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Expression profiling of muscles from Fukuyama-type congenital muscular dystrophy and laminin-α2 deficient congenital muscular dystrophy; is congenital muscular dystrophy a primary fibrotic disease? ☆

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Abstract

Fukuyama-type congenital muscular dystrophy (FCMD) and laminin-α2 deficient congenital muscular dystrophy (MDC1A) are congenital muscular dystrophies (CMDs) and they both are categorized into the same clinical entity of muscular dystrophy as Duchenne muscular dystrophy (DMD). All three disorders share a common etiologic defect in the dystrophin–glycoprotein complex, which connects muscle structural proteins with the extracellular basement membrane. To investigate the pathophysiology of these CMDs, we generated microarray gene expression profiles of skeletal muscle from patients in various clinical stages. Despite diverse pathological changes, the correlation coefficient of overall gene expression among these samples was considerably high. We performed a multi-dimensional statistical analysis, the Distillation, to extract determinant genes that distinguish CMD muscle from normal controls. Up-regulated genes were primarily extracellular matrix (ECM) components, whereas down-regulated genes included structural components of mature muscle. These observations reflect active interstitial fibrosis with less active regeneration of muscle cell components in the CMDs, characteristics that are clearly distinct from those of DMD. Although the severity of fibrosis varied among the specimens tested, ECM gene expression was consistently high without substantial changes through the clinical course. Further, in situ hybridization showed more prominent ECM gene expression on muscle cells than on interstitial tissue cells, suggesting that ECM components are induced by regeneration process rather than by 'dystrophy.' These data imply that the etiology of FCMD and MDC1A differs from that of the chronic phase of classical muscular dystrophy, and the major pathophysiologic change in CMDs might instead result from primary active fibrosis.

Keywords: Fukuyama-type congenital muscular dystrophy; Laminin-α2 deficient congenital muscular dystrophy; DNA microarray

☆ *Abbreviations:* FCMD, Fukuyama-type congenital muscular dystrophy; MDC1A, laminin-α2 deficient congenital muscular dystrophy; CMD, congenital muscular dystrophy; DMD, Duchenne muscular dystrophy; α-DG, α-dystroglycan; ECM, extracellular matrix.

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Fukuyama-type congenital muscular dystrophy (FCMD; MIM 253800) is an autosomal recessive disorder and the second most common muscular dystrophy, following DMD, in Japan [1]. Clinical manifestations of FCMD include severe muscle wasting from early infancy with malformation of the brain and eyes. We previously isolated the responsible gene for FCMD, termed *fukutin* [2,3]. Fukutin presumably modulates the glycosylation of α-dystroglycan (α-DG), one of the major components of the dystrophin–glycoprotein complex, since α-dystroglycan is hypoglycosylated in FCMD muscle [4,5]. Fukutin-mediated glycosylation is crucial for binding of α-DG to ECM proteins such as laminin, agrin, and perlecan, which are important for maintaining muscle cell integrity [5]. Hypoglycosylation of α-DG is likely to attenuate the stable connection between skeletal muscle sarcoplasmic membrane and the basement membrane. This architectural and functional instability of muscle fibers is presumed to induce severe muscular dystrophy in FCMD [4,6].

Accumulating evidence indicates that hypoglycosylation of α-DG underlies a number of muscular dystrophies. Hypoglycosylated α-DG provokes the post-translational disruption of dystroglycan-ligand interactions in skeletal muscle, leading to the severe phenotypes of congenital muscular dystrophies [7]. Mutations in glycosyltransferases have been linked to several muscular dystrophies, including POMGnT1 (protein *O*-mannose β-1, 2-*N*-acetylglucosaminyltransferase 1) with muscle–eye–brain disease; POMT1/2 (protein *O*-mannosyltransferase 1/2) with Walker–Warburg syndrome; FKRP (fukutin-related protein) with congenital muscular dystrophy type 1C (MDC1C). In addition, mutations in the putative glycosyltransferase LARGE correlate with myodystrophy in mice and human congenital muscular dystrophy 1D (MDC1D) [8–13].

