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Magnetic resonance imaging of muscle in congenital myopathies associated with *RYR1* mutations

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Abstract

Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene are associated with a wide range of phenotypes, comprising central core disease and distinct subgroups of multi-minicore disease. We report muscle MRI findings of 11 patients from eight families with *RYR1* mutations ($n=9$) or confirmed linkage to the *RYR1* locus ($n=2$). Patients had clinical features of a congenital myopathy with a wide variety of associated histopathological changes. Muscle MR images showed a consistent pattern characterized by (a) within the thigh: selective involvement of vasti, sartorius, adductor magnus and relative sparing of rectus, gracilis and adductor longus; (b) within the lower leg: selective involvement of soleus, gastrocnemii and peroneal group and relative sparing of the tibialis anterior. Our findings indicate that patients with *RYR1*-related congenital myopathies have a recognizable pattern of muscle involvement irrespective of the variability of associated histopathological findings. Muscle MRI may supplement clinical assessment and aid selection of genetic tests particularly in patients with non-diagnostic or equivocal histopathological features.

Keywords: Muscle MRI; *RYR1* gene; Central core disease; Multi-minicore disease

1. Introduction

Muscle magnetic resonance imaging (MRI) is a valuable tool for the study of healthy [1] and diseased muscle and has been systematically used in the assessment of the inflammatory myopathies [2] and the muscular dystrophies [3]. More recently, we could demonstrate consistent and distinct patterns of selective muscle involvement in autosomal-dominant Emery-Dreifuss muscular dystrophy due to lamin A/C gene mutations (LGMD1B) [4], congenital muscular dystrophy with early rigidity of the spine (RSMD1) due to recessive mutations in the selenoprotein N (*SEPN1*) gene [5], and autosomal-dominant Bethlem myopathy secondary to mutations in the COL6A1 gene [6].

The congenital myopathies are characterized by distinct histopathological changes on muscle biopsy and have now been attributed to a variety of genetic defects predominantly affecting sarcolemmal and sarcotubular proteins. Dominant and recessive mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene are associated with a wide range of

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phenotypes comprising central core disease (CCD) [7], CCD with additional nemaline rods [8], clinically distinct subgroups of minicore myopathy (Multi-minicore Disease, MmD) [9, 10] and the malignant hyperthermia susceptibility trait without abnormalities on muscle biopsy [7]. Some of the histopathological abnormalities associated with *RYR1* involvement may be caused by mutations in a number of different genes, confronting the clinician with the difficult task of choosing the appropriate confirmatory genetic test in patients with a rare disorder. Muscle MRI has been suggested as a useful ancillary tool to further inform this choice in some forms of the muscular dystrophies [11], but systematic MRI studies correlating genetic and imaging data in the congenital myopathies are currently not available.

The aim of the following study was to identify patterns of selective muscle involvement in patients with mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene and clinical and histopathological features of a congenital myopathy.

2. Patients and methods

2.1. Patients

During the period from July 1998 to October 2002, 11 patients from eight families (F1–F8) attending the Neuromuscular Unit at the Hammersmith Hospital in London were invited to take part in our study. Five patients were female and six patients were male. Mean age at the time of the muscle MRI scan was 16 years (median 13 years; range 4–39 years).

All 11 patients had a consistent clinical phenotype with proximal weakness predominantly affecting the hip girdle, only mild facial weakness, absence of extraocular involvement and no significant respiratory impairment.

The phenotype of each patient was classified depending on the degree of clinical severity: The *severe phenotype* ($n=4$) was characterized by predominant wheelchair dependence and the inability to walk unsupported for more than 10 steps. The *moderate phenotype* ($n=3$) was characterized by independent mobility but limitation of maximum walking distance. The *mild phenotype* ($n=4$) was characterized by onset in childhood, an unlimited walking distance but subtle signs of proximal weakness such as difficulties running or climbing stairs. There was marked variability in clinical severity, even between different members of the same family or unrelated individuals carrying the same mutation.

