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Myasthenic syndrome caused by plectinopathy

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ABSTRACT

Background: Plectin crosslinks intermediate filaments to their targets in different tissues. Defects in plectin cause epidermolysis bullosa simplex (EBS), muscular dystrophy (MD), and sometimes pyloric atresia. Association of EBS with a myasthenic syndrome (MyS) was documented in a single patient in 1999.

Objectives: To analyze the clinical, structural, and genetic aspects of a second and fatal case of EBS associated with a MyS and search for the genetic basis of the disease in a previously reported patient with EBS-MD-MyS.

Methods: Clinical observations; histochemical, immunocytochemical, and electron microscopy studies of skeletal muscle and neuromuscular junction; and mutation analysis.

Results: An African American man had EBS since early infancy, and progressive muscle weakness, hyperCKemia, and myasthenic symptoms refractory to therapy since age 3 years. Eventually he became motionless and died at age 42 years. At age 15 years, he had a marked EMG decrement, and a reduced miniature endplate potential amplitude. The myopathy was associated with dislocated muscle fiber organelles, structurally abnormal nuclei, focal plasmalemmal defects, and focal calcium ingress into muscle fibers. The neuromuscular junctions showed destruction of the junctional folds, and remodeling. Mutation analysis demonstrated a known p.Arg2319X and a novel c.12043dupG mutation in *PLEC1*. The EBS-MD-MyS patient reported in 1999 also carried c.12043dupG and a novel p.Gln2057X mutation. The novel mutations were absent in 200 Caucasian and 100 African American subjects.

Conclusions: The MyS in plectinopathy is attributed to destruction of the junctional folds and the myopathy to defective anchoring of muscle fiber organelles and defects in sarcolemmal integrity. ***Neurology*® 2011;76:327-336**

GLOSSARY

Ab = antibodies; **AChR** = acetylcholine receptor; **anti-C Ab** = antibody recognizing the C-terminal plectin domain; **anti-Rod Ab** = antibody recognizing the plectin rod domain; **EBS** = epidermolysis bullosa simplex; **EP** = endplate; **IF** = intermediate filament; **IgG** = immunoglobulin G; **MD** = muscular dystrophy; **MyS** = myasthenic syndrome; **P1** = patient 1; **P2** = patient 2.

Plectin is a ~500 kDa dumbbell-shaped molecule with a central coiled-coil rod domain flanked by globular N- and C-terminal domains. Owing to tissue and organelle-specific transcript isoforms, plectin is a versatile linker of cytoskeletal components to target organelles in cells of different tissues.[1–3] In skeletal muscle, multiple alternatively spliced transcripts of exon preceding common exon 2 link desmin intermediate filaments (IFs) to specific targets: the outer nuclear membrane (isoform 1), the outer mitochondrial membrane (isoform 1b), Z disks (isoform 1 d), and costameres in the sarcolemma (isoform 1f).[3] Plectin is also highly expressed at the neuromuscular junction where it provides crucial structural support for the junctional folds.[4] Plectin deficiency in muscle results in progressive muscular dystrophy (MD).[4–19] Plectin

Supplemental data at www.neurology.org

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is also highly expressed in intercalated disks in the heart but only a single patient with EBS/MD and cardiomyopathy was identified to date.[18] Plectin deficiency in skin causes epidermolysis bullosa simplex (EBS).[20] Some patients with EBS and MD (EBS-MD) also had symptoms suggesting a myasthenic disorder[9,21–23] but this was not suspected or confirmed by specific studies. The association of EBS-MD with a myasthenic syndrome (MyS) was well-documented in a single patient (P1) in 1999.[4] Although numerous autosomal recessive and one dominant mutation in *PLEC* have been detected,[20] the genetic basis of EBS-MD-MyS in P1 was not identified. We describe our findings in a second patient with EBS-MD-MyS (P2), report additional observations in P1, and identify the genetic basis of the disease in both patients.

METHODS All human studies described here were in accord with the guidelines of the Institutional Review Board of the Mayo Clinic.

