Novel TTN mutations and muscle imaging characteristics in congenital titinopathy

Meng Yu1*, Ying Zhu2*, Zhiying Xie1, Yiming Zheng1, Jiangxi Xiao2, Wei Zhang1, Ichizo Nishino3, Yun Yuan1 & Zhaoxia Wang1

1Department of Neurology, Peking University First Hospital, Beijing, China
2Department of Radiology, Peking University First Hospital, Beijing, China
3Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Kodaira, Japan

Abstract

Objective: We present clinical features, muscle imaging findings, and genetic characteristics of five unrelated Chinese patients with congenital titinopathy, emphasizing the diagnostic role of muscle MRI. Methods: Five patients who recessive titinopathies were recruited. All patients received muscle biopsies. Mutations were detected by panel massively parallel sequencing and confirmed by Sanger sequencing. Western blotting of muscle proteins was performed. Leg muscle MRIs were performed in four patients. Results: Four patients aged 1–4 years old showed delayed motor development from early infancy, while a 17-year-old boy showed only a 1-year history of exercise intolerance. Physical examination showed proximal weakness in three patients. Muscle biopsies demonstrated multiple myopathological changes, including increased internalized nuclei, multicores, central cores, and dystrophic changes. Genetic sequencing revealed compound heterozygous or homozygous novel TTN mutations, including six frameshift mutations, one nonsense mutation, two missense mutations, one splicing mutation, and one small nonframeshift deletion. Protein analyses revealed significant decrease of full-length titin in all patients. Thigh muscle MRIs in four patients showed prominent fatty infiltration in the upper portion of semitendinosus and the peripheral portion of gluteus medius, while the sartorius and gracilis were relatively preserved. Interpretation: These cases provided further evidence that TTN mutations are likely responsible for an increasing proportion of congenital myopathies than currently recognized. The novel mutations reported expand the mutation spectrum of the TTN gene. There is a characteristic pattern of muscle involvement in congenital titinopathy regardless of clinical or pathological phenotype, providing valuable clues for guiding a genetic diagnosis workup.

Introduction

Congenital myopathies are a heterogeneous group of disorders characterized by early-onset and distinctive myopathological changes on muscle biopsy, including central cores, multiniminicores, nemaline rods and central nuclei.1 The diagnosis of congenital myopathies mainly relies on clinical manifestations, muscle imaging, and histological features on muscle biopsy but is ultimately based on genetic data. Notably, muscle MRI has emerged as a useful tool for offering clinical clues in the differential diagnosis of congenital myopathy.2 With the advent of massively parallel sequencing, an increasing number of mutations in more than 20 genes have been identified in patients with congenital myopathies.3 Particularly, TTN gene has attracted considerable attention in recent years. Titin, the largest known protein, is encoded by the TTN gene (OMIM #188840). This protein is expressed in both cardiac and skeletal muscles of many different species including humans, spanning the whole distance from the Z-disc to the M-band and interacting with various proteins.4 The TTN gene consists of 364 exons (the first noncoding exon and 363 coding exons), and there are traditionally three main categories of titin isoforms
Mutations and Muscle MRI in Congenital Titinopathy

M. Yu et al.

– N2A, N2B, and N2BA, based on the presence of the N2A and N2B elements in the I-band region. The N2A isoforms (mainly expressed in the skeletal muscles) contain the N2A element but not the N2B element. The N2B isoforms only contain the N2B element, while the N2BA isoforms (mainly expressed in the heart) contain both the N2A and the N2B elements. There are also many other transcripts described (including Novex-1, Novex-2, Novex-3) and more yet to be identified. However, the inferred complete metatranscript (NM_001267550.1) has never been confirmed and specific exons included in this metatranscript are thought to be expressed only during embryonic development.6