2.2. Histopathological features

Nine patients had a diagnostic muscle biopsy and two patients were included in the study because of clinical features of a congenital myopathy and suggestive histopathological findings in a similarly affected relative. Biopsy samples were taken from the rectus femoris ($n=7$) and the vastus lateralis ($n=2$). Three patients (F4.1, F4.2, F8.1) from two autosomal-dominant families, one patient (F2.2) from an autosomal-recessive family and one sporadic case (F6.1) showed the typical histopathological appearance of CCD with marked type 1 predominance and well defined cores in the majority of muscle fibres (Fig. 1C). Two sporadic cases (F1.1, F3.1) reported previously [12] showed additional marked increase in fat and connective tissue on specimen obtained from the vastus lateralis (Fig. 1A). One patient (F5.1) from an autosomal-dominant pedigree had marked type 1 predominance, some minicores and unevenness of oxidative stain but no typical central cores on a biopsy sample obtained from the rectus femoris (Fig. 1B). One patient (F7.1) from an autosomal-recessive family reported previously [10] had a mixed histopathological picture with predominance of hypotrophic type 1 fibres, type 2 hypertrophy, minicores, central cores and few nemaline rods.

2.3. Genetic data

Mutations in the *RYR1* gene were identified in nine patients from seven families and have been partly reported

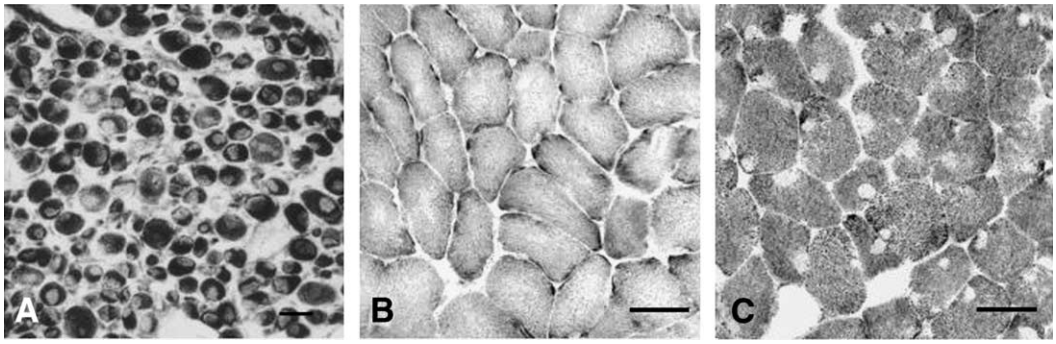

Fig. 1. Histopathological changes in patients with mutations in the *RYR1* gene. (A) Needle muscle biopsy from the vastus lateralis in an 11-year-old boy (Patient 3.1), NADH-TR: Predominance of high oxidative fibres with central cores and increase in fat and connective tissue. (B) Needle muscle biopsy from the rectus femoris in a 13 year old boy (Patient 5.1), NADH-TR: Predominance of high oxidative fibres with minicores and unevenness of stain but no typical central cores. (C) Needle muscle biopsy from the rectus femoris in a 12-year-old girl (Patient 4.1), NADH-TR: Predominance of high oxidative fibres with eccentrically located, well defined cores.

Table 1
Genetic data of 11 patients from 8 families included in the MRI study

Family	Patient	Origin	Consanguinity	Exon	Nucleotide change	Homozygous	Amino acid change	Inheritance
F1[a]	1.1	UK	No	101	C14581T	No	R4861C	Dominant (de novo)
F2[a]	2.1	Pakistan	Yes					Recessive[b]
	2.2							Recessive[b]
F3[a]	3.1	UK	No	101	C14581T	No	R4861C	Dominant (de novo)
F4	4.1	Malta	No	101	14587del18	No	4863del6	Dominant
	4.2			101	14587del18	No	4863del6	Dominant
F5	5.1	Saudi Arabia	Yes	102	A14740G	No	R4914G	Dominant
	5.2			102	A14740G	No	R4914G	Dominant
F6[a]	6.1	UK	No	101	C14581T	No	R4861C	Dominant (de novo)
F7[a]	7.1	UK	Yes	101	G14545A	Yes	V4849I	Recessive
F8	8.1	UK	No	102	G14678C	No	R4893Q	Dominant (de novo)

[a] Genetic data previously reported [10,13,14].
[b] Disease phenotype cosegregating with *RYR1* markers [10].

previously [10,13,14]. Mutational screening had focused on exons 98–103, encoding the C-terminal domain of the protein recently identified as a hotspot for *RYR1* mutations associated with a congenital myopathy phenotype [14]. One consanguineous family with two affected siblings (F2) was included in the study because of haplotyping results compatible with linkage to the *RYR1* locus on 19q13.1 [10]. Eight patients were heterozygous for a dominant *RYR1* missense mutation. In four of these patients there was a family history suggestive of autosomal-dominant inheritance (F4, F5) and in four sporadic cases the mutation had occurred de novo (F1, F3, F6, F8). One patient from a consanguineous English family (F7) was homozygous for a recessive *RYR1* mutation and both parents were unaffected carriers [10]. Mutations exclusively concerned exons 101 and 102 and the same C14581T base change was identified in three unrelated families. Genetic data for each family are summarized in Table 1.