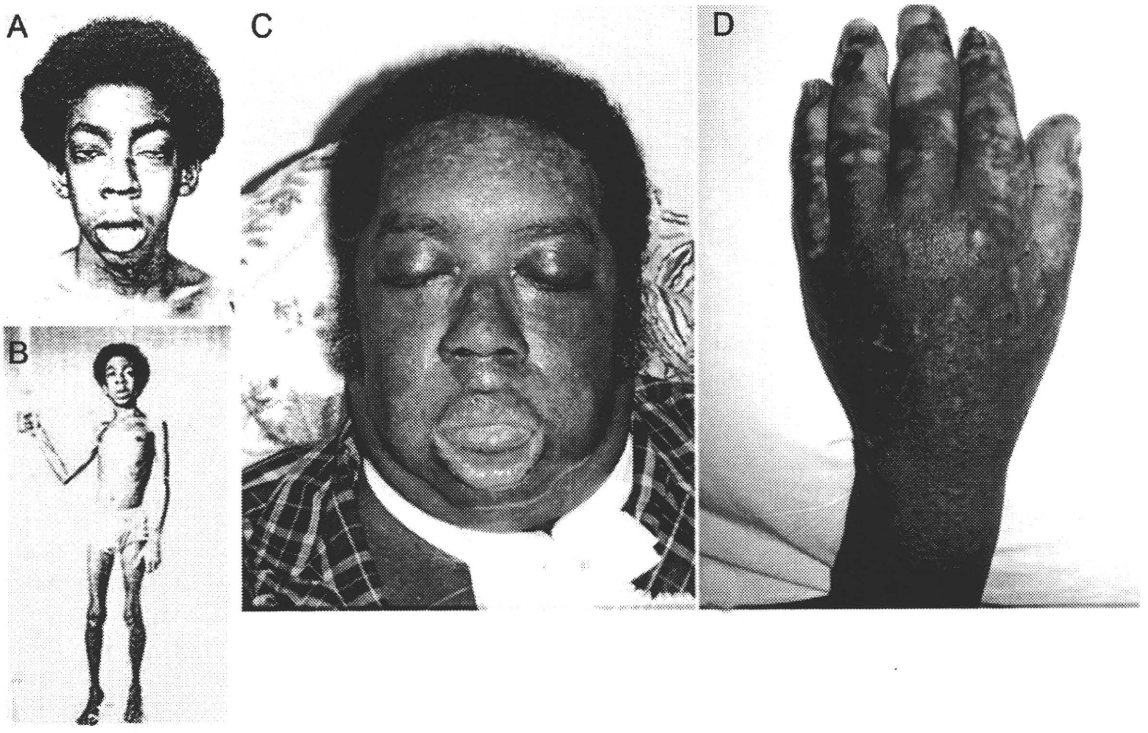
Structural observations. Routine histochemical studies on cryostat sections and electron microscopy studies were performed as previously described.[24] Immunoglobulin G and the C3 and C9 complement components were immunolocalized as previously reported.[25,26] We immunolocalized the last 50 C-terminal residues of plectin with 4 μg/mL goat polyclonal C-20 antibody (anti-C Ab), and the plectin rod domain with 4 μg/mL 10F6 mouse monoclonal antibody (anti-Rod Ab) (both from Santa Cruz Biotechnology), followed by 3 μg/mL biotinylated donkey antigoat or antimouse immunoglobulin G (IgG) (Jackson ImmunoResearch Laboratories) and the ABC peroxidase kit (Vector Laboratories). Intrafiber calcium excess was evaluated by the Alizarin red stain.[27] Synaptic contact regions were visualized on fixed, teased muscle fibers by a cytochemical reaction for acetylcholinesterase.[28] The acetylcholine receptor (AChR) and plectin were colocalized at endplates (EPs) with rhodamine-labeled α-bungarotoxin and the plectin anti-Rod Ab followed by fluorescent goat antimouse IgG. EPs were localized for electron microscopy[24] and quantitatively analyzed[29] by established methods. Peroxidase-labeled α-bungarotoxin was used for the ultrastructural localization of AChR.[30]

Molecular genetic studies. Genomic DNA was isolated from blood of P1 and muscle of P2 and mRNA from intercostal muscles of both by standard methods. *PLEC* nucleotides were numbered according to the mRNA sequence (GenBank reference no: NM_000445). We used PCR primer pairs to amplify and directly sequence the 32 exons and flanking noncoding regions of *PLEC* isoform 1 and also first exons of isoforms 1b, 1d, and 1f. We screened for the identified novel mutations in 200 Caucasian and 100 African American control subjects using allele-specific PCR. To estimate expression of the rodless isoform of *PLEC* at the mRNA level, we used real-time PCR and SYBR green I (Roche) with 5′ GTGTCATCCAGGAGTACGTG 3′ as the forward primer in exon 30, 5′ AGCGACAGCAGAGTGACCAT 3′ as the forward primer in exon 31 that encodes the rod domain, 5′ GCCTTCTCCTGCTCGATGAA 3′ as the reverse primer in exon 32 for both forward primers, and *GAPDH* as the housekeeping gene. All experiments were done in triplicate.

RESULTS Clinical observations. P1 is an African American woman. Her case was reported in 1999 when she was 23 years of age.[4] In brief, she was diagnosed with EBS as an infant and her myasthenic symptoms began around the age of 9 years. Since 1999, her weakness has worsened so she can now only take a few steps, has dysphagia, is dyspneic on slight exertion and at night, and is resistant to anticholinesterase drugs. However, her skin symptoms are mild, with new skin blisters appearing infrequently.

