CASE REPORT

RYR1-related central core myopathy in a Chinese adolescent boy

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Central core myopathy is a rare, inherited neuromuscular disorder with a wide spectrum of phenotypic presentations. It is also considered an allelic disease of malignant hyperthermia. We report a case of central core myopathy in a Chinese adolescent boy presenting with atypical clinical features and a moderately elevated serum creatine kinase level. The diagnosis was made from the histopathological findings of central cores on muscle biopsy, and confirmed by the molecular genetic testing for the *RYR1* gene mutation. This is the first case of central core myopathy confirmed by molecular study in our locality.

Introduction

Congenital myopathy is a heterogeneous group of inherited neuromuscular disorders categorised by characteristic histopathological features on muscle biopsy. Among different types, central core myopathy was the first described congenital myopathy in humans in 1956.[1] The clinical phenotype is highly variable, ranging from asymptomatic or mild muscle weakness to severe neuromuscular involvement leading to lack of independent ambulation and early infant death.[2,3] Central core myopathy is a rare disease, and is usually inherited in an autosomal dominant manner resulting from the ryanodine receptor type 1 *(RYR1)* gene mutation on chromosome 19q13.1.[4] Autosomal recessive inheritance has also been reported.[5,6] Classically it manifests as a slowly or non-progressive symmetrical proximal muscle weakness and infantile hypotonia that can persist into adulthood. Affected patients usually have delayed milestones for motor development and reduced muscle bulk. Orthopaedic manifestations such as congenital hip dislocation or kyphoscoliosis commonly coexist.[2,7] Here, we report a case of central core myopathy with atypical clinical presentations. The diagnosis is based on the histopathological findings of characteristic cores on muscle biopsy and molecular genetic testing for the *RYR1* gene mutation.

Case report

A 14-year-old Chinese boy was referred because of lower limb muscle atrophy and foot deformity. There was no family history of neuromuscular disease. He presented with delayed motor milestones and could not walk independently until 22 months old. He had always been poor at motor skills, never being able to squat and described as exhibiting clumsiness with easy falling. Aged 11 years, he had an episode of left knee injury with dislocation of patella, which was treated with a plaster for 2 months. Later, left thigh muscle atrophy was noted. He started to walk with a tip-toe gait and manifested bilateral foot deformities. There were no features of disordered sensory, bladder or bowel functions.

Examination revealed left lower limb muscle atrophy and bilateral pes cavus. He walked with tip-toe gait and an exaggerated lumbar lordosis. There was bilateral Achilles tendon tightness and weakness of ankle dorsiflexion, more severe on the left side. Deep tendon reflexes were normal, there was no abnormality in the upper limbs, and there was no ptosis, ophthalmoplegia or facial weakness. Investigations—including complete blood count, erythrocyte sedimentation rate, and tests for antinuclear antibody and thyroid disorders—yielded nil abnormal. Nerve conduction studies and magnetic resonance imaging (MRI) of the spine were entirely normal. However, his serum creatine kinase level was 2259 U/L. In view of the clinical features and the moderately high serum creatine kinase level, diagnoses including muscular dystrophies (such as the limb-girdle type) or other hereditary myopathies were suspected.

Muscle biopsy from the left vastus lateralis revealed skeletal muscle fibres with generally enlarged size, in the range of 120 to 130 μm (Fig 1). Internalised and central nuclei were readily seen. ATPase enzyme histochemistry showed indistinct fibre type discrimination, in keeping with myopathic changes. Staining for oxidative enzymes (NADH

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一名華籍青年患上與*RYR1*基因有關的肌中央軸空病

肌中央軸空病很罕見，是一種先天性神經肌肉障礙，也被認為是惡性高體溫的一種等位基因病。患者會出現多種臨床症狀。本文報告一名患有肌中央軸空病的華籍青年出現非典型臨床症狀，其血清肌酸激酶水平輕微上升。中央脊髓肌組織活檢顯示患者的病理組織，隨後利用分子基因檢測發現其*RYR1*基因突變才確診此症。這是本港利用分子檢測確診肌中央軸空病的首宗病例。

and SDH) demonstrated well-demarcated zones of clearing within a few muscle fibres, suggestive of central core formation. Gomori trichrome stain showed no nemaline rods or ragged red fibres. There was no significant atrophic change, fibrosis or muscle fibre necrosis. Immunohistochemical staining for various membrane proteins (dystrophin-1, -2, and -3, alpha-sarcoglycan and beta-sarcoglycan) showed normal sarcolemmal staining patterns. The biopsy findings were indicative of myopathic changes. The focal presence of central cores revealed by enzyme histochemical stains signalled the possibility of central core myopathy, although the morphological changes were rather subtle compared to typical finding in this condition.