2.4. Muscle magnetic resonance imaging

All patients were fully cooperative and no sedation or general anaesthesia was required for the MRI examination Muscle MRI was performed using conventional T1 weighted spin echos [TR = 500, TE = 20 ms] on a 1.0-Tesla HPQ system (Marconi Medical Systems, Cleveland, OH). Non contrast-enhanced images were obtained from pelvis and thighs and calves. The axial plane was selected with respect to the long axis of the body. This involved two sequential scans. We obtained 15 slices from each site. Slices were 5 mm thick and the gap between slices varied from 10 to 50 mm dependent on the site and on the size of the patient. Scanning time averaged 20 min for each patient.

Scans were assessed by two independent observers unaware of the genetic background of each patient. Scans were assessed for normal and abnormal signal intensity (consistent with fatty infiltration or replacement) within the different muscles.

The muscles examined were:

- *Thigh*: rectus femoris, vastus lateralis, intermedius and medialis, sartorius, gracilis; adductor longus and magnus; semimembranosus, semitendinosus, biceps femoris.
- *Lower leg*: gastrocnemius, soleus; tibialis anterior, tibialis posterior, extensor digitorum longus and peroneal group.

Signal intensity was scored on a 5-point scale using a modification of a scale reported by Lamminen [3]. The intensity of subcutaneous fat was used as a reference [15]. Signal intensity was classified as

0 = normal
1 = mild with only traces of increased signal intensity
2 = moderate with increased signal in less than 50% of affected muscle
3 = severe with increased signal intensity in more than 50% of affected muscle
4 = entire muscle replaced by abnormal signal

Mean signal intensity scores were calculated for each patient investigated. This allowed comparison of clinical severity and signal intensity changes.

3. Results

3.1. Muscle magnetic resonance imaging

All 11 patients had both thighs and lower leg muscles scanned. There was a consistent and recognizable pattern of selective muscle involvement with variations in signal intensity according to clinical severity. The typical pattern of selective involvement is shown in Fig. 2.

3.1.1. Thigh muscles

The adductor magnus was more severely affected than the adductor longus in all patients. Vasti were generally

