

Fukuyama-type Congenital Muscular Dystrophy and Abnormal Glycosylation of α -Dystroglycan

Tatsushi Toda, Kazuhiro Kobayashi, Satoshi Takeda⁽¹⁾, Junko Sasaki, Hiroki Kurahashi, Hiroki Kano, Masaji Tachikawa, Fan Wang, Yoshitaka Nagai, Kiyomi Taniguchi, Mariko Taniguchi, Yoshihide Sunada⁽²⁾, Toshio Terashima⁽³⁾, Tamao Endo⁽⁴⁾ and Kiichiro Matsumura⁽⁵⁾

Division of Functional Genomics, Department of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Suita, (1) Otsuka GEN Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, (2) Department of Neurology, Kawasaki Medical School, Kurashiki, (3) Department of Anatomy and Neurobiology, Kobe University Graduate School of Medicine, Kobe, (4) Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Tokyo and (5) Department of Neurology, Teikyo University School of Medicine, Tokyo, Japan

Abstract

Fukuyama-type congenital muscular dystrophy (FCMD), Walker-Warburg syndrome (WWS), and muscle-eye-brain (MEB) disease are clinically similar autosomal recessive disorders characterized by congenital muscular dystrophy, lissencephaly, and eye anomalies. We identified the gene for FCMD and MEB, which encodes the fukutin protein and the protein *O*-linked mannose β 1, 2-N-acetylglucosaminyltransferase (POMGnT1), respectively. α -dystroglycan is a key component of the dystrophin-glycoprotein-complex, providing a tight linkage between the cell and basement membranes by binding laminin via its carbohydrate residues. Recent studies have revealed that posttranslational modification of α -dystroglycan is associated with these congenital muscular dystrophies with brain malformations.

Key Words: Fukuyama congenital muscular dystrophy (FCMD), muscle-eye-brain (MEB) disease, fukutin, α -dystroglycan, glycosylation.

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Fukuyama-type congenital muscular dystrophy (FCMD) [10], Walker-Warburg syndrome (WWS) [8], and muscle-eye-brain (MEB) disease [28] are clinically similar autosomal recessive disorders characterized by congenital muscular dystrophy, lissencephaly, and eye anomalies. FCMD patients survive beyond infancy, and ocular manifestations are rare and usually mild. Patients with WWS are severely affected from birth, and few live beyond infancy. In MEB, the cerebral and ocular anomalies are severe, but some patients reach adulthood. While FCMD is frequent only in Japan, WWS has been found in many different nationalities, and MEB has been observed mainly in Finland, although we recently proposed that MEB has a worldwide distribution including Japan and Korea [31]. Structural defects in the dystrophin-glycoprotein-complex (DGC) can result in a loss of linkage between

laminin-2 (merosin) in the extracellular matrix and actin in the subsarcolemmal cytoskeleton, and this can lead to various muscular dystrophies [4]. Of these, α -dystroglycan is a heavily glycosylated mucin-type glycoprotein on the surface of muscle cells. It is the key component of the DGC, providing a tight linkage between the cell and basement membranes by binding laminin via its carbohydrate residues [5, 9, 18] (Figure 1). α -dystroglycan plays an active role in the basement membrane assembly itself [15]. Recent studies have revealed that posttranslational modification of α -dystroglycan is associated with congenital muscular dystrophy with brain malformations.

Clinical and Pathological Characteristic of Fukuyama CMD

FCMD, a relatively common autosomal recessive disorder in the Japanese population, is characterized by

congenital muscular dystrophy in combination with cortical dysgenesis (micropolygyria) and ocular abnormality [10]. It is the second most common form of childhood muscular dystrophy in Japan after Duchenne muscular dystrophy. Based on the averaged incidence of 3/100,000, one in ~90 persons could be a heterozygous carrier in Japan.

