

Novel heterozygous missense variants of CAPN3 gene found in a Chinese patient with LGMD phenotype (LGMD2A)

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Case report

A 48 year old man presented with adult-onset muscle weakness with progressive involvement and wasting of the pelvic and shoulder girdle muscles (Figure a) since 40 year old. There was presence of winging scapulae (Figure b), no facial, oculomotor or cardiac involvement and absence of contractures. He was born of non-consanguineous parents and there was no family history of neuromuscular disease.

His serum creatine kinase level was 30 folds higher than normal, electromyography studies revealed the presence of myopathy motor units. Muscle biopsy over right biceps showed dystrophic changes with lobulated muscle fibers (Figure c,d). Immunostudy showed normal sarcolemmal expression for dystrophin (I, II and III), sarcoglycan (alpha, beta, gamma and delta) and dysferlin (hamlet-1 and -2). Based on clinical phenotype and muscle biopsy findings, preliminary diagnosis suggests probable limb girdle muscular dystrophy (LGMD). LGMD2A is the most frequent type of LGMD worldwide and prevalent lobulated fiber in muscle biopsy is a common finding. Direct sequencing of the Calpain 3 (CAPN3) gene, responsible for LGMD2A, was performed and two heterozygous missense variants, c.1517T > C (p.I506T) & c.2120A > G (p. D707G), were detected.

Discussion

Limb girdle muscular dystrophies encompass a clinically heterogeneous group of inherited disorders characterized by progressive weakness of proximal muscles of the hip and shoulder girdles. Currently, there are 8 autosomal dominant (LGMD1A-1H) and 23 autosomal recessive (LGMD2A-2W) types of LGMD and the list is expanding. LGMD2A is the most prevalent type of recessive LGMD. The disease is due to loss-of-function mutations in the Calpain 3 (CAPN3) gene, which helps to maintain the integrity of the triad complex in skeletal muscle [1].

LGMD2A has a wide age onset from early childhood to late adulthood, affects female and male equally and manifests early pelvic girdle involvement with muscle atrophy, scapular wing and abdominal laxity, but relatively spares hip abductors. Facial,

extraocular and pharyngeal muscles are not involved. The disease progresses slowly as loss of independent ambulation usually occurs between 15 and 25 years after the onset of symptoms. Creatine kinase is always elevated with a wide range.

Although lobulated fiber is not a specific feature for any type of neuromuscular diseases, they are more pronounced in LGMD2A than in other forms of LGMD [2]. Other morphological features that characterize LGMD2A include significantly lower levels of regenerating fibers, type 1 fiber predominance and small fiber size [3].

Muscle immunohistochemistry or immunoblotting analysis can be used to detect calpain-3 deficiency when there is a complete or partial loss of the calpain-3 protein. However, about 30% of LGMD2A patients show a normal quantity of the protein due to gene mutations that inactivate the enzyme rather than its synthesis [4].

Genetic analysis can be relied upon to confirm the diagnosis in LGMD2A patients. To date, over 300 pathogenic variants have been identified and distributed throughout the entire CAPN3 gene. Using direct genomic Sanger sequencing, two heterozygous missense variants: c.1517T > C (p.I506T) & c.2120A > G (p. D707G) were detected in our patient. The c.2120A > G (p. D707G) is a known disease causing variant [5]. The 1517T > C (p.I506T) is a novel allele variant not previously reported in literature. The 1517T > C was absent from controls in 1000 Genomes Project or Exome Aggregation Consortium. It is located in a well-established functional domain of the CAPN3 protein. Multiple lines of computational evidence (PolyPhen-2, SIFT, and MutationTaster) suggest that 1517T > C would have a deleterious effect on protein function [6,7,8]. Additionally, the Ile residue at position 506 was found to be a highly conserved amino acid residue among different species, as assessed using Clustal Omega software, and so a variant of this residue is probably damaging [9].

In summary, LGMD2A has distinguishing patterns of muscle histopathological changes and muscle biopsy could provide a foundation for patient selection before proceeding to confirmatory gene analysis.

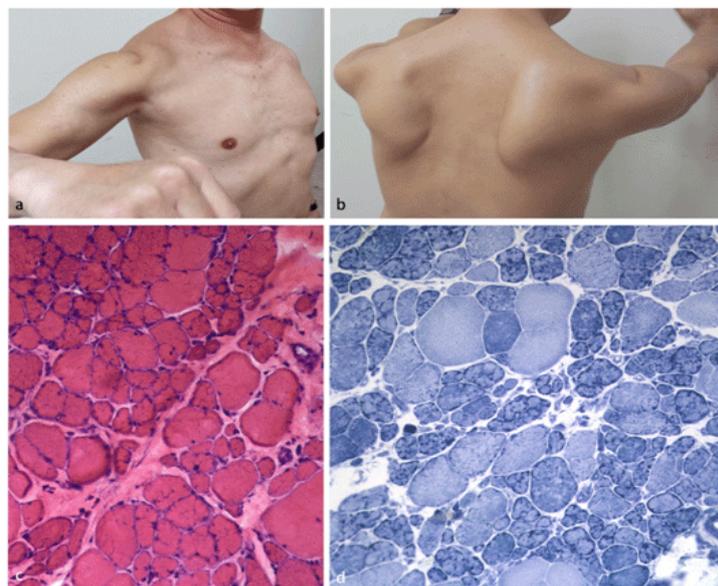


Figure (a,b,c,d): Muscle wasting noted over biceps muscle and there was bilateral winging of scapula (a, b). Haematoxylin and eosin stain revealed marked variation in muscle fiber sizes and increased connective tissue in endomysium 200x (c), while reduced nicotinamide adenine dinucleotide (NADH) stain highlights predominant lobulated type I fibers 200x (d).

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