

Abnormal Expression of Laminin Suggests Disturbance of Sarcolemma–Extracellular Matrix Interaction in Japanese Patients with Autosomal Recessive Muscular Dystrophy Deficient in Adhalin

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Abstract

Dystrophin is associated with several novel sarcolemmal proteins, including a laminin-binding extracellular glycoprotein of 156 kD (α -dystroglycan) and a transmembrane glycoprotein of 50 kD (adhalin). Deficiency of adhalin characterizes a severe autosomal recessive muscular dystrophy prevalent in Arabs. Here we report for the first time two mongoloid (Japanese) patients with autosomal recessive muscular dystrophy deficient in adhalin. Interestingly, adhalin was not completely absent and was faintly detectable in a patchy distribution along the sarcolemma in our patients. Although the M and B2 subunits of laminin were preserved, the B1 subunit was greatly reduced in the basal lamina surrounding muscle fibers. Our results raise a possibility that the deficiency of adhalin may be associated with the disturbance of sarcolemma–extracellular matrix interaction leading to sarcolemmal instability. (*J. Clin. Invest.* 1994; 94:601–606.) Key words: dystrophin–glycoprotein complex • laminin receptor • sarcolemma • basal lamina • extracellular matrix

Introduction

A severe autosomal recessive form of progressive muscular dystrophy resembling Duchenne/Becker muscular dystrophy (DMD/BMD)¹ in phenotype was first reported in Tunisia by Ben Hamida et al. (1) in 1983. This disease is now considered to be highly prevalent in Arabs of North Africa and the Middle East (2, 3). Dystrophin, the protein product of the *DMD* gene

(4), and utrophin, an autosomal homologue of dystrophin (5, 6), were shown to be expressed at normal levels in skeletal muscle biopsy specimens from Tunisian patients (7, 8).

In skeletal muscle, dystrophin exists in a large oligomeric complex tightly associated with several novel sarcolemmal proteins, including a transmembrane glycoprotein of 50 kD (adhalin) (9–15). The extracellular dystrophin-associated glycoprotein of 156 kD (α -dystroglycan) specifically binds to the extracellular matrix component laminin, indicating that the dystrophin–glycoprotein complex is a novel laminin–receptor in skeletal muscle (13, 14). Recently, we have demonstrated the specific deficiency of adhalin in one Lebanese and three Algerian patients afflicted with severe DMD-like muscular dystrophy (16). Since adhalin is also greatly reduced due to the absence of dystrophin in DMD patients, we postulated that the deficiency of adhalin may play an important role in the molecular pathogenesis leading to muscle fiber necrosis and, eventually, to DMD-like phenotype in these patients (16–18). However, the exact mechanism by which the deficiency of adhalin causes muscle fiber necrosis remains to be elucidated.

At present, it is unknown if adhalin deficiency is confined to specific human populations and how prevalent it is in various populations. Recently, adhalin deficiency was demonstrated in one Greek, one Italian, three French, and five Brazilian patients with severe muscular dystrophy (19, 20). These results indicate that adhalin deficiency exists in multiple human populations. So far, adhalin deficiency has not yet been reported in mongoloids. It is possible that adhalin deficiency may not exist in mongoloids. Alternatively, patients with adhalin deficiency may be misdiagnosed as DMD/BMD or symptomatic DMD carriers because of the phenotypic resemblance and potential reduction of dystrophin in the advanced stages (16).

In this paper, we report for the first time two mongoloid (Japanese) patients with adhalin deficiency. In addition, we demonstrate the abnormal expression of the laminin B1 subunit in the basal lamina surrounding muscle fibers, which implicates the disturbance of sarcolemma–extracellular matrix interaction in the molecular pathogenesis of muscle fiber necrosis in these patients. Our results show that the identification of adhalin deficiency, which is essential for both genetic counseling and potential future therapies, is possible by testing both dystrophin and adhalin in biopsied skeletal muscle, even in the populations in which adhalin deficiency has not yet been identified or is rare.

Methods

Case presentation

Patient 1. A 33-yr-old Japanese man comes from Kikai island in the southwest of Japan. His parents were second cousins. His father's uncle

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1. *Abbreviations used in this paper:* BMD, Becker muscular dystrophy; CK, creatine kinase; DMD, Duchenne muscular dystrophy; FCMD, Fukuyama-type congenital muscular dystrophy; 59DAP, 59-kD dystrophin-associated protein; 35DAG, 35-kD dystrophin-associated glycoprotein.