Patients with FCMD manifest weakness of facial and limb muscles, and general hypotonia that usually appears before nine months of age. The joints of affected individuals usually become contractive at an earlier stage than those in patients with DMD. Functional disability is more severe in FCMD patients than in DMD patients; usually the maximum level of motor function achieved is sliding while sitting on the buttocks, and most FCMD patients are never able to walk. Facial muscle involvement is characteristic. Patients usually become bedridden before 10 years of age due to generalized muscle atrophy and joint contracture, and most of them die by 20 years of age [10]. Another manifestation observed in all cases is severe mental retardation; IQ scores in most FCMD patients lie between 30 and 50. Seizures occur in nearly half of the cases, in association with abnormal EEGs [10]. Further, eye is also involved. The ophthalmologic lesions include myopia, cataract, abnormal eye movement, pale optic disc, retinal round lesion, and retinal detachment [27]. In skeletal muscle, there is a prominent necrotic and regenerating process, with dense fibrosis from early infancy. Small fibers are predominant in comparison with DMD. The most common and characteristic changes in the central nervous system are brain malformations, which include micropolygyria, pachygyria, and agyria of the cerebrum (cobblestone

cortex) lacking neuronal lamination of normal six-layered cortex. The formation of cerebellar foliation is disrupted, and beautiful 3-layered structures of the cerebellar cortex is regionally disorganized. In addition, focal interhemispheric fusion, fibroglial proliferation of the leptomeninges, mild to moderate ventricular dilatation, and hypoplasia of the corticospinal tracts are also often observed. Brain MRI shows always pachygyria in the cerebral cortex and transient T2-weighted high intensity in the white matter; there are sometimes hypoplasia of the pons and cerebellar cysts. The high intensity in the white matter is thought to be due to delayed myelination [10, 27].

Pathological study of brain tissue from FCMD fetuses revealed frequent breaks in the glia limitans and basement membrane complex. Because of this break, developing neurons overmigrate in the cerebrum, resulting in the failure to form a six-layered cortex [26]. Disruption of the basal lamina in FCMD muscle was also seen [19]. Thus, structural alteration of the basal lamina appears to play a key role in the pathophysiology of FCMD.

Fukutin Gene and Mouse Model

We previously identified on chromosome 9q31 the gene responsible for FCMD, which encodes a novel 461-amino-acid protein we have named fukutin [21, 32, 33]. The gene spans more than ~100 kb genomic DNA region. It is composed of 10 exons [22]. Most FCMD-bearing chromosomes (87%) have been derived from a single ancestral founder, who lived 2,000-2,500 years ago [6] and whose mutation consisted of a 3kb retrotransposal insertion in the 3' non-coding region of the fukutin gene. This insertion results in the reduction of mRNA. FCMD is the first known human disease to be caused by an ancient retrotransposal integration [21]. Point mutations have been seen to render the FCMD phenotype rather severe. Very few FCMD patients [29] have been identified with non-founder (point) mutations on both alleles, suggesting that such patients are embryonic-lethal and that fukutin is essential for normal development. This may explain why FCMD could occur only in the Japanese patients who have a milder retrotransposon mutation [23]. Fukutin is a protein of 461 amino acids with molecular weight of 53.7 kD. Fukutin is a novel protein and has an N-terminal hydrophobic region suggesting a signal sequence or a transmembrane domain.

There are no reported naturally occurring mice carrying mutations in the *fukutin* gene. Targeted homozygous mutation of the mouse *fukutin* gene [16] in mice leads to lethality at embryonic day 6.5-7.5, prior to development of skeletal muscle, cardiac muscle or mature neurons, suggesting that fukutin is essential for early embryonic development (Kurahashi *et al.*, unpublished data).