P2 is an African American man. He was a single child without similarly affected family members. He sucked poorly during infancy but this gradually improved. Since the age of 6 weeks, he had an intermittent vesicular eruption over his skin and oral mucosa and developed nail deformities. He attained his motor milestones on time, but had significant fatigue on exertion since age 3 years. At age 11 years he had difficulty running and rising from the floor and serum creatine kinase level was 827 U/L (normal <60 U/L). Prednisone therapy improved his strength but was discontinued because of abdominal pain. Nystatin therapy for thrush worsened the weakness. At age 12 years, a vastus medialis muscle specimen revealed a myopathy associated with necrotic and regenerating fibers, a sural nerve specimen was normal, and a skin biopsy showed EBS and secondary infection. In 1981, at age 15 years, the patient was evaluated at the Mayo Clinic. He now had reduced muscle bulk, bilateral eyelid ptosis, restricted eye movements, and mild facial and moderately severe diffuse cervical and limb muscle weakness, and was areflexic except at the ankles. Nerve conduction studies were normal. Repetitive stimulation at 2 Hz showed a decremental response (67% in hypothenar muscles) that was partially corrected by IV edrophonium chloride. Serratus anterior and intercostal muscles were biopsied. In vitro electrophysiology study of the intercostal specimen by Dr. Edward Lambert revealed reduction of the mean miniature endplate potential amplitude to 50% of normal; the quantal content of the endplate potential was in the low-normal range. Tests for anti-AChR antibodies were negative. A MyS was diagnosed but therapy with pyridostigmine bromide for a year was of no benefit. The weakness progressed more rapidly throughout adolescence and accelerated after routine illnesses. At age

Figure 1 **Patient photographs**



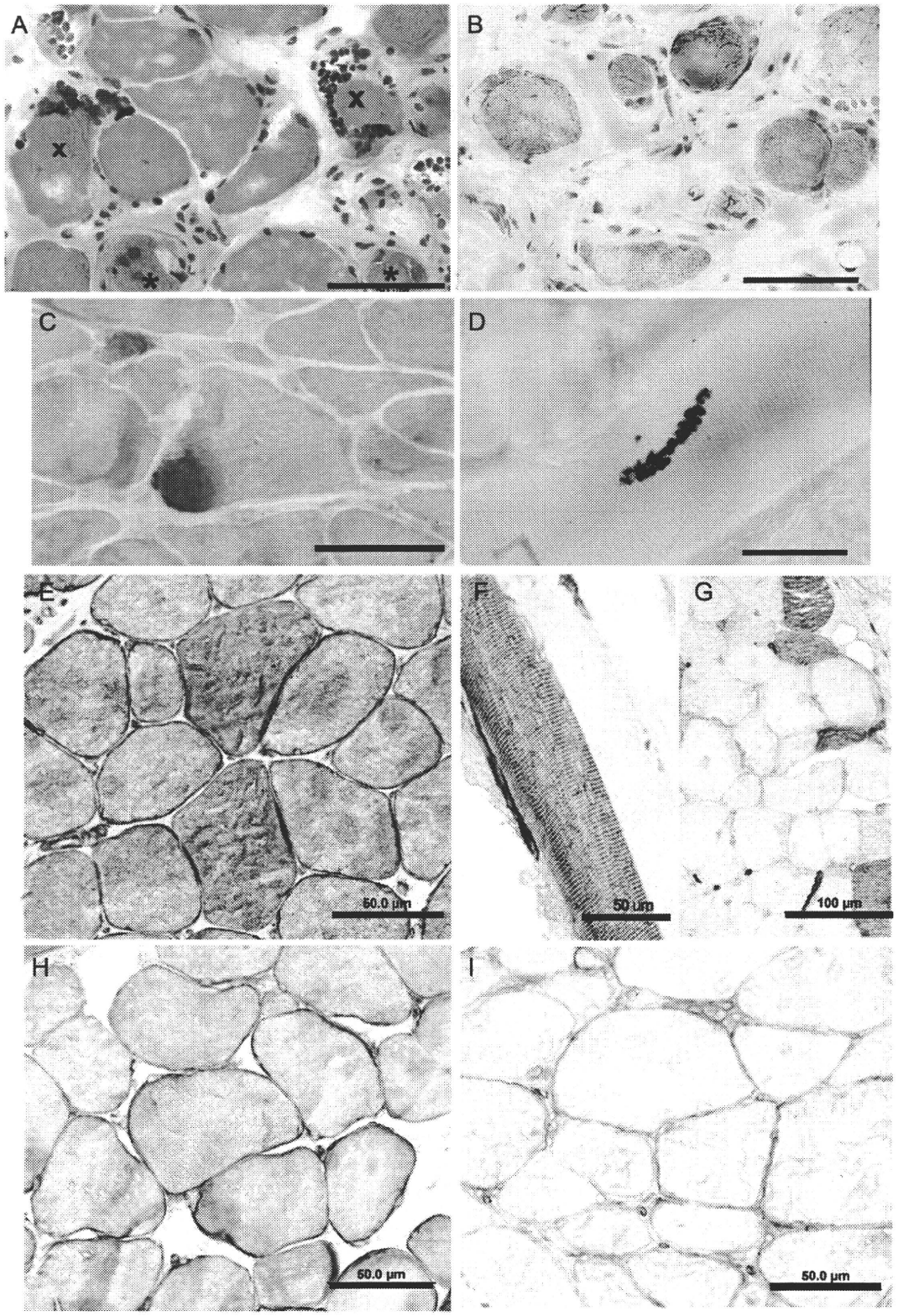
(A, B) Patient at age 17 years. Note severe asymmetric bilateral ptosis, hyperactive frontalis muscle, facial paresis, open mouth, cubitus valgus, Achilles tendon contractures, and diffuse muscle atrophy. (C, D) Patent at age 41 years. He has a tracheostomy, has facial diplegia, is unable to close his mouth or open his eyes, and shows the chronic skin changes of epidermolysis bullosa simplex. He also has blisters on his lip and tongue and oral moniliasis.