Another congenital muscular dystrophy, laminin-α2 deficient congenital muscular dystrophy (MDC1A), is one of the most common childhood congenital muscular dystrophies in the European population [14]. MDC1A is an autosomal recessive disorder that is caused by mutation of the *LAMA2* gene [15]. Clinical muscular features of MDC1A are grossly identical to the FCMD phenotype and characterized as severe neonatal hypotonia. Laminin-α2 is the main component of the basement membrane and plays an essential role in its formation, serving as a signaling molecule to interact with other ECM or sarcoplasmic membrane components [16]. α-DG is a primary receptor for laminin-α2 on the sarcoplasmic membrane [17,18]. Thus, attenuated connections between laminin and α-DG might account for muscular dystrophy in MDC1A.

Although DMD and these CMDs are categorized into the same clinical entity, 'muscular dystrophies,' manifestations of CMDs are known to differ from those of DMD in several ways. First, patients with CMDs show congenital anomalies in muscle pathology, implying developmental defects in CMD muscle [19]. Second, these CMDs show severe pathological changes in the early infantile period with almost fixed characteristics thereafter, unlike the later onset and progressive features of DMD [1]. Finally, the pathophysiology of CMD muscle is characterized by few active dystrophic and regenerating fibers, correlating with moderate increases in serum CK levels [1]. In contrast, muscle specimens from DMD patients show severe necrotic changes and active regeneration, both of which are evidenced by extremely high serum CK levels.

Dystrophic features in CMD have been defined by the observation of 'opaque fibers' in biopsied specimens, which are suggested to indicate pre-necrotic fibers like those often seen in DMD muscle. Opaque fibers in CMD biopsied muscle specimens are unexpectedly prominent, considering the moderate serum CK levels. However, we suspect that the opaque fibers are artificially generated by the biopsy itself, due to the technical tension placed on fragile CMD muscle membranes (unpublished data). Therefore, the pathological characteristics of these CMDs are not likely the same as those of late-stage DMD. The pathophysiological and molecular mechanisms of these CMDs deserve further investigation.

Although gene expression profiling of DMD skeletal muscle has been described previously [20–22], neither FCMD nor MDC1A has been characterized. To investigate the molecular mechanisms of CMDs relative to DMD, we profiled gene expression in FCMD and MDC1A muscle using a custom cDNA microarray. Profiles were analyzed by a multi-dimensional statistical analysis, the Distillation, to extract determinant genes, revealing a more detailed molecular mechanism for these CMDs.

Materials and methods

Patient materials. All clinical materials were collected for diagnostic purposes. Four muscle specimens (biceps branchii) from FCMD patients (F1–F4) and one from MDC1A (M1) were used in the analysis (Fig. 1). Genetic screening identified a homozygous retrotransposal insertion into the 3′ untranslated region of *fukutin* in all patients [3]. For MDC1A, the patient showed a typical disease onset, and the specimen was negative for anti-laminin-α2 staining. We used pooled muscle RNA (Origene Technologies) as an internal standard for microarray experiments. For age-matched non-dystrophic controls, muscle RNA from two children was used (C1 and C2, 1 year old, and 7 years old, respectively). These two samples were selected based on normal laboratory findings, normal plasma CK levels, and no histological findings of muscular dystrophy.

Histological analysis. Serial sections from the biopsied cryospecimen used for microarray experiments were stained with hematoxylin and eosin by standard methods. Scion Image Beta 3b (Scion) was used for the two-dimensional morphometric analysis of muscle specimen. For each cryospecimen, ten pictures were taken randomly. We measured the area of muscle tissue, adipose tissue, and the remaining interstitial tissues, and then measured the average cross-sectioned muscle fiber diameters. Statistical analysis was performed using Student's t test ($p < 0.005$).

RNA isolation and expression profiling. Generation of cDNA microarrays of skeletal muscle transcripts has been reported previously [22]. Using a similar method, we constructed a cDNA chip containing 5600 genes expressed in skeletal muscle. RNA isolation, hybridization, and detection methods also have been previously reported. Microarray experiments were carried out using a competitive hybridization method with two labeled targets: one for muscle RNAs from FCMD, MDC1A or normal controls, and another for pooled muscle RNA (Origene), which