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died at the age of 17 years after suffering from progressive muscle weakness. His parents, three brothers, and two sisters had no neuromuscular problems. The patient noticed difficulty in walking and myalgia in his calf muscles at 5 yr old. He became unable to climb stairs at the age of 9. He became wheelchair-bound at the age of 13. On the first admission at the age of 13, proximal dominant muscle atrophy and weakness, and hypertrophy of calf muscles were noted. Serum creatine kinase (CK) value was elevated to 940 U/l (normal upper limit, 195 U/l). Electromyography showed motor unit potentials of small amplitude and short duration. He was diagnosed as having progressive muscular dystrophy by a pediatrician. At 27 yr old, he was re-admitted with complaint of dyspnea. Upon examination, he had generalized muscle atrophy and weakness, scoliosis and contractures of hip, knee, and ankle joints. Deep tendon reflexes were absent. He had normal intelligence. Serum CK value was 335 U/l.

Electrocardiogram revealed left ventricular hypertrophy. Arterial blood gas analysis showed hypercapnia and hypoxemia. No abnormality was detected by multiplex PCR analysis of the dystrophin gene (not shown) (21). At 28 yr old, he was put on a ventilator due to respiratory failure.

At the age of 30, muscle biopsy was performed on the left tibialis anterior muscle (Fig. 1). Standard histochemical study demonstrated marked variation in size of muscle fibers. Splitting of hypertrophic muscle fibers was present. A few necrotic or regenerating muscle fibers were found. Cytoplasmic bodies were observed in a small number of muscle fibers. There was an increase of internal nuclei and of fibrous and fatty tissues in the interstitium.

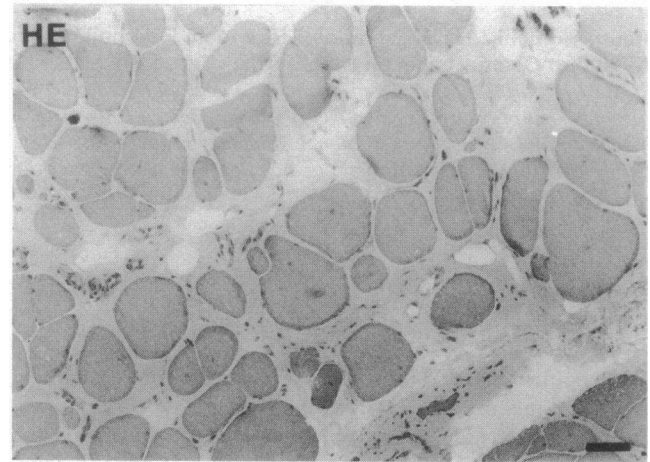
Patient 2. A 41-yr-old Japanese man comes from Kyushu, a southern island of Japan. His parents were first cousins. His older sister died at the age of 27 after suffering from progressive muscle weakness. The patient noticed difficulty in standing up from a chair at 10 yr old, in walking at 14, and in lifting his arms at 17, respectively. He was confined to a wheelchair at the age of 21. He was diagnosed as having progressive muscular dystrophy by a physician. On admission to our hospital at the age of 41, proximal dominant muscle atrophy and weakness, and contracture of knee joints were present. He had normal intelligence and had no calf hypertrophy. Serum CK value was elevated to 220 U/l. Electromyography showed motor unit potentials of small amplitude and short duration. No abnormality was detected by multiplex PCR analysis of the dystrophin gene (not shown) (21, 22).

At the age of 41, muscle biopsy was performed on the left gastrocnemius muscle (Fig. 1). Standard histochemical study demonstrated marked variation in size of muscle fibers. Splitting of hypertrophic muscle fibers was observed. A few necrotic or regenerating muscle fibers were found. There was an increase of internal nuclei and of fibrous and fatty tissues in the interstitium.