Chimeric mice generated using embryonic stem (ES) cells targeted for both *fukutin* alleles develop severe muscular dystrophy, with the selective deficiency of α -

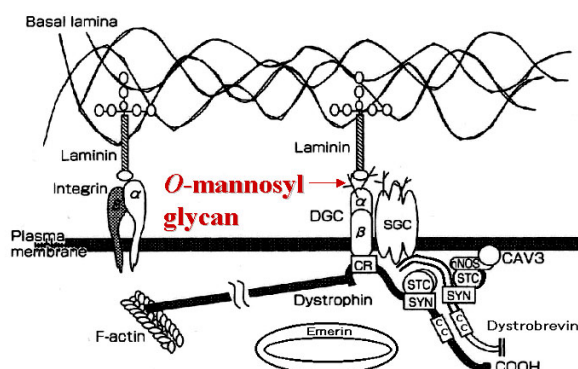


Figure 1. Dystrophin-glycoprotein-complex (DGC) and linkage between laminin-2 (merosin) in the extracellular matrix and actin in the subsarcolemmal cytoskeleton. α -dystroglycan is the key component of the DGC and is modified by O-linked mannosyl glycan and bind laminin via its glycan. Similar hypoglycosylation in FCMD, MEB, and WWS directly abolishes binding activity of dystroglycan for laminin. DGC, dystroglycan complex; SGC, sarcoglycan complex; and STC, syntrophin complex.

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dystroglycan and its laminin-binding activity (Figure 2). In addition, these mice showed laminar disorganization of the cortical structures in the brain with impaired laminin assembly, focal interhemispheric fusion, and hippocampal and cerebellar dysgenesis (Figure 3). Further, chimeric mice showed anomaly of the lens, loss of laminar structure in the retina, and retinal detachment. Injection of fukutin by electroporation resulted in restoration of α -dystroglycan.

These results indicate that fukutin is necessary for the maintenance of muscle integrity, cortical histogenesis, and normal ocular development and suggest the functional linkage between fukutin and α -dystroglycan. These mice are suitable models for studying the molecular pathogenesis and therapeutic approaches of the complex disorders consisting of the simultaneous occurrence of central nervous, ocular, and muscular abnormalities [30].

Abnormal Glycosylation of α -Dystroglycan

At present, the function of fukutin remains unknown, and the mechanism by which its deficiency causes defects in multiple organs has not been clarified. In this respect, it should be noted that sequence analysis predicts fukutin to be an enzyme that modifies cell-surface glycoproteins or glycolipids [1].

There are several lines of indirect but significant evidence to support this. First, it was reported that highly glycosylated α -dystroglycan was selectively deficient in the skeletal muscle of FCMD patients [14]. Second, we have reported that muscle-eye-brain disease (MEB), an autosomal recessive disorder having skeletal muscle, eye, and brain defects similar to FCMD, is caused by mutations in the gene encoding the protein *O*-linked mannose β 1, 2-N-acetylglucosaminyltransferase (POMGnT1), which cause the loss of the enzyme activity [34]. Moreover, we have found the selective deficiency of α -dystroglycan in the skeletal muscle of MEB patients [20]. Finally, defective glycosylation of

