Limb-Girdle Muscular Dystrophy Due to Emerin Gene Mutations

Shigehisa Ura, MD; Yukiko K. Hayashi, MD, PhD; Kanako Goto, MS; Mina Nolasco Astejada, MD; Terumi Murakami, MD; Masako Nagato, MD; Shigeru Ohta, MD; Yasuhisa Daimon, MD; Hidehiro Takekawa, MD, PhD; Koichi Hirata, MD, PhD; Ikuya Nonaka, MD, PhD; Masako Nagato, MD; Shigeru Ohta, MD; Yasuhisa Daimon, MD; Hidehiro Takekawa, MD, PhD; Koichi Hirata, MD, PhD; Ikuya Nonaka, MD, PhD; Satoru Noguchi, PhD; Ichizo Nishino, MD, PhD

Background: Emery-Dreifuss muscular dystrophy, caused by EMD gene mutations, is characterized by humeroperoneal muscular dystrophy, joint contractures, and conduction defects and is often associated with sudden cardiac death, even without prior cardiac symptoms.

Objective: To describe the clinical and molecular features of 2 patients with limb-girdle muscular dystrophy with mutations in EMD.

Design: Case reports.

Setting: Academic research.

Patients: Two male patients manifested proximal dominant muscle involvement, with minimal or no joint and cardiac involvement.

Main Outcome Measures: Muscle biopsy and mutation analysis results.

Results: Immunohistochemistry revealed an absence of emerin staining in muscle biopsy specimens. Mutation analysis identified nonsense mutations in EMD.

Conclusions: Mutations in EMD may indicate a limb-girdle muscular dystrophy phenotype. Identification of emerin deficiency among patients with limb-girdle muscular dystrophy is essential to prevent cardiac catastrophe.

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EMERY-DREIFUSS MUSCULAR dystrophy (EDMD) is a rare muscular dystrophy clinically characterized as a triad of (1) slowly progressive humeroperoneal muscular dystrophy; (2) early joint contractures of Achilles tendons, elbows, and hind neck, and (3) cardiomyopathy with conduction defects. The X-linked form of EDMD is caused by mutations in the emerin gene (EMD) or in STA on Xq28, whereas autosomal dominant and rare recessive forms of EDMD are caused by mutations in the lamin A/C gene (LMNA) on 1q21.1,2 EMD is composed of 6 small exons that encode emerin, a 34-kDa inner nuclear membrane protein.2,4 Immunohistochemical analysis is valuable for the diagnosis of the X-linked form of EDMD because most patients show a lack of emerin staining at the nuclear membrane of skin, leukocytes, and skeletal and cardiac muscles.5-7 LMNA encodes lamin A and C, which are major components of nuclear lamina. Emerin and lamin A/C are nuclear envelope proteins, and clinical features of the X-linked form of EDMD and autosomal dominant EDMD are similar and indistinguishable. However, mutations in LMNA are known to be associated with several other disorders collectively known as laminopathies, including limb-girdle muscular dystrophy (LGMD) type 1B, which is characterized by proximal dominant muscular dystrophy with atrioventricular conduction disturbance.8 To exclude EMD mutations in patients with LGMD, we examined emerin expression in muscle biopsy specimens and performed genetic screening.

METHODS

MUSCLE BIOPSY SPECIMENS

All clinical materials used in this study were obtained for diagnostic purposes following informed consent. Skeletal muscle biopsy specimens were flash frozen in isopentane chilled with liquid nitrogen. A panel of histochemical staining, including hematoxylin-eosin and modified Gomori trichrome, was performed to obtain the pathological diagnosis. Immunohistochemical and immunoblotting analyses
were conducted as previously described. Genomic DNA was extracted from muscle biopsy specimens or from peripheral lymphocytes using standard techniques.

We examined 94 patients who were clinically and pathologically diagnosed as having LGMD. Exclusion of LGMD types 1C, 2A to 2G, 2I, and 2K was performed by immunohistochemical and Western blotting analyses. In detecting emerin in skeletal muscle biopsy specimens, monoclonal antiemerin antibody (Novocastra Laboratories, New Castle upon Tyne, England) was used.

