

Novel Compound Heterozygous DYSF Mutations Lead to Dysferlinopathy

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Abstract

Here we report a 41-year-old patient who presented with a proximodistal onset limb weakness for 20 years at the administration. He was able to walk haltingly with a cane. On examination, his extremities muscle was wasting and strength was reduced. Laboratory examinations supported muscular disorders. Genetic testing demonstrated two novel pathogenic mutations c.2014-2020delATCGA-GA and c.5350 C>T in dysferlin gene. Muscle biopsy revealed lobulated muscle fibers, ragged red/blue fibers, lack of inflammation, and absence of dysferlin expression. Our case added further weight on the mitochondrial deficiency, rather than inflammatory response as the pathogenesis of dysferlinopathy.

Keywords: Limb-Girdle Muscular Dystrophy Type 2B; Miyoshi Myopathy; Dysferlinopathy; Compound Heterozygous Mutations; DYSF Gene

Introduction

Dysferlinopathy is composed of a spectrum of muscle disorders caused by loss function of dysferlin protein due to mutations in dysferlin (DYSF) gene. Clinically, it is characterised by two main phenotypes: Mioshi myopathy (MM) and limb-girdle muscular dystrophy type 2B (LGMD2B) with distinct initial muscle involvement: proximal (LGMD2B) and distal (MM) [1]. Here, we present a case with mixed phenotype of simultaneous involvement of proximal and distal weakness of the lower limbs and calf atrophy.

Case Report

Here we report a 41-year-old male with progressive limbs weakness for 20 years, who was admitted to Xi'an Gaoxin Hospital, China in August 2015. Initially, he noticed that he could not run as fast as past. The weakness was involved in both proximal and distal lower limbs. He occasionally tumbled in level walking, and mild calves atrophy was present one year later. Three years later, the patient had difficulty in climbing stairs and muscle atrophy in proximal lower and upper limbs was noticed. Seven years later, he had to stop and rest after level walking for 300 - 400 meters, and had difficulties in arising from a squatting position. He developed difficulty in taking off a sweater and holding a bowl steadily at table about 14 years later. At admission, the patient was able to walk haltingly with a cane, and reported being poorly able to wash his face and brush his teeth. No symptoms or signs of dyspnea, dyslalia or dysphagia were reported. The patient reported no family history of similar manifestations. Neurological examination revealed extremities muscle wasting, especially in the quadriceps femoris, the biceps and triceps brachii. Muscle tension was bilaterally decreased, and tendon reflex was absent in the lower and upper limbs. Muscle strength of the neck flexor was 2, according to Medical Research Council (MRC) scale, and shoulder elevation, arm extension and flexion, hand extension and flexion and finger extension were 3, 4+, 4-, 5, 5 and 5-, respectively, while hip

flexion, knee extension, foot extension and flexion, were 3-, 2, 2 and 4, respectively. Otherwise, nothing was remarkable. Laboratory tests showed significantly elevated serum creatine kinase (CK), and CKisoenzyme (CK-MB), alpha hydroxybutyrate dehydrogenase and lactate dehydrogenase. Electrocardiography and echocardiography were performed and showed within normal range, while electromyography showed myopathic changes.

Genetic testing on the entire coding region and intron/exon boundaries of the DYSF gene revealed two novel heterozygous compound mutations of c.5350 C>T and c.2014_2020delATCGAGA. The novel compound heterozygous mutations of DYSF gene were detected through next generation sequencing Panel (TruSight One, illumina) on Illumina NexSeq 500 sequencing system at KingMed Diagnostic Centre, Guangzhou, China. The average read depth was 100X and the read depth in the DYSF gene was 65X on average. The sequencing reads were aligned to the reference human genome (hg19) and variant calling was through GATK software (version 3.3-0-g37228af). Two mutations of c.5350 C>T mutation of exon 48 and c.2014_2020delATCGAGA deletion of exon 21 of DYSF gene were identified based on reference sequence NM_003494.3 (Figure 1).

