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Limb-girdle muscular dystrophy type 2I is not rare in Taiwan

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Abstract

Alpha-dystroglycanopathy is caused by the glycosylation defects of α-dystroglycan (α-DG). The clinical spectrum ranges from severe congenital muscular dystrophy (CMD) to later-onset limb girdle muscular dystrophy (LGMD). Among all α-dystroglycanopathies, LGMD type 2I caused by *FKRP* mutations is most commonly seen in Europe but appears to be rare in Asia. We screened uncategorized 40 LGMD and 10 CMD patients by immunohistochemistry for α-DG and found 7 with reduced α-DG immunostaining. Immunoblotting with laminin overlay assay confirmed the impaired glycosylation of α-DG. Among them, five LGMD patients harbored *FKRP* mutations leading to the diagnosis of LGMD2I. One common mutation, c.948delC, was identified and cardiomyopathy was found to be very common in our cohort. Muscle images showed severe involvement of gluteal muscles and posterior compartment at both thigh and calf levels, which is helpful for the differential diagnosis. Due to the higher frequency of LGMD2I with cardiomyopathy in our series, the early introduction of mutation analysis of *FKRP* in undiagnosed Taiwanese LGMD patients is highly recommended.

Keywords: Alpha-dystroglycan; Alpha-dystroglycanopathy; Limb-girdle muscular dystrophy type 2I; *FKRP*; Dilated cardiomyopathy; Glycosylation defect; Laminin binding; Muscle imaging

1. Introduction

Alpha-dystroglycanopathy is a group of muscular dystrophies caused by altered glycosylation of α-dystroglycan (α-DG), which is one of the components of dystrophin–glycoprotein complex [1,2]. The clinical phenotypes form a broad spectrum, ranging from severe congenital muscular dystrophy (CMD) with or without ocular and central nervous system involvement to later-onset limb girdle muscular dystrophy (LGMD) [3–5].

A number of genes have been reported to cause α-dystroglycanopathy, including *POMT1*, *POMT2*, *POMGnT1*, *FKTN*, *FKRP*, and *LARGE* that are known to be involved in glycosylation of α-DG, and *DAG1*, which encodes DG itself [6–11]. Recently, the number of genes associated with α-dystroglycanopathy has been increasing to include *ISPD*, *TMEM5*, *GTDC2*, *B3GNT1*, *DOLK*, *DPM2* and *DPM3* [12–19]. Patients with all

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kinds of α-dystroglycanopathy are inherited with autosomal recessive trait.

Among those causative genes for α-dystroglycanopathy, *FKRP* mutations are the most frequently seen in the Caucasian population, causing LGMD2I and congenital muscular dystrophy type 1C (MDC1C). In the Asian population, on the other hand, the most common α-dystroglycanopathy is Fukuyama congenital muscular dystrophy and LGMD2M caused by the mutations in *FKTN* [20–24]. This phenomenon may be caused by the founder effect of c.826C>A substitution in *FKRP* and the ancestral insertion of a SINE-VNTR-Alu (SVA) retrotransposon in *FKTN* in different geographic areas [21,25]. Recently, an increasing number of patients having *FKTN* mutations were identified outside Asia but so far few Asian patients with LGMD2I caused by *FKRP* mutations have been reported [26–29].

In this study, we found that LGMD2I is common in the Taiwanese patients with α-dystroglycanopathy due to a common mutation, c.948delC (p.Cys317Alafs∗111), which may cause more severe phenotype and cardiomyopathy.

2. Materials and methods

2.1. Patients

Forty patients clinically and pathologically diagnosed as LGMD and 10 patients with CMD who received muscle biopsy in Kaohsiung Medical University Hospital from January, 2008 to December, 2011 were enrolled. LGMD was defined as progressive proximal-dominant muscle weakness with characteristic dystrophic changes in muscle pathology. CMD was recognized as infantile floppiness with dystrophic muscle. Patients with deficiencies of dystrophin, sarcoglycans, dysferlin, merosin or collagen VI were excluded by immunohistochemistry beforehand. All merosin deficiency patients were confirmed to have *LAMA2* mutations [30]. This study was approved by the institutional review board of the Kaohsiung Medical University Hospital.

2.2. Histochemistry

Biopsied muscle specimens were frozen in isopentane cooled in liquid nitrogen. A serial frozen section was stained by a battery of histochemical methods including hematoxylin and eosin (H&E), modified Gomori-trichrome (mGt) and NADH-tetrazolium reductase (NADH-TR).

2.3. Immunohistochemistry

Frozen sections of 6 μm thickness were used for immunohistochemistry according to the standard protocols with Vantana Benchmark automated stainer. Primary antibodies used in this study were monoclonal anti-α-DG (VIA4-1; Upstate Biotechnology, Lake Placid, NY, USA) and anti-β-DG (43DAG1/8D5; Novocastra Laboratories, Newcastle upon Tyne, UK) antibodies.

2.4. Immunoblotting and laminin overlay assay

The detailed techniques of immunoblotting, and laminin overlay assay have been described previously [31]. The following antibodies were used for immunoblotting analysis: monoclonal anti-α-DG (VIA4-1) and polyclonal anti-α-DG (GT20ADG, kindly provided by Prof. K. Campbell, Iowa Univ.), polyclonal anti-laminin-1 (Sigma, St. Louis, MO, USA), and monoclonal anti-β-DG (43DAG1/8D5).

2.5. Mutation analyses of α-DGP associated genes

Genomic DNA was extracted from leukocytes in peripheral blood lymphocytes according to standard protocols. All exons and their flanking intronic regions of *FKRP* (NM_024301.4), *FKTN* (NM_001079802.1), *POMGnT1* (NM_001243766.1), *POMT1* (NM_007171.3), *POMT2* (NM_013382.5), and *LARGE* (NM_004737.4) were amplified and sequenced using an automated 3100 DNA sequencer (Applied Biosystems, Foster, CA, USA). Primer sequences are available upon request. DNA samples from 100 Taiwanese individuals without apparent neuromuscular disorders were analyzed as controls.

3. Results

3.1. Patients with α-DGP caused by FKRP mutations

Seven of 50 patients with unclassified LGMD and CMD had a reduced α-DG immunoreaction using VIA4-1antibody, which recognizes glycosylated forms of α-DG on muscles, and they were thus considered to have α-dystroglycanopathy (Fig. 1). Among these seven patients, six had LGMD phenotype and one was CMD. Mutation screening revealed that five LGMD patients from four families harbored *FKRP* mutations (Fig. 2). No mutation in *FKTN*, *POMGnT1*, *POMT1*, *POMT2* and *LARGE* was identified in these seven patients. The clinical, pathological and biochemical information of all five patients with *FKRP* mutations are summarized in Table 1 together with that of a previously reported Taiwanese LGMD2I patient who was the first reported case in East-Asia (Patient 6) [26]. The c.948delC (p.Cys317Alafs∗111) mutation was found heterozygously in four newly diagnosed patients (Patients 2, 3, 4 and 5) as well as in Patient 6. Patients 2, 3, and 4 carried a c.545A>G (p.TyrY182Cys) mutation, which was previously reported in two Brazilian patients, and a c.823C>T (p.Arg275Cys) mutation was identified in Patients 5 and 6. The compound heterozygous mutations for Patients 2, 5, and 6 were also found to lie on different parental alleles. Patient 1 bears two different novel