Both dominant and recessive TTN mutations have been reported to cause a wide spectrum of cardiac and skeletal muscle diseases. Dominant titinopathies include hereditary myopathy with early respiratory failure (HMERF) caused by mutations in exon 344, and late-onset tibial muscular dystrophy (TMD).7,8 Recessive titinopathies include limb-girdle muscular dystrophy 2J, young- or early-adult-onset distal titinopathy, Emery-Dreifuss-like myopathy without cardiomyopathy, and congenital myopathy with or without heart disease.9–14 The term “congenital titinopathy” has been suggested for patients with prenatal- or infant-onset form of titinopathy, including early-onset myopathy with fatal cardiomyopathy, centronuclear myopathy, core myopathy with heart diseases, and arthrogryposis multiplex congenita with myopathy.14 As the largest known protein, many mutations of the TTN gene have been detected by massively parallel sequencing, and meanwhile the spectrum of phenotypes of titinopathies is expanding. There was only one report of congenital titinopathy in Chinese with multimincore disease recently.15 In addition, the muscle MRI features have only been reported in several patients with congenital titinopathies.14 In this study, we describe five patients with infant-onset motor related symptoms or myopathological changes in accordance with congenital myopathy, and also summarize the muscle MRI features in these patients in order to further broaden our understanding of congenital titinopathy.

Methods

Patients recruitment

We retrospectively reviewed all of the patients who visited our neuromuscular clinic and received muscle biopsies. Patients with myopathological findings suggesting genetic diseases received genetic analyses with consent. Genomic DNA was extracted from peripheral blood samples of patients and their parents. A neuromuscular disease panel (Agilent, USA) was designed to capture the 420 genes known to be associated with known inherited neuromuscular disorders, as we have previously described. Samples were sequenced on an Illumina HiSeq 2500 Sequencer (Illumina, San Diego, CA) and the resulting data were analyzed using the same protocol as we have previously described.16 Variants with suspected pathogenicity were confirmed by Sanger sequencing. In addition, these variants were also tested in their parents by Sanger sequencing to confirm the segregation.

Five patients with myopathological changes suggesting congenital myopathies and genetic results showing recessive TTN mutations were enrolled for further analyses. Clinical information was collected, including natural histories of clinical symptoms, distributions of muscle weakness, evaluations of cardiac and pulmonary functions, as well as serum creatine kinase (CK) levels and electromyogram (EMG) findings. All patients received muscle biopsies from the biceps, and serial frozen sections of muscle tissues were stained by routine histological and histochemical methods. Variants in TTN gene are described referring to transcript variant-IC (NM_001267550.1). This study was approved by the Human Research Ethics Committee of Peking University First Hospital, and all participants provided written informed consent.

Western blot analysis

Western blotting (WB) of muscle biopsy samples were performed in all five patients. The detailed protocol was the same as previously reported,17 with titin N-terminal (Sigma SAB1400284) antibodies.

Muscle MRI

Muscle MRI using 1.5-T or 3.0-T magnetic resonance scanners (GE) of the thigh was performed in two patients, and of both the thigh and calf was performed in two patients. Axial scanning in conventional T1-weighted and short T1 inversion recovery (STIR) sequences was carried out and additional images were acquired in the coronal plane when necessary. The detailed protocol for the scanning was the same as our previous study.18 The MRI findings were independently interpreted by an experienced radiologist and a neurologist and any disagreements were resolved by consensus.

Results

Clinical and myopathological features

Clinical data from the five unrelated patients with recessive titinopathies are summarized in Table 1. There were three males and two females, aged between 1 and 17 years
old. All of the cases were sporadic, and the patients were born from nonconsanguineous parents. The ages of onset were infancy in four patients and 16 years old in patient 2. The main clinical symptoms of four patients (patients 1, 3, 4, and 5) were delayed early motor development, and of patient 2 were chest pain after intensive exercise accompanied by exercise intolerance, which worsened with time. Physical examinations showed neck flexor and proximal limb muscle weakness in patients 1, 4, and 5, while in patients 2 and 3 muscle strength was almost normal. High-arched palate, rigid spine, and contracture of the Achilles tendon were observed in patients 1, 4, and 5. No evidence of cardiac or respiratory involvement was found except for patient 4, who had congenital atrial septal aneurysm, which led to an atrial septal defect. The serum CK levels were normal in patients 1 and 5 and were mildly to moderately elevated in patients 2, 3, and 4 (200–2800 IU/L). Four patients received EMGs, and all showed myopathic changes.