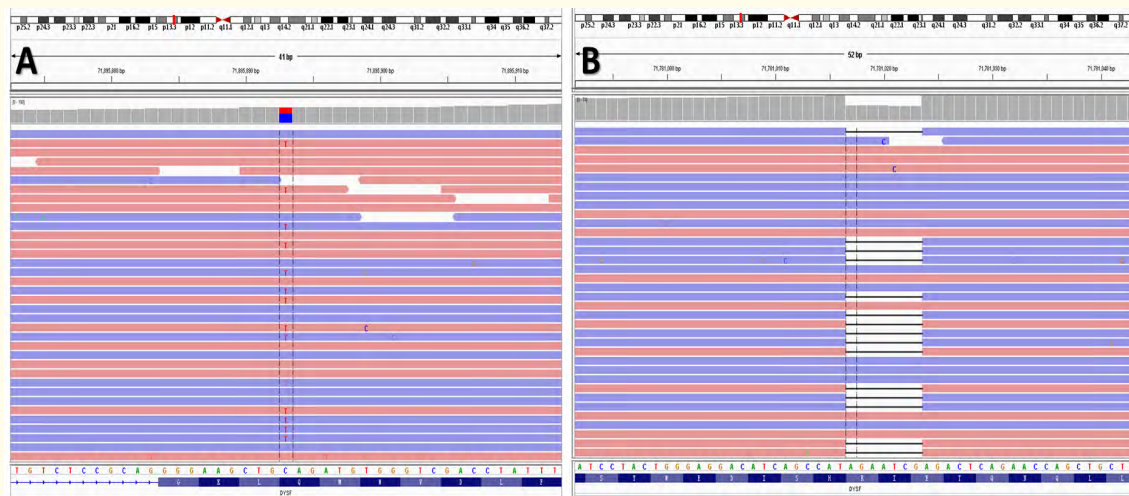


Figure 1: Novel compound heterozygous mutants of DYSF gene (A&B) were detected through next generation sequencing using TruSight One Sequencing Panel (illumina) on Illumina NexSeq 500 sequencing system (KingMed Diagnostic Centre, Guangzhou, China). Integrative Genomics Viewer (IGV) was used to analyse the generated data. c.5350 C>T mutation of exon 48 (A) and c.2014_2020delATCGAGA deletion of exon 21 (B) of DYSF gene were identified based on reference sequence NM_003494.3.

Muscle biopsy specimen from right biceps brachii was obtained and pathological examinations showed marked dystrophic changes including variable muscle fiber size (atrophic and hypertrophic fibers), moderate necrotic-degeneration, a few regenerating fibers, slit-like vacuoles within some muscle fibers, and an increase in connective tissue. Lobulated muscle fibers, ragged red or blue fibers were detected. There was limited inflammatory cells infiltration (Figure 2A-D). Immunohistochemistry showed total loss of dysferlin staining (Figure 2E, F).