**MUTATION ANALYSIS**

All 6 exons and their flanking intronic regions of EMD were directly sequenced using an automated sequencer (ABI PRISM 3100; PE Applied Biosystems, Foster City, California). Information about the primers used for the sequence analysis is available from the corresponding author. Sequence analysis of LMNA was also performed to exclude LGMD type 1B.

**RESULTS**

Among 94 patients with LGMD of unknown cause, we identified 2 patients with negative immunostaining for emerin in their skeletal muscles (Figure 1 and Figure 2).

**REPORT OF CASES**

Patient 1, a 9-year-old boy from nonconsanguineous parents, neither of whom had neuromuscular or cardiac disorders, was initially seen with proximal dominant muscle weakness and atrophy without joint contractures. Hematoxylin-eosin (HE) staining and emerin immunoreaction (Emerin) of skeletal muscle biopsy specimen. The HE staining shows fiber-size variation, regenerating fibers, and fibers with internalized nuclei. Immunoreaction of emerin is absent at the nuclear membrane. The bar indicates 50 µm. Inset, Emerin staining of control muscle is shown. C, Mutation analysis revealed a 1–base pair insertion at c.154 in exon 2.

**Figure 1.** Patient 1. A, A 9-year-old boy had proximal muscle atrophy without joint contractures. B, Hematoxylin-eosin (HE) staining and emerin immunoreaction (Emerin) of skeletal muscle biopsy specimen. The HE staining shows fiber-size variation, regenerating fibers, and fibers with internalized nuclei. Immunoreaction of emerin is absent at the nuclear membrane. The bar indicates 50 µm. Inset, Emerin staining of control muscle is shown. C, Mutation analysis revealed a 1–base pair insertion at c.154 in exon 2.

Patient 2, a 50-year-old man had proximal dominant muscle atrophy of the limbs with minimal joint contractures at the right elbow and Achilles tendon. Hematoxylin-eosin (HE) staining and emerin immunoreaction (Emerin) of muscle biopsy specimen. The HE staining shows fiber-size variation, regenerating fibers, and fibers with internalized nuclei. Immunoreaction of emerin is absent at the nuclear membrane. The bar indicates 50 µm. Inset, Emerin staining of control muscle is shown. C, Direct sequence analysis revealed a 4–base pair deletion at c.359-362 in exon 4.

**Figure 2.** Patient 2. A, A 50-year-old man had proximal dominant muscle atrophy of the limbs with minimal joint contractures at the right elbow and Achilles tendon. B, Hematoxylin-eosin (HE) staining and emerin immunoreaction (Emerin) of muscle biopsy specimen. The HE staining shows fiber-size variation, regenerating fibers, and fibers with internalized nuclei. Immunoreaction of emerin is absent at the nuclear membrane. The bar indicates 50 µm. Inset, Emerin staining of control muscle is shown. C, Direct sequence analysis revealed a 4–base pair deletion at c.359-362 in exon 4.
to microkatal per liter, multiply by 0.0167) (reference range, <180 U/L). Muscle biopsy was performed on the left biceps brachii, and the biopsy specimen showed moderate fiber size variation, with some regenerating fibers and several fibers with internalized nuclei (Figure 1). Results of dystrophin immunostaining were positive, and he was diagnosed as having LGMD. At age 9 years, he showed Gowers sign, waddling gait, and proximal dominant limb muscle weakness and atrophy. No joint contractures were observed (Figure 1). An electrocardiogram revealed transient sinus arrhythmia, but his echocardiogram was normal.

Patient 2, a 50-year-old man, initially was seen with proximal dominant muscle weakness and atrophy. His father had died of acute myocardial infarction; his mother was alive without symptoms of neuromuscular or cardiac disorders. He was healthy until age 35 years, when he noticed difficulty in going up and down stairs. During this time, he was incidentally found to have hypertension. Muscle weakness was progressive, and he had difficulty raising his arms by age 40 years. At age 49 years, he required support in climbing stairs and had difficulty in buttoning his clothes. Although his electrocardiogram was normal, an echocardiogram demonstrated moderate mitral and tricuspid insufficiency without dilatation of ventricles. Other than his father’s death from acute myocardial infarction, his family history was noncontributory. On physical examination, he had waddling gait, Gowers sign, and proximal dominant muscle weakness and atrophy. Only minimal joint contractures of the right elbow, bilateral Achilles tendons, and neck were observed (Figure 2). His serum creatine kinase level was elevated at 417 U/L. An electromyogram showed myopathy. Results of muscle computed tomography revealed proximal dominant muscle atrophy with fatty tissue replacement. A second electrocardiogram demonstrated complete atrioventricular conduction block. A muscle biopsy specimen was obtained from the left rectus femoris and showed marked fiber size variation, scattered regenerating fibers, and fibers with centrally placed nuclei (Figure 2).

