Clinical, Pathological, and Genetic Features of Two Chinese Cases with Filamin C Myopathy

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To the Editor: Filamin C (*FLNC*) myopathy is an autosomal dominant inherited myopathy caused by mutations in *FLNC*. *FLNC* belongs to the filamin superfamily and cross-links actin filaments to form a network, anchoring membrane protein to the cytoskeleton. Human protein filamins comprise three isoforms encoded by the human genome: filamins A, B, and C. Among these, *FLNC* is a Z-disk protein encoded by the *FLNC* gene and is mainly expressed in the skeletal and cardiac muscles. *FLNC* gene is on chromosome 7q32-q35, containing genomic DNA of about 29.5 kb length and 49 encoding exons. *FLNC* is an isoform of serine protease expressed primarily in the striated muscle, containing 2725 amino acids, at a molecular weight of 291,000. *FLNC* protein contains two domains. The amino terminus consists of two calponin homology domains that together constitute the actin-binding domain (ABD), followed by 24 filamin-type immunoglobulin (Ig-FLMN) repeated domains (rod-overlapping domains), the most carboxy-terminal of which is responsible for dimerization, assembly, and anchor filaments. Each rod-overlapping domain consists of seven β-chains, where 3–4 β-chains constitute a β-sheet, and the protein-binding interface is formed between the two β-sheets.

Three distinct types of myopathies have been described. The first *FLNC*-related disease was described in 2005 when a nonsense mutation (c.G8130A, p.W2710X) in the FLNC rod domain was shown to cause skeletal and cardiac myopathy in a large German myofibrillar myopathy (MFM) family.[1] A frameshift mutation in *FLNC* leads to haploinsufficiency in three presumably related Bulgarian families, as well as the missense mutations (p.A193T; p.M251T) in the ABD of filamin-C, resulting in increased actin binding affinity in families from Australia and Italy and causing distal myopathies.

In general, the skeletal muscle pathology of *FLNC* myopathy is mainly manifested as muscular dystrophy, with abnormal protein aggregation and rimmed vacuoles in muscle fibers, as well as lack of specificity in clinical symptoms. Here, we report two cases of sporadic *FLNC* myopathy describing their clinical, myopathological, and genetic features. This is the first report of *FLNC* myopathy in Chinese patients caused by missense mutations.

Two male patients had a disease-onset age at 25 and 47 years. Regarding initial symptoms, Case 1 was presented with weakness of right upper limb for 4 years, followed by progressive involvement of bilateral lower limbs for 2 years and left upper limb for 1 year. Case 2 manifested weakness of left lower limb accompanied by low voice for 2 years, and then followed by progressive weakness of bilateral upper limbs and right lower limb for 1 year. Muscle weakness in the two cases was more severe in lower limbs than upper limbs, and more so in proximal muscles than distal muscles, as well. A winged scapula and the weakness of neck flexor muscle could be observed in the two patients. Muscle volume reduced at various levels. Involvement of the heart and respiratory system was not manifested at the time of diagnosis. There was no evidence of positive family histories.

On physical examination, Case 1 showed weakness in the neck flexion; proximal muscle (IV−) and distal muscle (IV) of upper limbs; iliopsoas (II), quadriceps femoris (II), musculus biceps femoris (IV), and distal muscle (IV to V−) of lower limbs; normal limb muscle tension; and reduced muscle volume. Bilateral pathological sign was negative. Case 2 showed weakness in closing eye, blowing the gills, moving the tongue, neck flexing and turning the neck, winged scapula, bilateral deltoid (III+), humeral two, triceps and arm radial muscle (III), grip strength (V), the proximal muscle (II) and distal muscle (IV+) of lower limbs, and evident atrophy of proximal limb muscles and shoulder girdle muscles. Bilateral pathological sign was negative.

Laboratory tests revealed serum creatine kinase (CK) level at 812.5 U/L in Case 1 and 114.4 U/L in Case 2. Electromyography (EMG) indicated myogenic damage. The echocardiogram showed no abnormality.