Muscle biopsies revealed myopathic changes in all five patients with different features. The major myopathological changes were many fibers with multiple cores (multicores) in patient 1, a few fibers with central cores with ambiguous margins in patient 2, dystrophic changes with endomysial fibrosis and scattered regenerating fibers in patient 3, and increased internal nuclei in patients 4 and 5. In addition, other accompanying myopathological changes were also observed, including increased fiber size variation in all five patients, increased internal nuclei in patient 1 (seen in approximately 12% of muscle fibers) and patient 3 (seen in approximately 6% of muscle fibers), fiber type disproportion in patients 1 and 4, mild

Table 1. Summary of clinical features, muscle MRI manifestations, and myopathological changes

| Patient | 1 | 2 | 3 | 4 | 5 |
|---------|---|---|---|---|---|
| Gender/age at biopsy (years) | M/5 | M/17 | F/1 | M/3 | F/5 |
| Age at onset (years) | Infant | 16 | Infant | Infant | Infant |
| Symptoms | Delayed early motor development, unable to run or jump | Chest pain after exercise, with exercise intolerance | Delayed early motor development, difficulty in sucking | Delayed early motor development, difficulty in sucking | Delayed early motor development, exercise intolerance |
| Neck flexion | 2 | 5 | 5 | 3 | 2 |
| Shoulder abduction | 4 | 5 | 5 | 5 | 5 |
| Elbow flexion | 4 | 5 | 5 | 5 | 5 |
| Elbow extension | 4 | 5 | 5 | 5 | 5 |
| Gripping | 5 | 5 | 5 | 5 | 5 |
| Hip flexion | 5-4 | 5 | 5 | 5 | 5 |
| Knee flexion | 5 | 5 | 5 | 5 | 5 |
| Knee extension | 5 | 5 | 5 | 5 | 5 |
| Ankle dorsiflexion | 4 | 5 | 5 | 5 | 5 |
| Ankle extension | 5 | 5 | 5 | 5 | 5 |
| Rigid spine | Yes | No | No | Yes | Yes |
| Contractures | Achilles | No | No | Achilles | Achilles |
| High-arched palate | Yes | No | No | Yes | Yes |
| Cardiac/respiratory involvement | No/No | No/No | No/No | No/No | No/No |
| Serum CK level (IU/L) | Normal | Myopathic | 200–2000 | NA | 300–2800 |
| EMG | Normal | Myopathic | 400–600 | NA | Normal |
| Muscle fatty infiltration on MRI | ST, RF, SM, BF (thigh) SOL, GC, TA, TP (calf) | ST, BF, SM, VI | NA | Myopathic ST, BF, SM, RF (thigh) TP (calf) | NA |
| Muscle pathology | IFSV, increased internalized nuclei, multicores, FTD, subsarcolemmal mitochondrial accumulations | IFSV, center cores, fiber splitting, subsarcolemmal mitochondrial accumulations | IFSV, increased internalized nuclei, endomysial fibrosis, regenerating fibers, whorled fibers, MHC-I positive fibers | IFSV, increased internalized nuclei, FTD, regenerating fibers | IFSV, increased internalized nuclei, subsarcolemmal mitochondrial accumulations |

Abbreviation: MRI, magnetic resonance imaging; CK, creatine kinase; EMG, electromyography; NA, not available; ST, semitendinosus; RF, rectus femoris; SM, semimembranosus; BF, biceps femoris; SOL, soleus; GC, gastrocnemius; TA, tibialis anterior; TP, tibialis posterior; VI, vastus intermedius; MHC-I, major histocompatibility complex class I; FTD, fiber type disproportion; IFSV, increased fiber size variation.
subsarcolemmal mitochondrial accumulations in patients 1, 2, and 5, splitting fibers in patient 2, whorled fibers in patient 3, and major histocompatibility complex class I (MHC-I) positive fibers in patient 3. The characteristic myopathological changes are presented in Figure 1.