17 years, he could barely walk (figure 1, A and B). He was wheelchair-bound by age 18 years, and respirator-dependent by age 26 years. After age 35 years, he had dysarthria and dysphagia and needed a percutaneous gastrostomy. His cognitive functions and cardiac status remained normal. Subsequently, he became motionless (figure 1C), continued to have skin blisters (figure 1D), communicated with clicks and whispers, failed to respond to 3,4-diaminopyridine combined with pyridostigmine bromide, and died of pneumonia at age 42 years.

Histochemistry, P2. Serratus anterior and intercostal muscle specimens showed similar findings (figure 2, A and B). The muscle fiber diameters varied from 6 μm to ~120 μm. There was a mild to moderate increase of internal nuclei. Many nuclei were larger than normal and appeared in subsarcolemmal rows or clusters. Some fibers were necrotic or regenerating or subdividing by splitting, or displayed aberrant myofibrils. There was mild to marked (figure 2B) increase of perimysial and endomysial connective tissue. No immunoglobulin G, C3, or C9 deposits were present at patient endplates. In sections reacted for oxidative enzymes, some fibers showed attenuation or an irregular distribution of enzyme activity. Both muscle specimens showed type 1 fiber preponderance. Because plectin deficiency disconnects or weakens the link between the sarcolemma and the underlying cytoskeleton, it likely increases sarcolemmal vulnerability to mechanical stress. We therefore searched for signs of sarcolemmal injury evidenced by subsarcolemmal calcium deposits[27] and detected these in scattered fibers in both patients (figure 2C and figure e-1 [on the *Neurology*® Web site at www.neurology.org]).

Plectin immunostains. These were performed on 6- to 10-μm-thick acetone fixed frozen sections. In 1999, an antibody recognizing the rod domain of plectin (gift from Dr. Owaribe) showed no immunoreactivity in P1 muscle fibers. As this antibody was no longer available, we used the 10F6 antibody directed against the plectin rod domain (anti-Rod Ab), and a C-20 antibody raised against the last 50 C-terminal residues of plectin (anti-C Ab), and immunolocalized plectin in P1, P2, and normal muscle (see Methods). In normal muscle, both antibodies immunostained the sarcolemma, the intermyofibrillar network, capillaries, and vascular smooth muscle (figure 2, E and H); the C-20 Ab also immunostained perineurium and myelin-

Figure 2 **Histochemistry and plectin localization studies in patient 2**



(A, B) Note marked variation in fiber size, regenerating fiber elements (asterisks), endomysial fibrosis (B), and clusters of large nuclei at periphery of several fibers. (C) Alizarin red stain reveals focal calcium deposits in 2 fibers. (D) Multiple small cholinesterase-reactive endplate regions arrayed over an extended length of the fiber. Plectin was localized in normal control muscle (E, H) and patient intercostal muscle (F, G, I) with antibody recognizing the plectin rod domain (anti-Rod Ab) (E–G) and antibody recognizing the C-terminal plectin domain (anti-C Ab) (H, I). (E, H) In normal muscle, plectin is localized to the sarcolemma and sarcoplasm with both Abs. The anti-Rod Ab shows plectin-depleted and plectin-positive muscle fibers (F, G), whereas the anti-C Ab shows sarcoplasmic loss and slight sarcolemmal expression of plectin in all muscle fibers (I). Bars indicate 50 μm in all panels except in (G), where they indicate 100 μm.