On muscle biopsy, hematoxylin and eosin (H & E) staining displayed inhomogeneous sizes of muscle fibers in Case 1. Rimmed vacuoles

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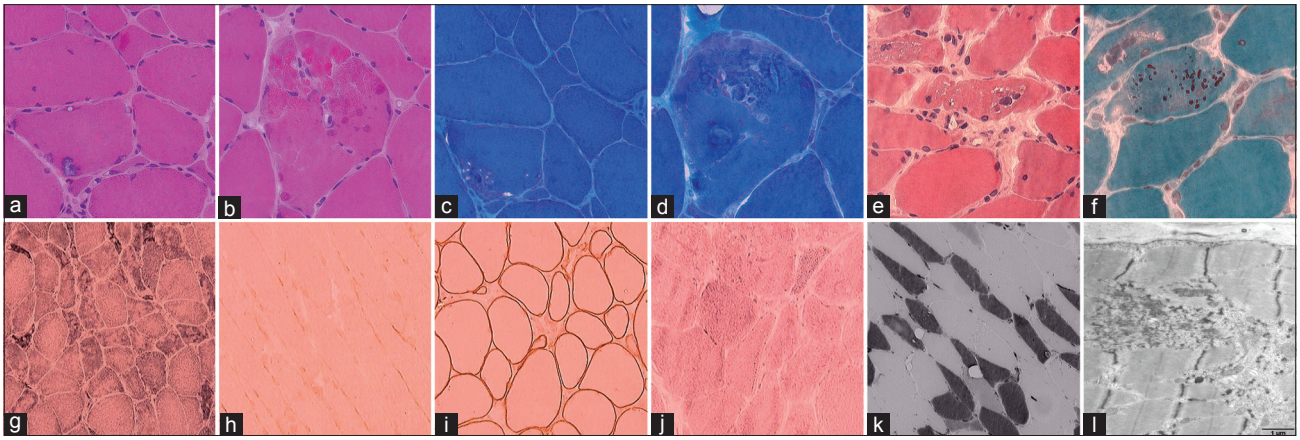

Figure 1: Pathologic pictures of the patients. In Case 1, H & E staining showed rimmed vacuoles with basophilic inclusion bodies and numerous eosinophilic bodies appearing in fibers (a and b, ×400). MGT staining exhibited blue-colored amorphous deposits in fibers (c and d, ×400). In Case 2, H & E staining exhibited orange-colored inclusion bodies inside a number of diseased fibers (e, ×400), and these inclusion bodies appeared red in MGT staining (f, ×400). NADH staining demonstrated the structural damage of large amounts of muscle fibers (g, ×400). Immunohistochemistry staining detected γ-sarcoglycan (−) (h, ×100) and its normal control was provided (i, ×400). The succinate dehydrogenase staining (j, ×400) and adenosine triphosphatase staining (pH at 4.5) (k, ×400) was normal. Ultrastructural analysis of skeletal muscle showed the presence of Z-disk and Z-disk streaming (l, ×15,000). H & E: Hematoxylin and eosin; MGT: Modified gomori trichrome; NADH: Nicotinamide adenine dinucleotide dehydrogenase.

appeared in some atrophy fibers in various amounts and shapes, and the particles in vacuoles were basophilic [Figure 1a]. Some fibers showed numerous eosinophilic bodies and amorphous deposits [Figure 1b]. Modified gomori trichrome (MGT) staining exhibited blue-colored amorphous deposits without ragged-red fiber [Figure 1c and 1d]. In Case 2, H & E staining exhibited degeneration of vacuoles and fragmentation in large amounts of muscle fibers, with orange-colored inclusion bodies inside lots of diseased fibers [Figure 1e]. These inclusion bodies appear red in MGT staining [Figure 1f]. Fibers with rimmed vacuoles were not seen. Nicotinamide adenine dinucleotide dehydrogenase staining demonstrated structural damage of large amounts of muscle fibers [Figure 1g]. Immunohistochemistry staining detected γ-sarcoglycan (−) [Figure 1h] and its normal control was provided [Figure 1i]. Other kinds of immunohistochemistry staining sections were normal [Figure 1j and 1k]. An ultrastructural analysis of skeletal muscle showed the presence of Z-disk and Z-disk streaming [Figure 1l].