**Molecular genetics**

DNA sequencing and family co-segregation analysis proved compound heterozygous mutations or homozygous mutations of the \( TTN \) gene in all five patients (Table 2). None of the mutations were detected in various population databases or have been reported in the Human Gene Mutation Database (HGMD). The most common type of mutation was a frameshift mutation, which appeared in 6/11 of the mutated alleles. Other types of mutations included nonsense mutations (1/11), missense mutations (2/11), splicing mutations (1/11) and small nonframeshift deletions (1/11). None of the mutations have been reported, and are novel. Three mutations were located in exon 358, while the other mutations were located in different exons. When referring to different transcripts, all mutations were predicted to impact both N2BA and N2B cardiac isoforms except for three mutations. The c.25165_25176del mutation carried by patient 1 and c.29708_29809insTGAT mutation carried by patient 2 were predicted to impact only the N2BA cardiac isoform and the N2A skeletal muscle isoform but not to impact the N2B cardiac isoform. Meanwhile, the c.35730delA mutation carried by patient 2 was predicted to be a metatranscript-only mutation, which was not included in either the N2A skeletal muscle isoform or the N2B cardiac isoform.

**Protein analyses**

The expression of titin proteins was analyzed by using biopsy muscle samples from all five patients (Fig. 2). WB

![Figure 1](image_url)

*Figure 1.* Myopathological and ultrastructural changes in this group of patients with congenital titinopathy. A–D belonging to patient 1 show very few centralized nuclei and peripheral basophilic deposits on hematoxylin and eosin (H&E) staining (A), multicores as well as peripheral dark deposits on reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) staining (B) and succinate dehydrogenase (SDH) staining (C), and type 2 fibers significantly larger than type 1 fibers on ATPase pH 10.85 staining (D). E belonging to patient 2 shows a few center cores on NADH-TR staining. F and G belonging to patient 3 show abundant of small fibers as well as necrotic and regenerated fibers on H&E staining (F), and extremely small fibers of both types confirmed on ATPase pH 10.6 staining (G). H and I belonging to patient 4 shows centralized nuclei on H&E staining (H), and type 2 fibers significantly larger than type 1 fibers on ATPase pH 10.6 staining (I). J–L belonging to patient 5 show centralized nuclei and peripheral basophilic deposits on H&E staining (J), which are dark on NADH-TR staining (K) and SDH staining (L). Scar bars of A–C, F, H are 50 μm, of D, E, G, I–L are 100 μm.
results showed significant decrease of full-length titin proteins in patients compared with healthy controls. The splicing isoforms of titin proteins did not show any difference between patients and healthy controls.

**Muscle MRI**

In patient 1, a thigh muscle MRI showed fatty infiltration in the gluteus medius (mainly the peripheral part), rectus femoris, semitendinosus, semimembranosus, and biceps femoris. The upper part of semitendinosus was more severely involved compared to the lower part. The calf muscle MRI showed fatty infiltration of the soleus, gastrocnemius, tibialis anterior, and tibialis posterior. Edema was not observed in either the thigh or calf. The thigh muscle MRI of patient 2 showed fatty infiltration of the semimembranosus, biceps femoris, vastus intermedius, and semitendinosus, which also showed a more severe distribution in the upper part. No edema was found in the thigh muscles.

The thigh muscle MRI of patient 4 showed fatty infiltration of the gluteus maximus, gluteus medius (mainly the peripheral part), and the upper part of the semitendinosus. Edema of muscles was observed in the semimembranosus, adductor magnus, and rectus femoris.

