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Congenital muscular dystrophy with glycosylation defects of α-dystroglycan in Japan

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Abstract

Glycosylation defects of α-dystroglycan (α-DG) cause various muscular dystrophies. We performed clinical, pathological and genetic analyses of 62 Japanese patients with congenital muscular dystrophy, whose skeletal muscle showed deficiency of glycosylated form of α-DG. We found, the first Japanese patient with congenital muscular dystrophy 1C with a novel compound heterozygous mutation in the fukutin-related protein gene. Fukuyama-type congenital muscular dystrophy was genetically confirmed in 54 of 62 patients. Two patients with muscle–eye–brain disease and one Walker-Warburg syndrome were also genetically confirmed. Four patients had no mutation in any known genes associated with glycosylation of α-DG. Interestingly, the molecular mass of α-DG in the skeletal muscle was similar and was reduced to ~90 kDa among these patients, even though the causative gene and the clinico-pathological severity were different. This result suggests that other factors can modify clinical features of the patients with glycosylation defects of α-DG.

Keywords: α-dystroglycan (α-DG); Fukuyama-type congenital muscular dystrophy (FCMD); Congenital muscular dystrophy 1C (MDC1C); Muscle-eye-brain disease (MEB); Walker-Warburg syndrome (WWS); Glycosylation; Fukutin; FKRP; POMGnT1; POMT1; LARGE

1. Introduction

Recent advances demonstrated that glycosylation defects of cell surface membrane protein, α-dystroglycan (α-DG) cause a group of muscular dystrophy, including Fukuyama-type congenital muscular dystrophy (FCMD), muscle–eye–brain disease (MEB), Walker-Warburg syndrome (WWS), congenital muscular dystrophy 1C (MDC1C) and its allelic limb-girdle muscular dystrophy (LGMD) 2I, and congenital muscular dystrophy 1D (MDC1D) [1–8]. Some of these forms are associated with neuronal migration disorder in brain and ocular abnormalities, and others with normal brain and eyes. Characteristically, they all show abnormally glycosylated α-DG with preserved core structure in the muscle sarcolemma [9]. From this result, the responsible gene products of these diseases are thought to have a role in the glycosylation process of α-DG. In fact, mutations in the glycosyltransferase genes of protein *O*-mannose β 1,2-*N*-acetylglucosaminyltransferase 1 (*POMGnT1*) and protein *O*-mannosyltransferase 1 (*POMT1*) have been identified in patients with MEB and WWS, respectively [3,4]. In addition, other responsible gene products of fukutin, fukutin-related protein (FKRP), and LARGE are also predicted to have structural similarity to glycosyltransferases [10].

In Japan, FCMD is the most common form of congenital muscular dystrophy (CMD) [11], whereas merosin-deficient

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CMD (MDC1A), which is common in European countries, MEB, and WWS were rarely seen [12,13]. Patients with MDC1C and MDC1D have not been identified yet in Japan. To know more about the CMD patients with glycosylation defects of α-DG in Japan, we performed detailed genetic and clinico-pathological analyses on 62 patients.

2. Materials and Methods

2.1. Clinical materials

All clinical materials were obtained for diagnostic purposes with informed consent. We analyzed a total of 62 patients whose limb-muscle specimens showed altered glycosylation of α-DG. The clinical diagnoses of the 62 patients are shown in Table 1. The muscle samples were flash-frozen in isopentane chilled with liquid nitrogen.

2.2. Immunohistochemistry, immunoblotting, and laminin overlay assay

The following antibodies were used for immunohistochemical and immunoblotting analyses: monoclonal anti-α-DG (VIA4-1, Upstate Biotechnology), polyclonal goat anti-α-DG (GT20ADG) [9], monoclonal anti-laminin α2 chain (5H2, Chemicon), polyclonal anti-laminin-1 (Sigma), and monoclonal anti-β-DG (43DAG1/8D5, Novocastra Laboratories). The detailed techniques of the immunohistochemistry, immunoblotting and laminin overlay assay have been described previously [1,9].

2.3. Genetic analyses of fukutin, FKRP, POMGnT1, POMT1, and LARGE

DNA was isolated from skeletal muscle or peripheral lymphocytes using a standard technique.

To detect the 3-kb retroransposal insertion in *fukutin*, the genomic PCR was performed using two primer sets; one is designed to amplify a 375 bp product containing a part of retrotransposal insertion and the other is designed to amplify a normal 157 bp fragment (the primers were designed by Dr Toda, Osaka University). All exons and their flanking intronic regions of *fukutin* [14] were directly sequenced in patients without homozygous retrotransposal insertion using an ABI PRISM 3100 automated sequencer (PE Applied Biosystems).

Mutation analysis of *FKRP* was performed using the primers reported elsewhere [15].

Mutation analysis of *POMGnT1*, *POMT1*, and *LARGE* was performed by directly sequencing all exons and their flanking introns. The information on primer sequence and PCR conditions is available upon request. To detect the mutation in exon 11 of *POMGnT1* in patient 2, primers F (5′-CATTCACCTCTGTGGGTAAGC) and R (5′-AGGCC TTCACATTTCACAGC) were used.

Table 1
Clinical and genetic diagnosis of 62 patients

Genetic diagnosis		Clinical diagnosis	
FCMD	54	FCMD	53
		MEB	1
MEB	2	FCMD	1
		CMD	1
WWS	1	WWS	1
MDC1C	1	FCMD	1
Unknown	4	MEB	1
		WWS	3

2.4. Single-strand conformation polymorphism (SSCP) analysis of FKRP

To exclude the possibility of polymorphism, we performed SSCP analysis for the missense mutation identified in *FKRP* in patient 1, using Gene Gel Excel (Pharmacia Biotech). The amplified genomic DNA fragments using a set of primers (4-2F and 4-2R [15]) including the site of the missense mutation was electrophoresed for 600 mA at 10 °C in a Gene Phor Electrophoresis Unit (Pharmacia Biotech). One hundred chromosomes from healthy individuals were analyzed as control.

3. Results

We found the first patient with MDC1C (patient 1) in the oriental countries. *Fukutin* mutations were found in 87% of the patients examined, and two MEB (patients 2 and 3) and one WWS (patient 4) were genetically confirmed. Four patients had no mutation in the known genes associated with glycosylation defects of α-DG (Table 1).

3.1. Clinical features of the patients

Patient 1 (MDC1C) was a Japanese girl and first admitted to a hospital at 12-months old. She was the first child of nonconsanguineous healthy Japanese parents. From at birth, left eye strabismus was seen, and the floppiness and delayed motor milestones became apparent in growing. She was able to sit at 7 months, but unable to crawl or stand up at 12 months of age. She spoke some meaningful words, and no mental retardation was observed. Serum creatine kinase (CK) level was 6429 IU/l. Muscle biopsy was performed at 12 months of age and showed dystrophic changes with marked variation in fiber size, active necrotic and regenerating process, and dense interstitial fibrosis (Fig. 1A). She was diagnosed to have FCMD. At 6-years-old, generalized muscular atrophy was marked, but she could move by herself using her wheelchair. Facial and calf muscles were mildly hypertrophic and high-arched palate was seen. Tongue hypertrophy was not apparent. Cardiac dysfunction was not detected from the chest radiograph or electrocardiogram. Joint contractures