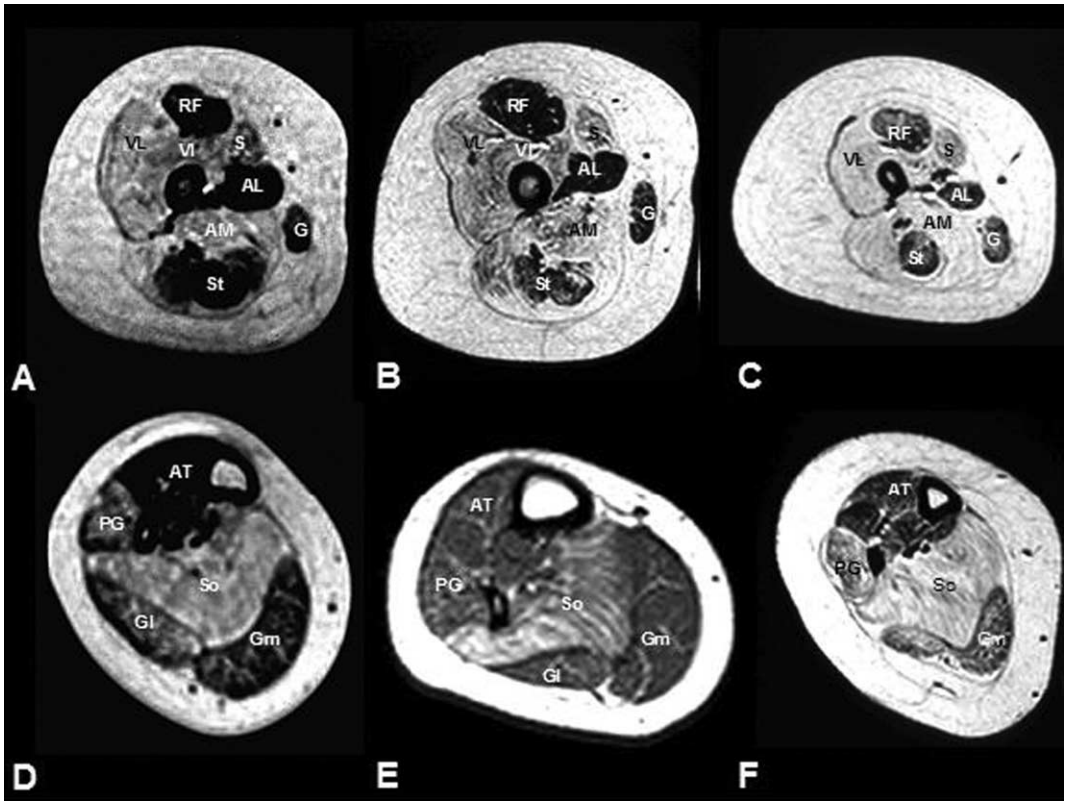

Fig. 2. Muscle involvement in the lower limbs in congenital myopathies secondary to mutations in the *RYR1* gene: T1-weighted MR imaging, transverse sections of the proximal thigh (A–C) and the proximal lower leg (D–F) in an 11 (Patient 3.1) (A) and a 13-year-old boy (Patient 5.1) (B,E), and a 12 (Patient 4.1) (C,F) and 17-year-old girl (Patient 6.1) (D). In the thigh (A–C), there is marked increase in abnormal signal within vasti, sartorius and adductor magnus with relative sparing of rectus femoris, adductor longus, gracilis and semitendinosus. In the lower leg, there is increase in abnormal signal in soleus (D–F), and-in more severe cases (E–F) peroneal group and gastrocnemius medialis. Tibialis anterior and gastrocnemius lateralis are relatively spared. (VL, vastus lateralis; VI, vastus intermedius; VM, vastus medialis; RF, rectus femoris; AL, adductor longus; AM, adductor magnus; S, sartorius; G, gracilis; St, semitendinosus; AT, tibialis anterior; PG, peroneal group; So, Soleus; Gm, gastrocnemius medialis; Gl, gastrocnemius lateralis).

more affected than the rectus femoris. In 10 patients vastus lateralis was more severely affected than rectus femoris and only in one mild case (F8.1) both muscles were equally involved. Sartorius was more severely affected than gracilis in eight cases. Moderate involvement of sartorius and gracilis and mild involvement of the adductor magnus were the earliest sign in the mildest case (F8.1).

Hamstring muscles were less severely affected compared to most muscle groups in the anterior thigh. Signal intensity scores in the semitendinosus were lower compared to the vastus lateralis in nine patients. In more than half of all patients, the semimembranosus was more severely affected compared to the semitendinosus muscle.

3.1.2. Lower leg muscles

Changes were less pronounced compared to the thigh muscles. There was marked differential involvement of calf muscles with the soleus being more severely affected than the medial gastrocnemius in all patients. In eight patients, the lateral gastrocnemius was more severely involved than the medial gastrocnemius. Within the anterior compartment, the peroneal group was more severely affected than the tibialis anterior in nine patients.

3.2. Clinical phenotypes and muscle MRI findings

Mean signal intensity scores for individual patients ranged from 1.1 to 1.3 (mean 1.2; median 1.2) in patients with a mild clinical phenotype, from 1.3 to 2.3 (mean 1.9; median 2.1) in patients with a moderate clinical phenotype and from 1.9 to 2.8 (mean 2.5; median 2.6) in patients with a severe clinical phenotype All patients with a severe but none of the patients with a mild clinical phenotype showed complete abnormal signal replacement within the vastus lateralis, the adductor magnus and the soleus.

3.3. Histopathological changes and muscle MRI findings

The pattern of selective muscle involvement was consistent despite marked variability of histopathological findings. Patients with type 1 predominance as the main abnormal finding had the same pattern of selective muscle involvement as patients with the ‘classical’ histopathological appearance of central core disease or minicores as the predominant abnormality.

Histopathological changes (Fig. 1A–C) depended on the biopsy site and corresponded closely to muscle MRI

findings (Fig. 2A–C). Severe increases in abnormal signal intensity in the vastus lateralis (Fig. 2A) were associated with increases in fat and connective tissue when this site was sampled (Fig. 1A). Connective and fatty tissue increases were not seen in samples taken from the rectus femoris (Fig. 1B and C), typically not or only mildly affected on muscle MRI (Fig. 2B and C).