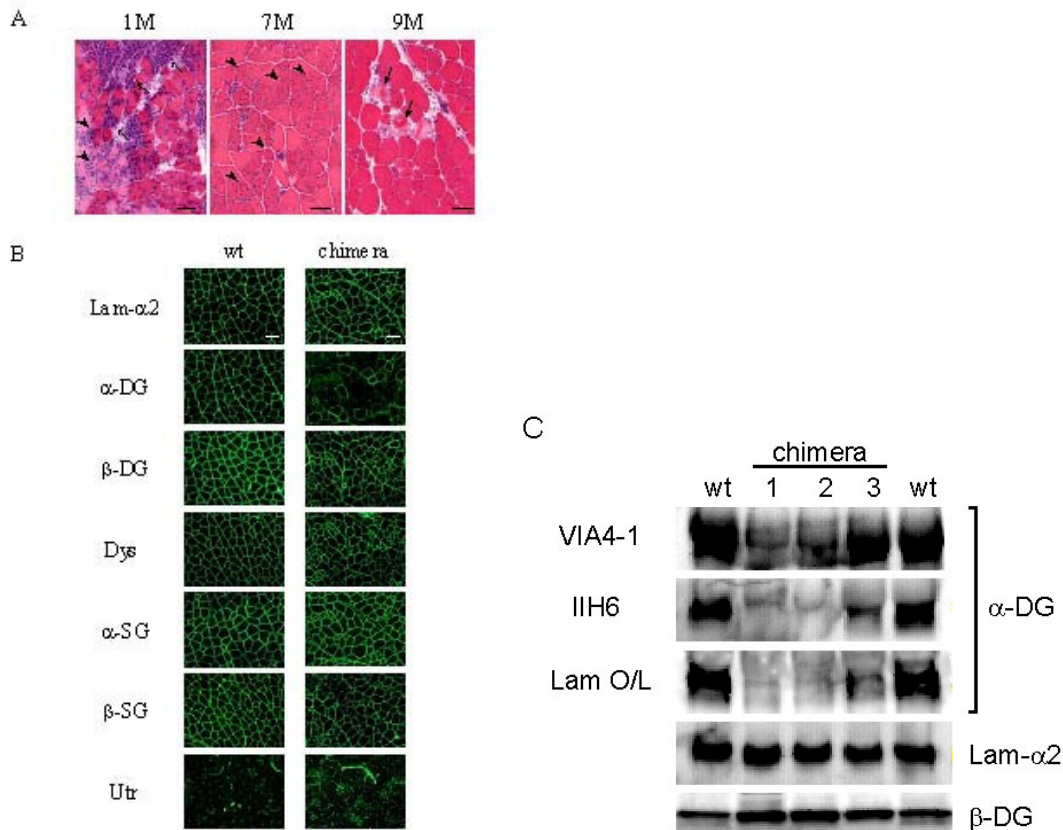


Figure 2. Muscular dystrophic changes in fukutin-deficient chimeric mice. (A) HE stained quadriceps muscle of chimeric mice. Massive necrosis with phagocytosis (asterisk), mononuclear cell infiltration (arrowhead), basophilic regenerating fibers (arrow), and an increase in connective tissue mass was present at 1 month of age. At the late stages of 7 to 9 months, a large number of small sized fibers were found to have central nuclei (arrowhead), while a small number of fibers were undergoing active degeneration (arrow). Scale bar, 100 μ m. (B) Immunohistochemical analysis of sarcolemmal proteins in quadriceps muscle from normal (wt) and chimeric mice. Lam- α 2, laminin α 2 chain; DG, dystroglycan; Dys, dystrophin; SG, sarcoglycan; Utr, utrophin. Selective and scattered deficiency of α -dystroglycan was observed in chimeric mice, while the other proteins were preserved. Scale bar, 100 μ m. (C) Immunoblot and laminin blot overlay analyses of quadriceps muscle from the two normal (wt) and three chimeric mice. α -dystroglycan immunoreactivity, as well as its laminin-binding activity, was greatly reduced in chimeric mice, while β -dystroglycan and laminin α 2 chain were preserved. The deficiency of α -dystroglycan revealed by laminin blot overlay paralleled that revealed by monoclonal antibody IIH6C4 and was more prominent than that revealed by monoclonal antibody VIA4-1.

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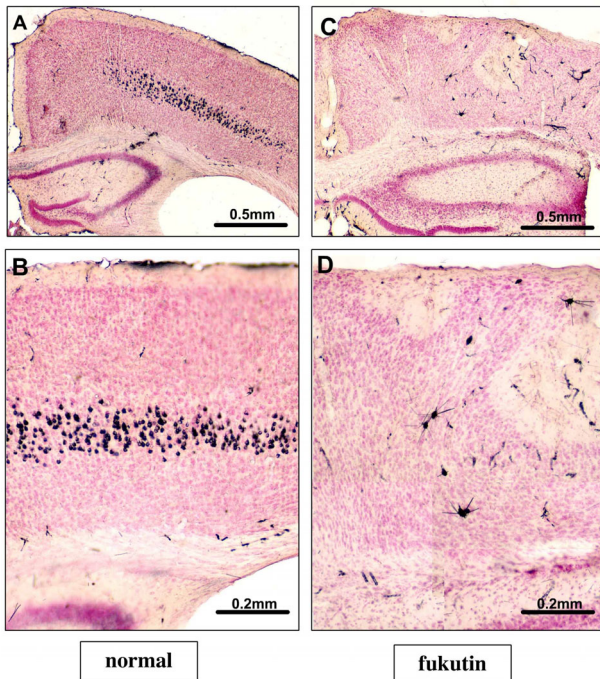


Figure 3. Brain anomalies in fukutin-deficient chimeric mice. HRP retrograde labeling of cerebral cortex from normal (**A** and **B**) and chimeric (**C** and **D**) mice. In chimeric mice, HRP-labeled corticospinal neurons were not localized in layer V but scattered throughout all the depths of the motor cortex.

