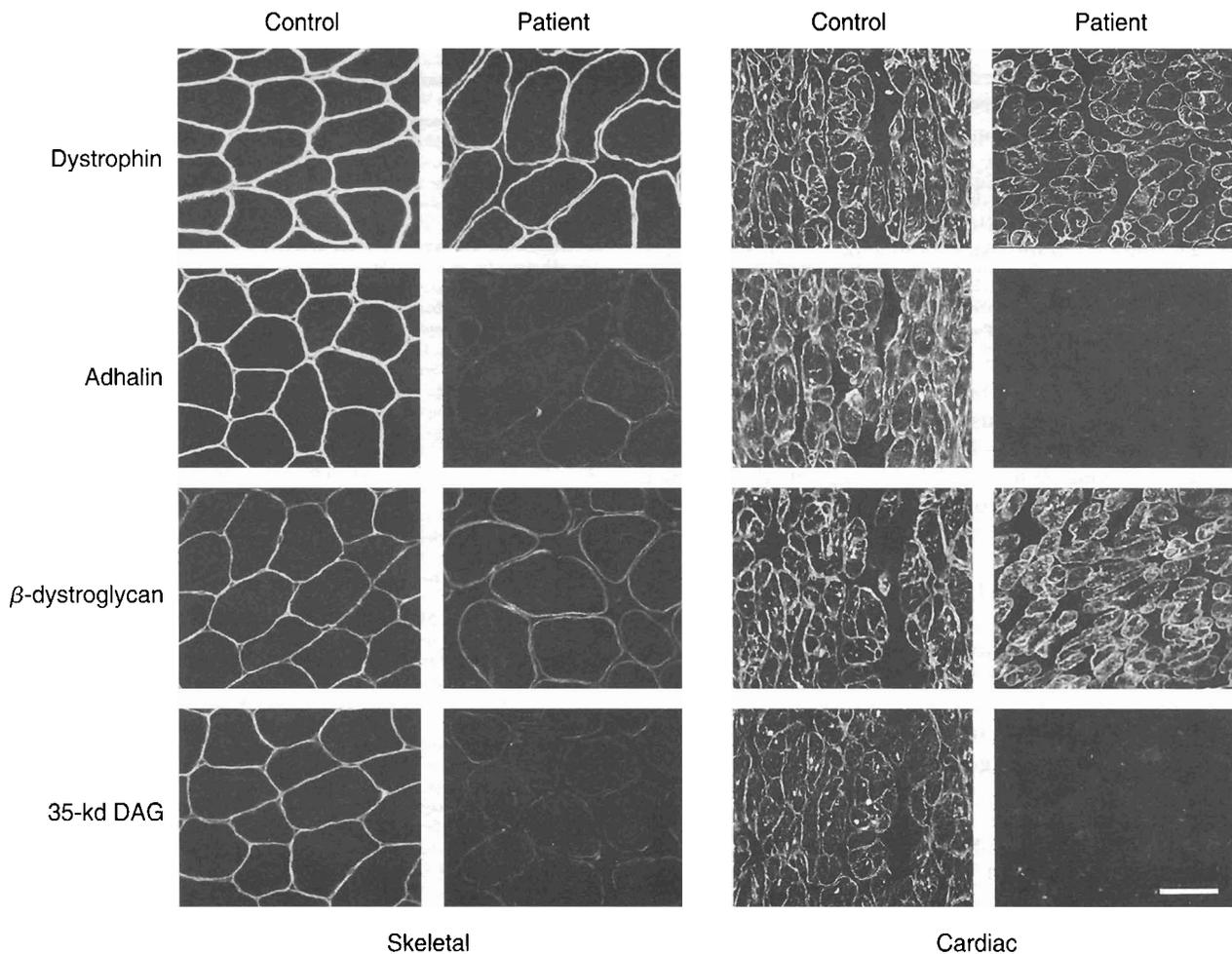


Figure 2. Skeletal and Cardiac Muscle from a Control and from the Patient Immunostained with Antibodies against Dystrophin, Adhalin,  $\beta$ -Dystroglycan, and 35-kd Dystrophin-Associated Glycoprotein (35-kd DAG).