Immunohistochemical analysis of dystrophin, the dystrophin-associated proteins and laminin subunits in biopsied skeletal muscle

Immunohistochemistry of biopsied skeletal muscle was performed as described previously (6, 16–18). Monoclonal antibodies against dystrophin and adhalin, and affinity-purified sheep antibodies against α -dystroglycan, the 59-kD dystrophin-associated protein (59DAP), the 43-kD dystrophin-associated glycoprotein (β -dystroglycan), and the 35-kD dystrophin-associated glycoprotein (35DAG) were characterized previously (6, 10, 16–18). Adhalin fusion protein G (FP-G) corresponding to amino acid residues 217–289 and FP-H corresponding to amino acid residues 312–387 were produced as described (15) and injected into individual sheep. Each antiserum cross-reacted with a single protein of 50 kD on immunoblots of rabbit skeletal muscle membranes (not shown). The affinity-purified rabbit anti-COOH-terminal adhalin antibody has been characterized previously (15). Immunohistochemical analysis of laminin subunits were performed using monoclonal antibodies 2G9 against human M, 11D5 against human A, 4E10 against human B1 and 2E8 against human B2 subunits of laminin (GIBCO BRL, Gaithersburg, MD).

Patient 1



Patient 2

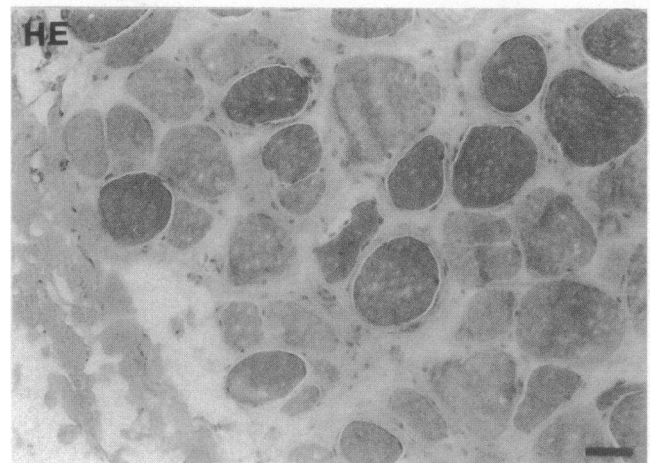


Figure 1. Histochemistry of biopsied skeletal muscle from patients 1 and 2. Hematoxylin and eosin (HE) and Gomori-Trichrome (GT) staining are shown. There was marked variation in the size of muscle fibers in both patients 1 and 2. Splitting of hypertrophic muscle fibers was present. There was an increase of internal nuclei and of fibrous and fatty tissues in the interstitium. In patient 1, cytoplasmic bodies were observed in a small number of muscle fibers (arrow in panel GT). Bar, 50 μ m.