The thigh muscle MRI of patient 5 showed fatty infiltration of the gluteus medius (mainly the peripheral part),

| Patient | Nucleotide change | Protein change | Mutation Taster | PolyPhen-2 | HumVar | SIFT | Global allele frequency | ExAC | gnomAD | ESP6500 | TGP | Exome Sequencing Project, 1000 Genomes Project |
|---------|-------------------|----------------|-----------------|------------|--------|------|------------------------|------|---------|----------|-----|-----------------------------------------|
| 1       | c.25165_25176del  | p.8389_8392del | M               | absent     | absent  | absent| Disease causing         | /C0  | /C0     | /C0     | /C0 | Disease causing                        |
| 2       | c.51504G>A        | p.W17168*     | P               | 272        | absent  | absent| Disease causing         | /C0  | /C0     | /C0     | /C0 | Disease causing                        |
| 3       | c.35730delA       | p.G11911Afs   | M               | 105        | absent  | absent| Disease causing         | /C0  | /C0     | /C0     | /C0 | Disease causing                        |
| 4       | c.109300_109301delCA | p.T35267Rfs | M               | 31         | absent  | absent| Disease causing         | /C0  | /C0     | /C0     | /C0 | Disease causing                        |
| 5       | c.91916T>C        | p.L30639P     | M               | 162        | absent  | absent| Disease causing         | /C0  | /C0     | /C0     | /C0 | Disease causing                        |

**Table 2.** Variants of the patients, allele frequencies in various population databases and in silico analysis of novel candidate variants

**Table 2** shows the variants of the patients, allele frequencies in various population databases and in silico analysis of novel candidate variants.
semimembranosus, biceps femoris, and upper part of the semitendinosus, and edema of the quadriceps femoris. The calf muscle MRI showed only the tibialis posterior was affected with slight fatty infiltration.

The fatty infiltration patterns of the thigh muscles are summarized in Table 1 and presented in Figure 3.

**Discussion**

In this study, we reported five cases with congenital recessive titinopathies that were supported by clinical features, myopathological changes, and genetic data. Clinically, four patients younger than five years old presented with delayed motor development since early infancy. Except for the 1-year-old infant who had basically normal muscle strengths, neck flexion weakness was prominent in the three other patients, which was in keeping with previous reports. Furthermore, rigid spine, contractures of Achilles, and high-arched palate were all observed in these three patients, showing high clinical consistency in clinical phenotypes with a higher prevalence than previous reports. However, facial weakness was quite common in previous reports of patients with centronuclear myopathies caused by TTN mutations but was not found in either of the two patients with centronuclear pathological changes in our study. It is noteworthy that the 17-year-
old patient in our cohort presented with only mild motor symptoms until his adolescence, and his muscle strengths were essentially normal with no specific physical signs. However, both the myopathological and genetic data from this patient support the diagnosis of congenital titinopathy, which further expand the phenotype of congenital titinopathy.

Cardiac or respiratory involvement was very rare in this group of patients, and only one patient was found to have an atrial septal defect caused by a congenital atrial septal aneurysm. However, only one mutation from patient 1 and one from patient 2 were predicted not to impact the N2B cardiac isoform, while the other mutation of patient 2 was predicted to be a metatranscript-only mutation. The relatively low prevalence of cardiac involvement and the inconsistency of isoform analysis, which is contrary to previous research, might be partly due to the relatively young ages of the patients at the time of visit. Further close follow-ups to monitor cardiac function should be performed.