4. Discussion

Systematic muscle MRI data have not previously been reported in congenital myopathies associated with mutations in the *RYR1* gene. Differential involvement of the quadriceps with relative sparing of the rectus femoris was already suggested in early ultrasound studies of CCD [16]. In a combined ultrasound and CT study of two cases from an autosomal-dominant CCD pedigree, Arai and co-workers [17] described markedly decreased attenuation values within the soleus but relative sparing of rectus femoris and foot dorsiflexors. A similar pattern has been reported more recently in a family with recessive CCD transiently presenting as multi-minicore disease [9].

The data presented in this study of 11 patients with molecularly confirmed *RYR1* involvement, including one recessive case, expand these early observations and suggest that the distinct pattern of selective muscle involvement due to mutations in the *RYR1* gene may be more consistent than associated histopathological changes. Moreover, the pattern of differential muscle involvement observed in congenital myopathies secondary to *RYR1* mutations is distinct from the pattern reported in other neuromuscular disorders with similar clinical or histopathological features.

The clinical phenotype of central core disease (CCD) is relatively unspecific and a similar distribution of weakness may be observed in Becker muscular dystrophy, some of the limb girdle muscular dystrophies, milder forms of spinal muscular atrophy or other congenital myopathies. Although relative sparing of adductor longus, gracilis and semitendinosus may be observed in Becker muscular dystrophy, the rectus femoris tends to be involved severely and gastrocnemii are typically more prominently involved than soleus [18]. Case studies in the limb girdle muscular dystrophies indicate that the clinical and genetic heterogeneity of these conditions is reflected in a variety of distinct pattern of differential involvement on muscle MRI associated with specific genetic defects [19–20]. The limited muscle MRI data available for nemaline myopathy [21] due to mutations in the nebulin (*NEB*) gene suggest prominent involvement of the lower leg with marked signal increases within the tibialis anterior, further supporting the hypothesis of a distinct pattern of selective muscle involvement in genetically heterogeneous conditions. Nemaline rods have now also been observed as an additional finding in patients with proven *RYR1* mutations [8], emphasizing the potential role of muscle MRI in the assessment of patients with equivocal histopathological features. One of our patients had a mixed histopathological picture featuring minicores, central cores and nemaline rods, and screening of the *RYR1* gene was only considered because of suggestive features on muscle MRI [10].

Minicore myopathy (Multi-minicore disease, MmD) is a clinically and genetically heterogeneous condition, showing considerable overlap with other congenital myopathies and a subset of the congenital muscular dystrophies. Recessive mutations in both the selenoprotein N *(SEPN1)* gene [22]-also implicated in congenital muscular dystrophy with early rigidity of the spine (RSMD1)-and the *RYR1* gene [9–10] have now been identified in clinically distinct subsets of patients with minicores on muscle biopsy. Muscle MR imaging of patients with *SEPN1* gene mutations is distinct from the findings presented in this paper and demonstrates diffuse involvement of the adductors in the thigh and predominant involvement of the gastrocnemii compared to the soleus in the leg [5], whereas the pattern in MmD patients with confirmed *RYR1* involvement is indistinguishable from patients with typical CCD [10]. Muscle MRI in MmD may therefore show important differences depending on the underlying genetic mechanism.

Our data demonstrate correspondence between histopathological and MRI changes in individual muscles. Histopathological changes in cases where the vastus lateralis was biopsied were more severe than usually observed in the congenital myopathies, and corresponded to marked increases in abnormal signal in the same muscle on MRI. These findings indicate that muscle biopsy findings associated with mutations in the *RYR1* gene may vary greatly even in the same patient depending on the selected muscle biopsy site.

The degree of clinical severity in our patients corresponded to the changes observed on muscle MRI. Although individual muscles such as the vastus lateralis were often markedly affected even in clinically moderate cases, sparing of others was prominent and included the rectus femoris, adductor longus and tibialis anterior. Compensatory synergist hypertrophy evolving over time may therefore explain the occasionally observed ‘functional improvement’ in patients with central core disease, and the only slowly progressive course of this condition even over prolonged periods of follow-up [23].

The findings presented in this study have important implications for the differential diagnosis of the congenital myopathies. Muscle MR imaging indicated *RYR1* involvement even at an early stage in cases where type 1 predominance was the predominant histopathological finding and oxidative stain abnormalities were non-diagnostic. Our data therefore suggest that muscle MRI can be a useful diagnostic tool in patients with congenital myopathies secondary to *RYR1* gene involvement, particularly in sporadic cases or patients with equivocal histopathological findings.