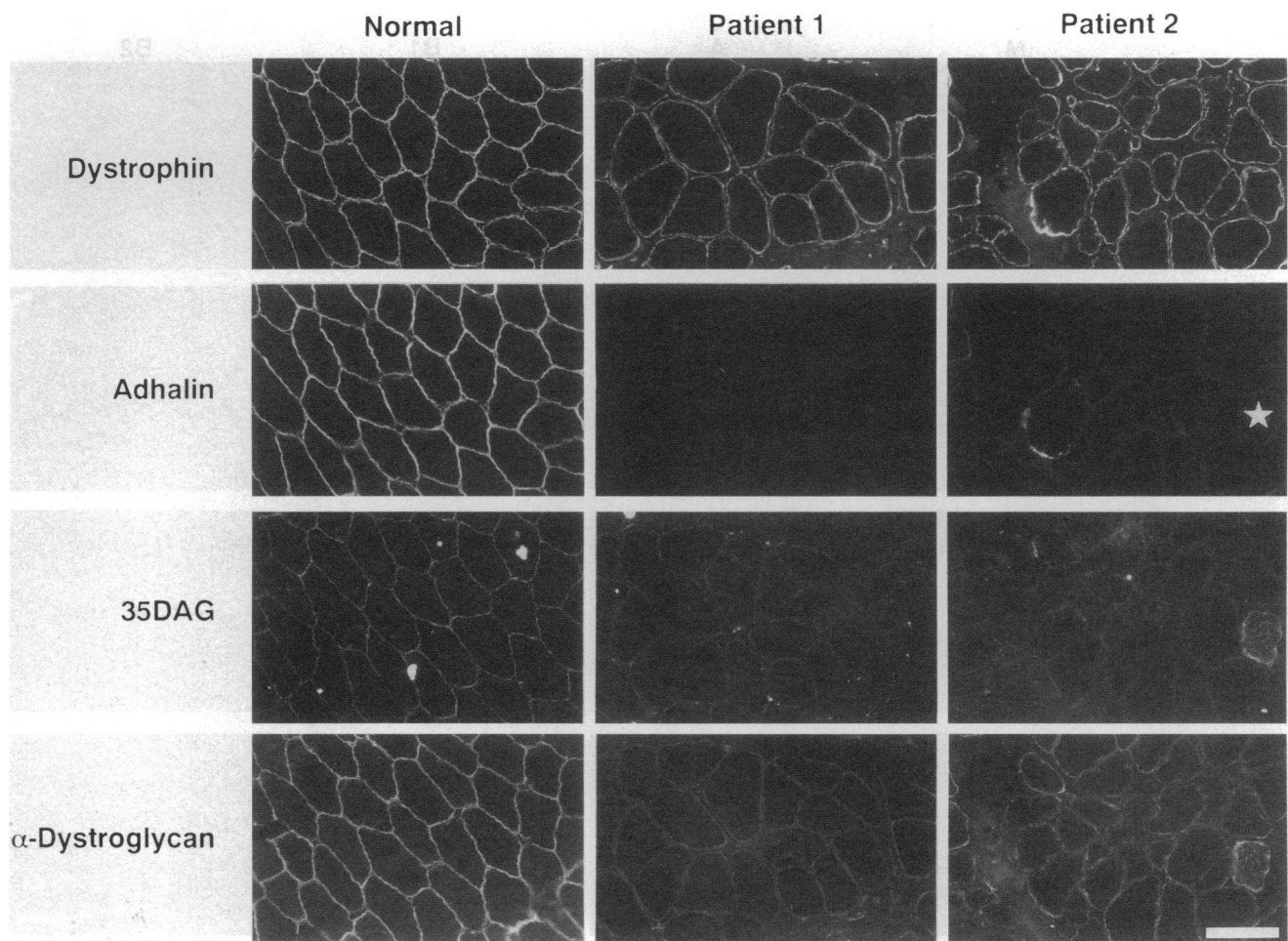


Figure 2. Immunohistochemical analysis of dystrophin and the dystrophin-associated proteins in biopsied skeletal muscle from patients 1 and 2. In patients 1 and 2, dystrophin and laminin-binding α -dystroglycan were expressed at near-normal levels in the sarcolemma. β -Dystroglycan and the 59DAP were also preserved in the sarcolemma (not shown). Adhalin was drastically reduced in the sarcolemma in both patients. The reduction of adhalin in these two patients was far more prominent than in DMD muscle immunostained simultaneously on the same microscopy slide (not shown). Adhalin was not completely absent and was preserved in an irregular and patchy distribution in the sarcolemma. The reduction of adhalin was more severe in patient 1 than in patient 2. The 35DAG was moderately reduced in these two patients compared with normal muscle immunostained simultaneously but the reduction was not as severe as in DMD muscle (not shown). One muscle fiber in the whole field (indicated by an asterisk) showed diffuse cytoplasmic staining for α/β -dystroglycan, the 59DAP (not shown) and the 35DAG. Bar, 100 μ m.

Results

The results of immunohistochemical analysis of dystrophin and the dystrophin-associated proteins are shown in Fig. 2. In patients 1 and 2, dystrophin and laminin-binding α -dystroglycan were expressed at near-normal levels in the sarcolemma. β -Dystroglycan and the 59DAP were also preserved in the sarcolemma (not shown). However, adhalin was drastically reduced in the sarcolemma in both patients. The reduction of adhalin in these two patients was far more prominent than in DMD muscle immunostained simultaneously on the same microscopy slide (not shown). Interestingly, adhalin was not completely absent and was faintly detectable in an irregular and patchy distribution in the sarcolemma. The reduction of adhalin was more severe in patient 1 than in patient 2. Similar results were obtained using sheep antiserum against FP-G, sheep antiserum against FP-H and affinity-purified rabbit anti-COOH-terminal adhalin

antibody (not shown) (15). The 35DAG was moderately reduced in these two patients compared with normal muscle immunostained simultaneously, but the reduction was not as severe as in DMD muscle (not shown).