In the two cases, the application of target region sequence capture and next-generation sequencing (NGS) led to the detection of two heterozygous mutations in *FLNC* gene: p.D1691N in Case 1 and p.D648Y in Case 2. These were further confirmed by Sanger sequencing. Segregation studies in the families were conducted, but the parents of the two patients did not carry the same mutations as their child.

FLNC gene trait is usually autosomal dominant, and the *FLNC* protein plays an essential role in myofibrillar development. *FLNC*-related myopathies are composed of three allelopathic, MFM Type 5, distal myopathy Type 4 (William myopathy), and familial hypertrophic cardiomyopathy. Disease onset of MFMs occurs mostly in adult life, which clinically manifests as a slowly progressive weakness of limbs, mostly affecting proximal and distal muscles, with the involvement of the peripheral nerve, cardiac muscle, or respiratory system in a few patients. Individual clinical features are observed in different types of MFMs, of which *FLNC* myopathy mainly presents with weakness of proximal muscles, peculiarly limb girdle weakness.[1] We reported here that two cases of sporadic *FLNC*-related myopathy were characterized by weakness and atrophy of proximal limbs, especially limb girdle weakness, and winged scapula – features that are consistent with international reports. Therefore, they were considered as MFM5.

FLNC is expressed in cardiac and skeletal muscles. According to reports of known *FLNC* myopathy families, myocardial involvement occurs in one out of every three *FLNC* myopathy patients with a W2710X mutation.[2] In China, myocardial involvement occurs in 50% of familial patients with K899-V904 deletion mutation combined with V899-C900 insertion mutation, and one-third of them have diarrhea.[3] No involvement of the cardiac muscle is reported in c.8107delG and V930-T933 del mutations. In this study, muscle weakness in these two cases is more severe in lower limbs than upper limbs, as well as being more severe in proximity than in distal extremities, which is consistent with previous reports where weakness is dominantly shown in proximal muscles. Both patients denied family history, without symptoms affecting the heart or respiratory system. According to a previous report on *FLNC* myopathy, right bundle branch block occurs at the early disease course,[1] while other cardiac lesions mainly take place in patients with the long-term disease course (>20 years).[3] In our study, the disease course was 4 years for Case 1 and 2 years for Case 2, and no symptoms of myocardial involvement were manifested, which can be related to the mutation site or type. This is possibly attributable to the shortness of disease course. The patients were followed up for the elucidation of the question.

The level of serum CK was 812.5 U/L for Case 1 and 114.4 U/L for Case 2, similar to previous reports on *FLNC* myopathy patients, which was normal or slightly elevated, with the highest value less than tenfold of the normal range.[2] Both cases also showed myogenic damage by EMG, but Vorgerd *et al.*'s study showed that peripheral nerve damage could occur on *FLNC* myopathy, as well.[1] The shared pathological feature of MFMs is the presence of abnormal protein aggregates associated with the sarcomeric Z-line in diseased muscle fibers. Morphologically, it manifests inhomogeneous sizes of muscle fibers, possibly with atrophy, hypertrophy, and internal nuclei fibers. Rimmed vacuoles, inclusion bodies, or amorphous substance likely exist in the subsarcolemmal and sarcoplasm of some muscle fibers. In our study, muscle histopathological examination by H & E staining exhibited rimmed