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References

[1] Fleckenstein JL, Weatherall PT, Bertocci LA, et al. Locomotor system assessment by muscle magnetic resonance imaging. Magn Reson Q 1991;7:79–103.
[2] Fraser DD, Frank JA, Dalakas MC. Inflammatory myopathies: MR imaging and spectroscopy. Radiology 1991;179:341–4.
[3] Lamminen AE. Magnetic resonance imaging of primary skeletal muscle diseases: patterns of distribution and severity of involvement. Br J Radiol 1990;63:946–50.
[4] Mercuri E, Counsell S, Allsop J, et al. Selective muscle involvement on magnetic resonance imaging in autosomal dominant Emery-Dreifuss muscular dystrophy. Neuropediatrics 2002;33:10–14.
[5] Mercuri E, Talim B, Moghdaszadeh B, et al. Clinical and imaging findings in six cases of congenital muscular dystrophy with rigid spine syndrome linked to chromosome 1p (RSMD1). Neuromusc Disord 2002;12:631–8.
[6] Mercuri E, Cini C, Counsell S, et al. Muscle MRI findings in a three-generation family affected by Bethlem myopathy. Eur J Paediatr Neurol 2002;6:309–14.
[7] McCarthy T, Quane KA, Lynch PJ. Ryanodine receptor mutations in malignant hyperthermia and central core disease. Human Mutat 2000; 15:410–7.
[8] Monnier N, Romero NB, Lerale J, et al. An autosomal dominant congenital myopathy with cores and rods is associated with a neomutation in the RYR1 encoding the skeletal muscle ryanodine receptor. Hum Mol Genet 2000;9:2599–608.
[9] Ferreiro A, Monnier N, Romero NB, et al. A recessive form of central core disease, transiently presenting as multi-minicore disease, is associated with a homozygous mutation in the ryanodine receptor type 1 gene. Annu Neurol 2002;51:750–9.
[10] Jungbluth H, Müller CR, Halliger-Keller B, et al. Autosomal-recessive inheritance of RYR1 mutations in a congenital myopathy with cores. Neurology 2002;59:284–7.
[11] Mercuri E, Pichiecchio A, Counsell S, et al. A short protocol for muscle MRI in children with muscular dystrophies. Eur J Paediatr Neurol 2002;6:305–7.
[12] Manzur AY, Sewry CA, Ziprin J, Dubowitz V, Muntoni F. A severe clinical variant of central core disease with possible recessive inheritance. Neuromusc Disord 1998;8:467–73.
[13] Tilgen N, Zorzato F, Halliger-Keller B, et al. Identification of four novel mutations in the C-terminal membrane spanning domain of the ryanodine receptor 1: association with central core disease and alteration of calcium homeostasis. Hum Mol Genet 2001;10:2879–87.
[14] Davis MR, Haan E, Jungbluth H, et al. Principal mutation hotspot for muscle cetral core pathology in the C-terminal transmembrane region of the RYR1 gene. Neuromusc Disord 2003;13:151–7.
[15] Dooms CG, Hricak H, Margulis AR, de Greer G. MR imaging of fat. Radiology 1986;158:51–4.
[16] Heckmatt JZ, Dubowitz V. Ultrasound imaging and directed needle biopsy in the diagnosis of selective involvement in muscle disease. J Child Neurol 1987;2:205–13.
[17] Arai Y, Sumida S, Osawa M, et al. Skeletal muscle CT scan and ultrasound imaging in two siblings with central core disease. No To Hattatsu 1990;22:55–60.
[18] de Visser M, Verbeeten Jr B. Computed tomography of the skeletal musculature in Becker-type muscular dystrophy and benign infantile spinal muscular atrophy. Muscle Nerve 1985;8:435–44.
[19] Meola G, Sansone V, Rotondo G, Jabbour A. Computerized tomography and magnetic resonance muscle imaging in Miyoshi's myopathy. Muscle Nerve 1996;19:1476–80.
[20] Topaloglu H, Dincer P, Richard I, et al. Calpain-3 deficiency causes a mild muscular dystrophy in childhood. Neuropediatrics 1997;28: 212–6.
[21] Wallgren-Petterson C, Kivi L, Jääskeläinen J, et al. Ultrasonography, CT, and MRI of muscles in congenital nemaline myopathy. Pediatr Neurol 1990;6:20–8.
[22] Ferreiro A, Quijano-Roy S, Pichereau C, et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. Am J Hum Genet 2002;71:739–49.
[23] Lamont PJ, Dubowitz V, Landon DN, Davis M, Morgan-Hughes JA. Fifty year follow-up of a patient with central core disease shows slow but definite progression. Neuromusc Disord 1998;8:385–91.