Based on the recent findings that the dystrophin-glycoprotein complex is a laminin-receptor in the sarcolemma (13, 14), we hypothesized that the deficiency of adhalin might disturb the function of the dystrophin-glycoprotein complex and cause abnormal expression of laminin subunits in the basal lamina. Thus, we investigated the status of expression of laminin subunits in our patients. The results of immunohistochemical analysis of laminin subunits are shown in Fig. 3. In normal and DMD muscle, the M, B1 and B2 subunits of laminin were distributed continuously in the basal lamina surrounding muscle fibers. While A was distributed only around blood vessels in normal muscle, it was faintly detectable in the basal lamina surrounding DMD muscle fibers as reported previously (23). In patients 1

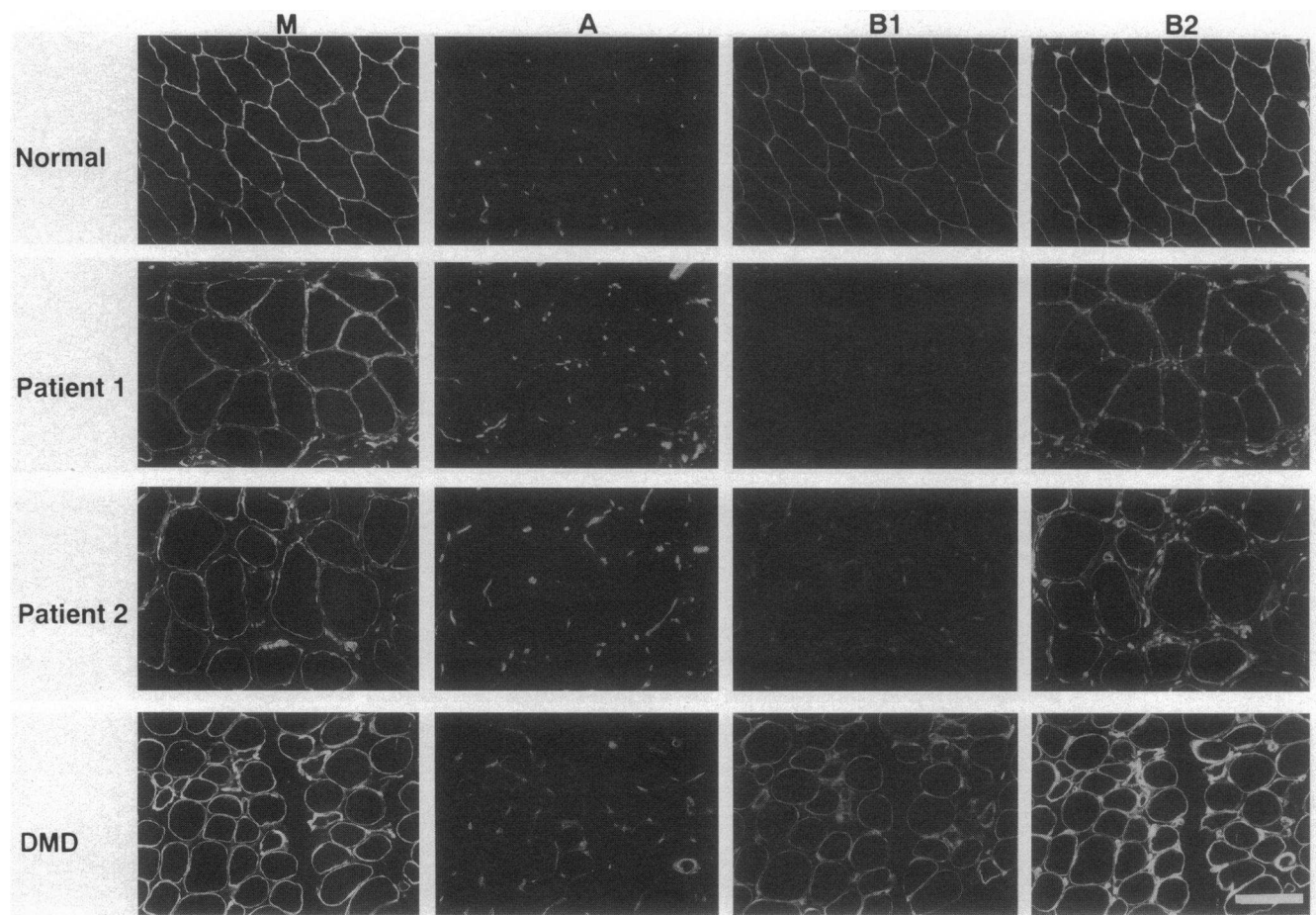


Figure 3. Immunohistochemical analysis of laminin subunits in biopsied skeletal muscle from patients 1 and 2. In normal and DMD muscle, the M, B1 and B2 subunits of laminin were distributed continuously in the basal lamina surrounding muscle fibers. While A was distributed only around blood vessels in normal muscle, it was faintly detectable in the basal lamina surrounding DMD muscle fibers. In patients 1 and 2, M and B2 were well preserved in the basal lamina surrounding muscle fibers. A was detected only around blood vessels. B1 was greatly reduced in the basal lamina surrounding muscle fibers in both patients. The distribution was irregular and patchy. The reduction of B1 was more severe in patient 1 than in patient 2. B1 was well preserved around capillaries in both patients. Bar, 100 μ m.

and 2, M and B2 were well preserved in the basal lamina surrounding muscle fibers and A was detected only around blood vessels as in normal muscle. Surprisingly, B1 was greatly reduced in the basal lamina surrounding muscle fibers in both patients. The distribution was irregular and patchy. The reduction of B1 was more severe in patient 1 than in patient 2. On the other hand, B1 was well preserved around capillaries in both patients.

Discussion

Here we have reported two Japanese patients with adhalin deficiency. Patient 1 presented with a severe and progressive muscular dystrophy which was difficult to distinguish from DMD/severe BMD clinically. Myalgia of calf muscles, which is frequently observed in DMD/BMD patients, was also present in this patient. On the other hand, patient 2 suffered from a much milder muscular dystrophy which was quite different from DMD clinically. This indicates that the phenotype of adhalin deficiency varies considerably among patients and that the pa-