Adhalin staining is dramatically reduced in the patient's skeletal muscle and absent in the cardiac muscle. There is a secondary decrease in staining for 35-kd dystrophin-associated glycoprotein in the patient's skeletal and cardiac muscle. The scale bar represents 500  $\mu$ m.

but our patient is noteworthy because he had end-stage cardiomyopathy related to a deficiency of adhalin in the myocardium. The immunohistochemical findings in the skeletal and cardiac muscle from our patient are similar to those in cardiomyopathic hamsters, in which both cardiomyopathy and skeletal myopathy are related to adhalin deficiency.<sup>31</sup> In our patient and in the animal model, inheritance is probably autosomal recessive.

Severe childhood autosomal recessive muscular dystrophy is phenotypically very similar to Duchenne's and Becker's muscular dystrophies, but it affects both males and females. It has a higher variability in the severity of the clinical course from one sibling to another, and symptoms suggestive of nervous system involvement have not been described.<sup>19</sup> Becker's muscular dystrophy was originally diagnosed in our patient on the basis of his clinical presentation, creatine kinase levels, and skeletal-muscle pathological findings. However, as demonstrated in our patient, the diagnosis of a dystro-

phinopathy requires confirmation of dystrophin abnormalities by immunohistochemical means.

Several types of muscular dystrophy have been related to the disruption of different components of the link between the muscle cytoskeleton and the extracellular matrix.<sup>9,11-13,16,18</sup> It has been postulated that the structurally weakened sarcolemma ruptures under mechanical stress, allowing uncontrolled ingress of extracellular-fluid components, particularly calcium, which triggers several processes that eventually result in necrosis of muscle fibers. This common pathophysiologic mechanism may explain the histologic similarities found among biopsy specimens of skeletal muscle from patients with congenital muscular dystrophies, dystrophinopathies, and severe childhood autosomal recessive muscular dystrophy. Degenerative changes in cardiac-muscle fibers and foci of fibrosis have been described both in dystrophinopathies<sup>1,32</sup> and in Fukuyama-type congenital muscular dystrophy.<sup>33</sup> The findings in our patient were similar. Why no necrotic

fibers have been identified in cardiac muscle remains to be explained.

In dystrophinopathies, the cardiomyopathy appears to be unrelated to the course or severity of the skeletal myopathy. This observation is especially relevant to Becker's muscular dystrophy, in which severe myocardial involvement may be the presenting symptom.<sup>34-37</sup> At the other end of the spectrum is X-linked cardiomyopathy, which is linked to the dystrophin gene but presents without clinical muscle weakness.<sup>38-40</sup> The findings in some patients with X-linked cardiomyopathy suggest that deletions around exon 1 of the dystrophin gene may severely damage the function or expression of dystrophin in cardiac but not in skeletal muscle.<sup>39,40</sup> The differences in skeletal- and cardiac-muscle involvement in patients with dystrophinopathies may be explained by different underlying mutations. Interestingly, Michels et al.<sup>41</sup> did not find the common dystrophin-gene defect in 51 patients with idiopathic dilated cardiomyopathy, either familial or nonfamilial.

In our patient, dilated cardiomyopathy was related to adhalin deficiency. One might speculate that a defect in any of the dystrophin-associated proteins may present as a cardiomyopathy. Adhalin deficiency presents as an autosomal recessive condition,<sup>19</sup> and only sporadic cases have been reported in countries with a low consanguinity rate. It is also genetically heterogeneous, since the defective gene was mapped to the pericentromeric region of chromosome 13q<sup>20</sup> in the North African series, whereas the adhalin gene has been mapped to chromosome 17q12-21.33.<sup>25,26</sup> Conversely, Fukuyama-type congenital muscular dystrophy has been mapped to chromosome 9q31-33,<sup>13</sup> and merosin-negative congenital muscular dystrophy to chromosome 6q2.<sup>17</sup> A missense mutation in the adhalin gene may be the cause of adhalin deficiency in some families,<sup>26,27</sup> but different mechanisms are probably involved, since the linkage studies of the North African families indicate a locus on chromosome 13q.

Because dystrophinopathies and adhalin deficiency may be indistinguishable in terms of clinical presentation and pathological features of muscle, the diagnosis of Duchenne's or Becker's muscular dystrophy should be based on histochemically determined abnormalities of dystrophin. We propose that constituents of the dystrophin-glycoprotein complex and merosin be analyzed in all patients with the histologic findings suggestive of a dystrophinopathy and with normal results of muscle dystrophin analysis. Analysis of the dystrophin-glycoprotein complex should also be considered in patients with sporadic or familial non-X-linked dilated cardiomyopathy.

We are indebted to Masami Sunada and Sia Nizamuddin for expert technical assistance, and to Jo Ann Zaborowski for assistance in preparing the manuscript.

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