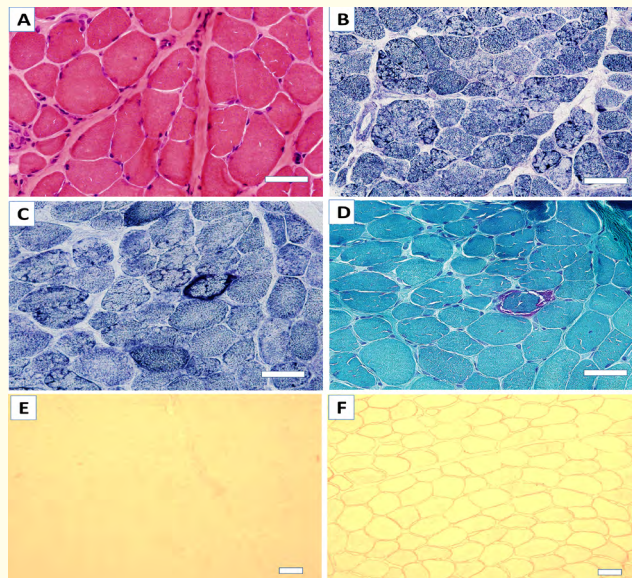


Figure 2: Muscle tissue was obtained by muscle biopsy. A serial of 6 μ m sections was cut from frozen tissue sample for histo- and immunohisto- chemistry. Hematoxylin and eosin (H&E) staining shows scattered atrophic fibers, fiber splitting and fibers replaced by fibrosis (A). Nicotinamide adenine dinucleotide – tetrazolium reductase (NADH-TR) staining shows misaligned intermyofibrillar networks and lobulated muscle fibers (B). Succinate dehydrogenase (SDH) staining shows muscle fiber with mitochondrial proliferation (C). Modified Gomori's trichrome (MGT) staining shows 'ragged red fiber' (D). Monoclonal anti-dysferlin antibody NCL-Hamlet (1:20, Novocastra, Newcastle, UK) was used for immunohistochemistry, which shows diminished dysferlin staining in this case (E) in comparison to a normal control (F). Scale bars are equal in all panels (A-F) = 50 μ m.

Discussion

This is the first case report of proximodistal phenotype of dysferlinopathy from China, although a few Chinese cases with dysferlinopathy have been reported [2-5]. After 20 years of disease progression, the patient presented with rather typical LGMD phenotype during examination. This is consistent with a Swiss study that all patients manifested a typical LGMD phenotype in the late disease course, regardless of initial onset [6].

The patient carries novel compound heterozygous mutations of DYSF gene, located in exon 21 (c.2014_2020delATCGAGA, p.Ile672fs) and exon 48 (c.5350C>T, p.Gln1784*). One mutated allele (p.Gln1784*) losses a 297aa fragment of protein and another mutated allele (p.Ile672fs) causes a frame shift at the position p.672, and the product of the mutated allele losses a 1408aa protein fragment. Although both of the mutations are not reported in the literature, they meet the criteria of pathogenic mutation according to the American College of Medical Genetics and Genomics (ACMG) guidelines [7]. Compound heterozygous DYSF gene mutations leading to dysferlinopathy had been reported [1], in accordance with hyperCKemia, which is similar to our case.

Lobulated fibers, ragged red and blue fibers, suggestive features of mitochondrial abnormalities in dystrophic myopathy, had been found in different patients with dysferlinopathy [1,8]. Dysferlin protein complex is related to membrane repair and maintenance of Ca²⁺ homeostasis of mitochondria. Inflammation had been considered as a potent component in dysferlinopathy [1,8]. However, other reports

argue lack of obvious inflammatory changes, which could be attributed to the failure of immunosuppressive treatment [1,5]. Our case added weight to mitochondrial deficit rather than inflammatory reaction in the pathogenesis of dysferlinopathy.

In summary, our case presented with proximodistal phenotype dysferlinopathy, carrying on novel compound heterozygous DYSF gene mutations, and demonstrated mitochondrial abnormalities in atrophic muscle fibers, indicating mitochondrial function restore might be the key for the treatment of dysferlinopathy.

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Bibliography

1. Nguyen K., *et al.* "Phenotypic study in 40 patients with dysferlin gene mutations: high frequency of atypical phenotypes". *Archives of Neurology* 64.8 (2007): 1176-1182.
2. Xi J., *et al.* "Clinical heterogeneity and a high proportion of novel mutations in a Chinese cohort of patients with dysferlinopathy". *Neurol India* 62.6 (2014): 635-639.
3. Shunchang S., *et al.* "Dysferlin mutation in a Chinese pedigree with Miyoshi myopathy". *Clinical Neurology and Neurosurgery* 108.4 (2006): 369-373.
4. Ro LS., *et al.* "Phenotypic features and genetic findings in 2 chinese families with Miyoshi distal myopathy". *Archives of Neurology* 61.10 (2004): 1594-1599.
5. Zhao Z., *et al.* "DYSF mutation analysis in a group of Chinese patients with dysferlinopathy". *Clinical Neurology and Neurosurgery* 115.8 (2013): 1234-1237.
6. Petersen JA., *et al.* "Dysferlinopathy in Switzerland: clinical phenotypes and potential founder effects". *BMC Neurology* 15 (2015): 182.
7. Richards S., *et al.* "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology". *Genetics in Medicine* 17.5 (2015): 405-424.
8. Park HJ., *et al.* "Heterogeneous characteristics of Korean patients with dysferlinopathy". *Journal of Korean Medical Science* 27.4 (2012): 423-429.

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