**COMMENT**

Herein, we demonstrate an expanded clinical spectrum associated with EMD mutations, from the X-linked EDMD phenotype to the X-linked LGMD phenotype. The differences between EDMD and LGMD relate to the distribution of affected muscles and to the presence of early joint contractures. From childhood, patients with EDMD having an EMD or LMNA mutation may demonstrate slow progressively weak and wasting of humerorouneal muscles. Early contracture of the elbows, Achilles tendons, and posterior cervical muscles is another characteristic cardinal feature. Mutations in LMNA are associated with variable disorders, including EDMD, LGMD type 1B, peripheral neuropathy, progeria syndrome, lipodystrophy syndrome, and cardiomyopathy with conduction defects. Several overlapping clinical conditions are likewise observed, including an intermediate phenotype of EDMD and LGMD type 1B manifesting as proximal limb muscle involvement with early joint contractures. In contrast, mutations in EMD have been associated with only the EDMD phenotype.

A previously described 2½-year-old boy had a condition resembling LGMD with contracture of the right ankle joint requiring Achilles tendon lengthening; this patient had an absence of emerin and had a mutation in EMD. Both patients described herein demonstrated proximal muscle weakness with minimal or no joint contractures due to mutations in EMD. Patient 1 showed proximal muscular dystrophy without joint contractures and demonstrated only minimal cardiac involvement as transient sinus arrhythmia. However, joint contractures and a severe cardiac condition may develop in this patient. Patient 2 had unusual clinical findings manifesting as adult-onset LGMD. Minimal joint contractures were noticed only after careful physical examination at the age of 50 years. A conduction defect was observed during the course of his cardiac follow-up for valvular insufficiency. It remains unclear whether the absence of emerin has a role in the development of valvular insufficiency observed in this patient. The same mutation identified in patient 2 was previously reported in a patient with typical EMD. Additional unknown factors may cause different clinical phenotypes in patients harboring identical mutations in the same gene.

Based on these findings, mutations in EMD may cause the clinical phenotype of LGMD and the overlapping state of LGMD and EDMD, as seen in patients with mutations in LMNA. Cardiac involvement is the most important clinical symptom among patients with EMD mutations. Lethal conduction defects with cardiomyopathy have been observed not only in male patients but also in female carriers, at an older age compared with male patients. Careful follow-up of cardiac function is essential, including female family members of patients even in the absence of overt clinical signs or the unavailability of genetic information. Diagnosis of emerin deficiency can be easily performed by immunohistochemical analysis using several tissue specimens. Our results demonstrate the importance of identifying emerin deficiency in patients with LGMD to provide prompt cardiac intervention and to avoid unexpected sudden cardiac death.

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**Correspondence:** Yukiko K. Hayashi, MD, PhD, Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan (hayashi_y@ncnp.go.jp).

**Author Contributions:** Study concept and design: Ura, Hayashi, Ohta, and Nishino. Acquisition of data: Ura, Hayashi, Ohta, Astejada, Murakami, Nagato, Ohta, Daimon, Takekawa, and Hirata. Analysis and interpretation of data: Ura, Hayashi, Nonaka, Noguchi, and Nishino. Drafting of the manuscript: Ura, Nagato, Ohta, Daimon, Takekawa, Hirata, and Noguchi. Critical revision of the manuscript for important intellectual content: Hayashi, Goto, Astejada, Murakami, Nonaka, and Nishino. Obtained funding: Hayashi and Nishino. Administrative, technical, and material support: Hayashi, Goto, Nonaka, and Noguchi.
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