All mutations in this study are novel, which might be due to the different ethnic background of the enrolled patients in our study. Truncating mutations were the most common type in this study. According to the algorithm for the clinical interpretation of genetic findings in titin proposed by Savarese et al., patients with biallelic truncation mutations can be directly diagnosed as titinopathy. In this way, four patients in our study belonged to this group. For patient 5, a single truncating mutation caused by splicing a mutation together with a missense mutation were detected on the paternal allele, while a different missense mutation was detected on the maternal allele. As the clinical and pathological features strongly supported the diagnosis of congenital titinopathy, the missense mutation c.91916T>C on the maternal allele was thought to be pathogenic. Further assessment with a computational tool called TITINdb showed mCSM score −1.424 and destabilizing, PolyPhen-2 score 0.982, and Condel score 0.563794569134, which was deleterious. Besides, this variant was neither found in ExAC nor in 1000G, and the amino acid affected was extremely conserved among species, all suggesting its pathogenicity. However, the pathogenicity of the c.59864A>T variant is unclear and needs further confirmation. Western blotting demonstrated significant decrease of the full-length titin protein in all five patients, further proving the pathogenicity of the mutations. The mutations detected in our study were distributed nearly throughout the whole TTN gene, from the N-terminal to the C-terminal with a weak tendency of focus in the C-terminal region.

The muscle MRI results in our study showed that the upper portion of the semitendinosus and the peripheral portion of gluteus medius were the most commonly affected in all patients. Although the clinical phenotypes and myopathological changes varied between the different patients, the muscle MRI shared similar patterns of involvement. Muscle MRI studies of congenital titinopathies are limited, and in one previous study, the semitendinosus, together with gluteal and other hamstring muscles, was also involved in all three patients who received lower limb MRIs. Other thigh muscles were involved to different degrees in different patients, while the adductors, sartorius, and gracilis were relatively preserved, which is also in accordance with previous studies. In previous reports of patients with HMERF, the involvement of semitendinosus also showed high sensitivity, which varied between 93 and 100%, 7,23,24 However, involvement of the sartorius and gracilis in HMERF might be distinct from congenital titinopathy, but comparative studies in a larger cohort are needed. In addition, in TMD patients, involvement of the semitendinosus was also observed in all 14 of 22 patients with both thigh and leg muscle involvement. The relatively similar distribution pattern on the thigh muscle MRI of TTN-related myopathies indicates that common muscle MRI features might be shared independent of the clinical and pathological phenotypes.

On the other hand, congenital myopathies with the same myopathological changes caused by mutations of different genes also exhibited different patterns of muscle MRI features. For instance, in multiminicore disease, selective involvement of the vasti, sartorius, and adductor magnus and relative sparing of the rectus, gracilis and adductor longus was a specific feature in congenital myopathies caused by recessive RYR1 mutations, while in SPEN1-related multiminicore diseases, atrophy of the semimembranosus and fatty infiltration of adductor magnus and the short head of biceps femoris were characteristic. Although patients with congenital myopathies with different gene mutations might share common clinical features and similar myopathological changes, muscle MRI could assist in the differential diagnosis of different genotypes.

Our findings provided further evidence that TTN mutations are likely responsible for a greater proportion of congenital myopathies than previously understood, including central core myopathy and central nuclei myopathy. The novel mutations reported here expand the mutational spectrum of the TTN gene. Fatty infiltration of the upper portion of semitendinosus and the peripheral portion of the gluteus medius on a thigh MRI is a characteristic pattern of muscle involvement in congenital titinopathy regardless of clinical or pathological phenotype, which could provide valuable clues for guiding genetic diagnostic workup.
Acknowledgments

The authors acknowledge Y. Zuo and J. Liu (Peking University First Hospital) for their works in preparations for pathological sections.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Reference