α -dystroglycan has also been reported in congenital muscular dystrophy with secondary meosin deficiency 2 (MDC1C), caused by mutations in the gene encoding the putative glycosyltransferase named FKR (fukutin-related protein) [3] and in myodystrophy (*myd*) mice, an animal model of congenital muscular dystrophy, caused by the mutation in the gene encoding a putative glycosyltransferase named Large [13], although brain and eye anomalies are not the hallmarks of MDC1C. Recently 20% of WWS patients has been found to have mutations in POMT1, a putative human counterpart of yeast O-mannosyltransferase [2] (Figures 1 and 4, Table 1).

Moreover, Michele *et al.* showed, in MEB, FCMD, and *myd* mice, that α -dystroglycan is expressed at the muscle membrane, but similar hypoglycosylation in the diseases directly abolishes binding activity of dystroglycan for the ligands laminin, neurexin, and agrin [24] (Figure 1). Together with the present results, these findings suggest that defective glycosylation of α -dystroglycan due to the primary genetic defects of glycosyltransferases may be the common denominator causing muscle cell degeneration in these diseases. Fukutin may be involved in the modification of laminin-binding carbohydrate residues in α -dystroglycan, and, in the absence of fukutin, the linkage between laminin and α -dystroglycan may never be established on the

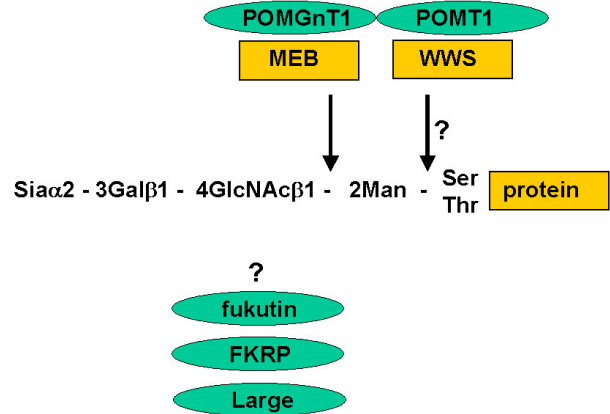


Figure 4. O-mannosyl glycan found in brain, nerve, and muscle and its synthesis by POMGnT1 and POMT1. Only POMGnT1 for MEB has a demonstrated enzymatic activity.

muscle cell surface. This scenario is also consistent with the report that chimeric mice lacking skeletal muscle dystroglycan [7] developed muscular dystrophy similar to the fukutin-deficient chimeric mice [30].

Finally, the role of dystroglycan in the pathogenesis of brain and eye defects in FCMD remains unclear. Brain and/or eye defects similar to those reported here have recently been observed in mice lacking dystroglycan in the brain via Cre/loxP-mediated gene inactivation [25] and in *myd* mice, although the retina was apparently morphologically normal [17]. Interestingly, brain and/or eye defects similar to those reported here have also been observed in mice lacking integrin β 1 subunit in brain via Cre/loxP-mediated gene inactivation [12] and in mice carrying a targeted mutation in the integrin α 6 subunit gene [11], respectively. We can envisage that the defects in dystroglycan have similar consequences as those caused by integrin deficiency.

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Address correspondence to:

Dr. Tatsushi Toda, Professor and Head, Division of Functional Genomics, Department of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, 2-2-B9 Yamadaoka, Suita, Osaka 565-0871, Japan, tel. +81-6-6879-3380, fax. +81-6-6879-3389, Email toda@clgene.med.osaka-u.ac.jp

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