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Case report

A Portuguese case of Fukuyama congenital muscular dystrophy caused by a multi-exonic duplication in the fukutin gene

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Abstract

Fukuyama congenital muscular dystrophy (FCMD) is one of the most common autosomal recessive diseases among the Japanese population, due to a founder mutation of the fukutin gene (*FKTN*). Mutations in *FKTN* are now being described in an increasing number of non-Japanese patients. We report a Portuguese child with FCMD. The diagnosis was supported by clinical, histological, magnetic resonance imaging (MRI) and genetic studies. Genetic analysis of *FKTN* by Multiplex Ligation Probe Amplification (MLPA) revealed a homozygous duplication from exon 4 to exon 7. This in-frame duplication was confirmed by cDNA analysis. To our knowledge this is the first report of a FCMD case caused by an intragenic gross exonic duplication in the *FKTN* gene. This report widens the clinical and mutational spectrum in FCMD and corroborates the importance of screening for large deletions and duplications in CMD patients.

Keywords: Duplication; Fukutin; FKTN; Fukuyama congenital muscular; Dystrophy; α-Dystroglycanopathies

1. Introduction

Congenital muscular dystrophies (CMDs) with hypoglycosylation of α-dystroglycan (α-DG) are clinically and genetically heterogeneous disorders often involving brain, eyes, and muscle [1]. Causal mutations have been identified in eight glycosyltransferase genes: protein-*O*-mannosyl transferase 1 (*POMT1*) [2], protein-*O*-mannosyl transferase 2 (*POMT2*) [3], protein-*O*-mannose1,2-*N*-acetylglucosaminyltransferase 1 (*POMGNT1*) [4], fukutin (*FKTN*) [5], fukutin-related protein (*FKRP*) [6], like-glycosyltransferase (*LARGE*) [7], isoprenoid synthase domain-containing protein (*ISPD*) [8,9] glycosyltransferase-like domain containing 2 (*GTDC2*) [10]. Mutations in each of these genes have been associated with a wide variety of phenotypes ranging from the severe Walker–Warburg syndrome (WWS) to milder variants of limb girdle muscle dystrophy.

Fukuyama congenital muscular dystrophy (FCMD), is the second most common form of muscular dystrophy in the Japanese population, after Duchenne muscular dystrophy, with a carrier frequency of one in 88 [11], an estimated incidence of 1 in 10,000 births [12] and is one of the most common autosomal recessive disorders in

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Japan. The high incidence is related to a founder mutation located in the 3'UTR of *FKTN* [5]. Seventy-five percent of Japanese patients are homozygous for this ancestral mutation [13]. Clinically, the classical Japanese FCMD is characterized by an early onset with hypotonia, symmetric generalized muscle weakness and structural brain malformations and mental retardation. Affected individuals have contractures of the hips, knees, and interphalangeal joints. Later features include myopathic facial appearance, pseudohypertrophy of the calves and forearms, convulsions (febrile or afebrile), ophthalmologic abnormalities including visual impairment and retinal dysplasia, and progressive cardiac involvement in individuals in the second decade of life. A static course is frequent until early childhood followed by extensive muscle wasting, most prominent proximally, and later progression of joint contractures. Most patients are never able to walk independently [11,14]. The most common brain malformations in FCMD include cobblestone lissencephaly of the cerebrum and cerebellum, but also posterior fossa malformations (cerebellar polymicrogyria, vermis hypoplasy and cysts) may be present [15].

Non-Japanese patients with *FKTN* mutations have been described for the first time in 2003 by Silan et al. [16] and de Bernabe et al. [17]. These two reports described Turkish infants homozygous for frameshift mutations associated with a severe CMD phenotype matching the established criteria for WWS. In fact, besides WWS, non-Japanese *FKTN* mutations can cause a wide clinical spectrum including FCMD, muscle-eye-brain disease (MEB, OMIM#253800), a congenital form without mental retardation (MDDGB4, OMIM#613152), a milder limb-girdle form (MDDGC4, OMIM#611588) also designated LGMD2M, and dilated cardiomyopathy (CMD1X, OMIM#611615) with mild or no limb-girdle muscle involvement. In particular, Godfrey et al. [18] defined FCMD/MEB as congenital onset muscular dystrophy with fronto-parietal pachygyria, cerebellar dysplasia and frequent flattening of the pons and brainstem. Eye abnormalities are often seen, and rare patients may acquire the ability to walk or to learn a few words.

We report a CMD patient with central nervous system (CNS) changes and no ocular involvement, where the genetic study revealed a new, large, in-frame duplication in the *FKTN* gene.

2. Case report

A seventeen-month-old Portuguese female child was referred for evaluation due to psychomotor retardation and hypotonia, after having been treated for hip dislocation in our hospital. She was the first child of young, healthy and non-consanguineous parents and no significant family history was registered. She was born at term and pregnancy and delivery were unremarkable.

The physical examination showed head circumference normal for the age, facial diplegia with an open mouth and drooling but without major dimorphisms, strabismus or ophtalmoparesis. The patient had axial weakness with poor head control and was unable to sit without support; a symmetric flaccid tetraparesis predominately proximal and brachial; contractures of hips, knees, ankles and interphalangeal joints of the hands; a rigid spine and absent tendon reflexes. She evidenced a mental disability with a developmental quotient of 50. At the present age (3 and a half years) she is able to sit without support and has some antigravity movements of the four limbs, but she cannot stand independently or hold any objects (Fig. 1A). For the time being ophthalmologic and cardiac examinations remain normal.

The investigation revealed elevation of creatine kinase (CK) values [6777U/l(17mo)/5084U/l(19mo)]. Muscular histology revealed small round muscle fibers of variable size along with necrotic and regenerative changes. The hematoxylin staining showed end-stage muscular dystrophy with atrophic fibers and replacement with connective tissue (Fig. 1B). Immunohistochemical analysis revealed major loss of α-dystroglycan (Fig. 1C) as compared to control (Fig. 1D).

Brain magnetic resonance imaging (MRI) at the age of 20 months illustrated an extensive bilateral frontal, temporal and parietal dysplasia, with preservation of posterior temporal and occipital regions; significant anomalies of the supratentorial white matter; cystic degeneration of white matter and multiple cerebellar cysts (Fig. 2A–D).

Molecular analysis was initially performed for *LAMA2*, *FKRP, POMT1* and *POMT2* genes and no mutations were found by direct genomic sequencing. Considering the presence of cobblestone lissencephaly with cerebellar cysts and muscular dystrophy, the diagnosis of FCMD was considered and a genetic test for the *FKTN* gene was performed. All 11 exons and flanking intronic regions were analyzed by direct sequencing (reference sequences with accession number NM_001079802.1). Only three previously known homozygous polymorphisms were detected: c.608G>A, c.1026C>A and c.1044+44A>G. In order to identify potential large deletions or duplications in *FKTN*, a quantitative study was performed using the Multiplex Ligation Probe Amplification (MLPA) technique. We used the commercial kit from MRC-Holland that includes probes for intron 1 and exons 4, 6, 7, 8, and 11 of *FKTN*. This analysis revealed that the patient has a duplication involving exons 4 to 7 of *FKTN* (Fig. 3A). Results also show that this mutation is present in a homozygous state, considering the probe ratios (~2.0) of the duplicated regions and that both parents are carriers of the same duplication (Fig. 3B). In order to determine the impact of this mutation at the cDNA level, expression studies were conducted using mRNA obtained from a cryopreserved muscle specimen of the patient. Amplification and sequencing of *FKTN* transcripts