1. Jungbluth H, Treves S, Zorzato F, et al. Congenital myopathies: disorders of excitation-contraction coupling and muscle contraction. Nat Rev Neurol 2018;14:151–67.
2. Jungbluth H. Myopathology in times of modern imaging. Neuropathol Appl Neurobiol 2017;43:24–43.
3. North KN, Wang CH, Clarke N, et al. Approach to the diagnosis of congenital myopathies. Neuromuscul Disord 2014;24:97–116.
4. Bang ML, Centner T, Fornoff F, et al. The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. Circ Res 2001;89:1065–1072.
5. Freiburg A, Trombitas K, Hell W, et al. Series of exon-skipping events in the elastic spring region of titin as the structural basis for myofibrillar elastic diversity. Circ Res 2000;86:1114–1121.
6. Savarese M, Jonson PH, Huovinen S, et al. The complexity of titin splicing pattern in human adult skeletal muscles. Skelet Muscle 2018;8:11.
7. Palmio J, Evila A, Chapon F, et al. Hereditary myopathy with early respiratory failure: occurrence in various populations. J Neurol Neurosurg Psychiatry 2014;85:345–353.
8. Hackman P, Vihola A, Haravuori H, et al. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. Am J Hum Genet 2002;71:492–500.
9. Chauveau C, Rowell J, Ferreiro A. A rising titan: TTN review and mutation update. Hum Mutat 2014;35:1046–1059.
10. Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, et al. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. Neurology 2013;81:1205–1214.
11. Carmignac V, Salih MA, Quijano-Roy S, et al. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. Ann Neurol 2007;61:340–351.
12. Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol 2014;33:1–12.
13. De Cid R, Ben Yaou R, Roudaut C, et al. A new titinopathy: Childhood-juvenile onset Emery-Dreifuss-like phenotype without cardiomyopathy. Neurology 2015;85:2126–2135.
14. Oates EC, Jones KJ, Donkervoort S, et al. Congenital titinopathy: comprehensive characterization and pathogenic insights. Ann Neurol 2018;83:1105–1124.
15. Ge L, Fu X, Zhang W, et al. Recessive mutations in proximal I-band of TTN gene cause severe congenital multi-minicore disease without cardiac involvement. Neuromuscul. Disord. 2019;29:350–7.
16. Yu M, Zheng Y, Jin S, et al. Mutational spectrum of Chinese LGMD patients by targeted next-generation sequencing. PLoS ONE 2017;12:e0175343.
17. Evila A, Vihola A, Sarparanta J, et al. Atypical phenotypes in titinopathies explained by second titin mutations. Ann Neurol 2014;75:230–240.
18. Xie Z, Xiao J, Zheng Y, et al. magnetic resonance imaging findings in the muscle tissue of patients with limb girdle muscular dystrophy type 2I harboring the founder mutation c.545A>G in the FKRP gene. Biomed Res Int 2018;2018:3710814.
19. Kasinathan A, Sankhyan N, Singh P. Novel TTN mutation causing congenital myopathy. J Clin Neuromuscul Dis 2018;19:232.
20. Savarese M, Maggi L, Vihola A, et al. Interpreting genetic variants in titin in patients with muscle disorders. JAMA Neurol 2018;75:557–565.
21. Chauveau C, Bonnemann CG, Julien C, et al. Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. Hum Mol Genet 2014;23:980–991.
22. Laddach A, Gautel M, Fraternali F. TITINdb—a computational tool to assess titin’s role as a disease gene. Bioinformatics 2017;33:3482–3485.
23. Uruha A, Hayashi YK, Oya Y, et al. Necklace cytoplasmic bodies in hereditary myopathy with early respiratory failure. J Neurol Neurosurg Psychiatry 2015;86:483–489.
24. Ohlsson M, Hedberg C, Bradvik B, et al. Hereditary myopathy with early respiratory failure associated with a mutation in A-band titin. Brain 2012;135(Pt 6):1682–1694.
25. Mahjneh I, Lamminen AE, Udd B, et al. Muscle magnetic resonance imaging shows distinct diagnostic patterns in Wanelander and tibial muscular dystrophy. Acta Neurol Scand 2004;110:87–93.
26. Jungbluth H, Davis MR, Muller C, et al. Magnetic resonance imaging of muscle in congenital myopathies associated with RYR1 mutations. Neuromuscul Disord 2004;14:785–790.
27. Hankiewicz K, Carlier RY, Lazaro L, et al. Whole-body muscle magnetic resonance imaging in SEPN1-related myopathy shows a homogeneous and recognizable pattern. Muscle Nerve 2015;52:728–735.