vacuoles and inclusion bodies in Case 1, without infiltration of inflammatory cells. The presence of rimmed vacuoles and inclusion bodies likely indicates the involvement of pathological mechanisms of autophagy and protein misfolding, further extending the disease spectrum for rimmed vacuole myopathies. In Case 2, H & E staining displayed vacuolation and fragmentation of muscle fibers, with orange-colored inclusion bodies in a significant amount of degenerated fibers, and immunohistochemistry staining revealed the absence of membrane protein of sarcoglycan. γ, δ-sarcoglycan is a dystrophin-associated glycoprotein complex, and its expression is respectively deficient in limb girdle muscular dystrophy 2C and 2F. A previous study revealed that *FLNC* protein interacted with γ, δ-sarcoglycan complex, and coexpression of three proteins was detected *in vitro*.[4] Kley *et al*. from German once reported cases of *FLNC* myopathy associated with ectopic accumulation of the γ, δ-sarcoglycan protein.[2] Studies from Löwe *et al*. demonstrated the ectopic accumulation of γ, δ-sarcoglycan, resulting in an impaired connection between myofibrillar filaments and the extracellular matrix,[5] as well as affecting signal transduction in the dystrophin-associated pathways. In Case 2, muscle immunohistochemistry staining revealed deficiency of membrane protein γ-sarcoglycan, but no ectopic accumulation. It remains to be determined whether the *FLNC* mutation, c.7012G>A, causes focal changes in the Ig-like rod repeating domain, thus influencing the expression of membrane protein γ-sarcoglycan.

FLNC mutations from previous reports are mostly localized in the rod-overlapping domain, where mutations primarily result in MFMs with the primary symptom of proximal muscle weakness. Missense mutations in the ABD mostly lead to distal myopathy, but proximal weakness predominance has also been reported. The application of NGS and confirmation by Sanger sequencing in our study detected two *FLNC* mutations: p.D1691N and p.D648Y. Both mutations were located in the rod-overlapping domain. They were not recorded in the Human Gene Mutation Database professional or in the Exome Aggregation Consortium database. Moreover, the carrying rate of normal population was zero. Since the parents of the two patients did not carry the same mutations as their child, the mutations detected were *de novo* mutations, signifying the alteration in a gene resulted from a mutation in a germ cell of the parent or in the fertilized egg itself. Limb weakness dominance in both of the cases is consistent with reports from abroad that *FLNC* mutations in the rod-overlapping domain cause proximal weakness myopathy. Since the clinical manifestations and histopathological features in these two cases meet the diagnosis criteria for MFMs, in addition to the genetic detection supporting this further, the diagnosis of *FLNC* myopathy can be made for them. Owing to the fact that patients enrolled in this study sporadically, along with variation in the baseline information and inadequate duration of follow-up, long-term observations are necessary to obtain data of changes in clinical manifestations.

For the first time, this report describes two sporadic cases of *FLNC* myopathy caused by missense mutations in China. The typical presenting characteristics in both cases were weakness and atrophy of proximal limbs. Two *de novo FLNC* mutations – p.D1691N and p.D648Y – were discovered.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Vorgerd M, van der Ven PF, Bruchertseifer V, Löwe T, Kley RA, Schröder R, *et al.* A mutation in the dimerization domain of filamin c causes a novel type of autosomal dominant myofibrillar myopathy. Am J Hum Genet 2005;77:297-304. doi: 10.1086/431959.
2. Kley RA, Hellenbroich Y, van der Ven PF, Fürst DO, Huebner A, Bruchertseifer V, *et al.* Clinical and morphological phenotype of the filamin myopathy: A study of 31 German patients. Brain 2007;130:3250-64. doi: 10.1093/brain/awm271.
3. Luan X, Hong D, Zhang W, Wang Z, Yuan Y. A novel heterozygous deletion-insertion mutation (2695-2712 del/GTTTGT ins) in exon 18 of the filamin C gene causes filaminopathy in a large Chinese family. Neuromuscul Disord 2010;20:390-6. doi: 10.1016/j.nmd.2010.03.009.
4. Thompson TG, Chan YM, Hack AA, Brosius M, Rajala M, Lidov HG, *et al.* Filamin 2 (FLN2): A muscle-specific sarcoglycan interacting protein. J Cell Biol 2000;148:115-26.
5. Löwe T, Kley RA, van der Ven PF, Himmel M, Huebner A, Vorgerd M, *et al.* The pathomechanism of filaminopathy: Altered biochemical properties explain the cellular phenotype of a protein aggregation myopathy. Hum Mol Genet 2007;16:1351-8. doi: 10.1093/hmg/ddm085.