tients with adhalin deficiency may be misdiagnosed as having other diseases unless tested for both dystrophin and adhalin. The elevation of serum CK value in our patients was mild compared with the reported cases of adhalin deficiency of comparable age (19). Although the peak levels of serum CK value are unknown in our patients, our results suggest that the absence of marked elevation of serum CK value may not rule out adhalin deficiency.

In contrast to the patients who have no detectable levels of adhalin, adhalin was not completely absent and was faintly detectable in an irregular and patchy distribution along the sarcolemma in our patients. This may be comparable with the status of dystrophin expression in DMD/BMD muscles (24), and raises an intriguing possibility that the severity of adhalin deficiency might vary depending on the underlying genetic defect in the individual. The defective gene(s) causing adhalin deficiency are unknown at present. Recently, Ben Othmane et al. (25) localized the causative gene of severe muscular dystrophy affecting Tunisian families to chromosome 13q. It is not known if adhalin was deficient in these families. Subsequently, adhalin

deficiency in Algerian families was linked to the same locus (26). However, Brazilian families with adhalin deficiency have been reported that are not linked to chromosome 13q (20). This suggests that there may be multiple genetic defects causing adhalin deficiency and that the deficiency of adhalin may be a common denominator leading to muscle fiber necrosis in multiple forms of autosomal recessive muscular dystrophies. In this respect, it is interesting that the 35DAG is reproducibly reduced in abundance in adhalin-deficient muscle as in our patient (16, 19). Biochemical data suggest that adhalin and the 35DAG may exist in a subcomplex in the dystrophin–glycoprotein complex (6). Thus, both the adhalin and 35DAG genes are reasonable candidate genes for phenotypically similar forms of autosomal recessive muscular dystrophies. This might explain the apparent variation among different patients in the severity of adhalin deficiency as discussed above. Furthermore, underlying genetic defects of adhalin deficiency might vary from one population to another. Thus, it is of utmost importance to identify the defective gene(s) causing adhalin deficiency in various populations.

In the present study, we investigated for the first time the status of expression of laminin subunits in adhalin deficiency, based on the hypothesis that the deficiency of adhalin may cause the dysfunction of the dystrophin–glycoprotein complex as a laminin-receptor in the sarcolemma. Laminin is involved in a number of important cellular functions including cell adhesion, differentiation, migration, and proliferation, in addition to being a true structural component of the basement membrane meshwork (27). Laminin molecule is a cross-shaped heterotrimer made up of three subunits of types A, B1 and B2 (27). In the skeletal muscle form of laminin called merosin, A is replaced by its isoform, M (27–29). It has been shown recently that α -dystroglycan binds to the globular domain at the end of the long arm of laminin which is predominantly contributed to by M in the case of merosin (14, 30). Thus, the aforementioned hypothesis suggested a possibility that the expression of M might be affected by the deficiency of adhalin more than B1 or B2. Surprisingly, we found the specific reduction of B1 in our patients. In this respect, it is noteworthy that B1, but not M/A or B2, was shown to be immunoprecipitated by an antibody against dystrophin from the solubilized myotube extracts (31), raising the possibility that B1 itself might be associated with the dystrophin–glycoprotein complex, independent of other subunits. Recently, the primary structure of adhalin was published and it was suggested that adhalin might also be involved in the cell–extracellular matrix interaction, in addition to α -dystroglycan (15). Taking these findings into consideration, our results raise an intriguing possibility that the deficiency of adhalin/35DAG may cause the dysfunction/disruption of the dystrophin–glycoprotein complex as a laminin receptor. This may cause the abnormal expression of the laminin B1 subunit in the basal lamina and the disturbance of sarcolemma–extracellular matrix interaction, which may lead to sarcolemmal instability and eventually to muscle fiber degeneration (Fig. 4).

Our results suggest that the composition of laminin heterotrimer is disturbed due to the selective reduction of B1, unless an immunologically distinct isoform of B1 is expressed to replace B1 in our patients. It has been shown recently that M is greatly reduced and A is expressed in the basal lamina surrounding muscle fibers in Fukuyama-type congenital muscular dystrophy (FCMD) (23). This suggests that the reduction of

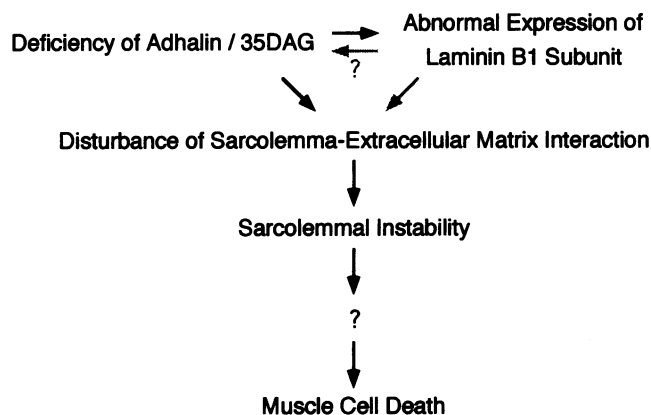


Figure 4. Hypothetical scheme on the molecular pathogenesis of muscle fiber necrosis in adhalin deficiency. The deficiency of adhalin/35DAG may cause the dysfunction/disruption of the dystrophin–glycoprotein complex as a laminin receptor. This may cause the abnormal expression of the laminin B1 subunit in the basal lamina and the disturbance of sarcolemma–extracellular matrix interaction, which may lead to sarcolemmal instability and eventually to muscle cell death. Alternatively, the deficiency of adhalin may be caused by the abnormal expression of B1. However, the finding that B1 was relatively well preserved around capillaries in both patients 1 and 2 suggests that this may not be the case.

M may be a trigger for the upregulation of A in FCMD. In muscular dystrophies other than FCMD and DMD, no abnormality was reported in the expression of laminin subunits (23). In any case, the putative dysfunction/disruption of the sarcolemma–extracellular matrix interaction in adhalin deficiency must be elucidated by further investigation. It would be interesting to see if the reduction of B1 is common to all the patients with adhalin deficiency regardless of geographical origin, and if its severity is correlated with the clinical severity/progression of the disease.

Finally, our Japanese patients with adhalin deficiency demonstrate a hitherto unrecognized worldwide distribution of this condition. This is important since genetic diseases which specifically affect certain populations exist. For instance, FCMD is second to DMD in frequency as a childhood form of muscular dystrophy in Japanese, while it is almost non-existent in Europeans (32). Our results emphasize the necessity of clinicians to consider adhalin deficiency in all regions of the world, since this genetically heterogeneous condition has a phenotype which varies considerably among patients and may easily be misdiagnosed.

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