Subsequent mutation analysis of the *RYR1* gene was performed (Fig 2). Peripheral blood samples were collected after informed consent. The *RYR1* gene is a very large gene with 106 exons and most mutations localise between exons 39 to 48.[7] These selected coding exons and their flanking introns were amplified using polymerase chain reaction. The primer sequences and analysis protocol are available upon request. A de-novo heterozygous known disease-causing mutation of the *RYR1* gene arginine-to-histidine substitution at codon 2508 (NM_000540.2:c.7523G>A; NP_000531.2:p.R2508H) was identified in the index patient, while in the parents, such a specific mutation was absent.

The patient's clinical condition was stable. Genetic counselling together with the possibility of malignant hyperthermia susceptibility (MHS) were explained to the patient and the family.

Discussion

Central core myopathy is a rare neuromuscular disease with a wide phenotypic spectrum, and is considered an allelic disease of malignant hyperthermia (MH). The diagnosis depends on the presence of characteristic core areas devoid of mitochondria and oxidative enzyme stains in muscle biopsy specimens, and is confirmed by molecular genetic testing for an *RYR1* gene mutation.[2,3] These gene mutations were firstly discovered in 1990 as the cause of MH, and were subsequently detected in central core disease, multi-minicore disease, congenital myopathies with cores and rods, and congenital neuromuscular disease with uniform type 1 fibre.[4,8] To date, over 280 *RYR1* mutations have been identified.[7,8] *RYR1*-related myopathies typically present in infancy or childhood. More severe cases with antenatal onset have been reported, in which case they are recessively inherited or due to de-novo dominant mutations.[9] Recently, onset in late adulthood and predominant axial involvement has been reported.[10]

RYR1 is one of the largest described genes in humans. The ryanodine receptor is the principal sarcoplasmic reticulum calcium release channel. The mutated codons giving rise to central core disease are clustered in three restricted regions of proteins

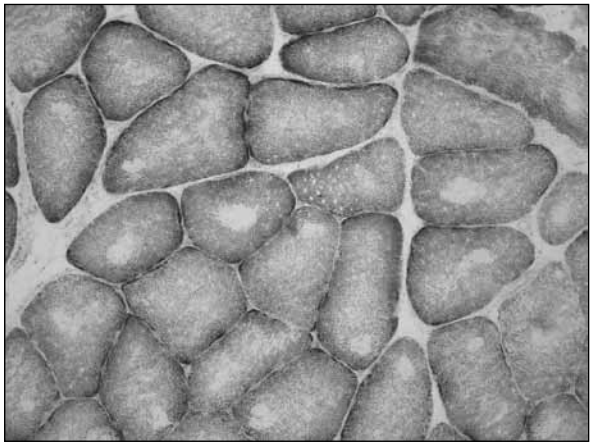

FIG 1. Muscle biopsy taken from left vastus lateralis of the patient

Succinic dehydrogenase stain shows occasional well-delineated zones of clearing within some of the skeletal muscle fibres, suggestive of "central core" formation (× 200)

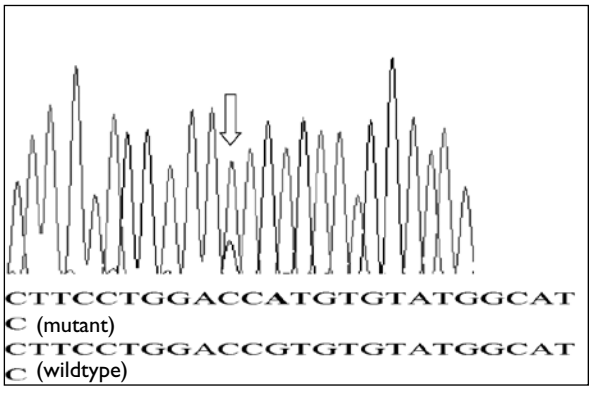

FIG 2. Mutation analysis of the ryanodine receptor type 1 (*RYR1* gene)

Electropherogram in sense direction showing the heterozygous mutation of *RYR1* NM_000540.2: c.7523G>A; NP_000531.2: p.R2508H as indicated by the arrow. The mutation site is indicated by the arrow with the mutant nucleotide in bold

or 'hot-spots': N-terminal (amino acids p.M1-p.R614), central (p.R2163-p.R2458), and C-terminal (p.R4136-p.P4973). Most of the central core disease mutations are clustered in the C-terminal region. Because of the large size of the *RYR1* gene, most routine screenings have been limited to the three 'hot-spots' or even to the C-terminal region alone (containing 47-67% *RYR1* mutations).[2,11,12] In 2006, Wu et al[7] sequenced all the 106 exons encoding *RYR1* among 27 unrelated Japanese central core disease patients, of whom more than 90% had *RYR1* mutations, which was a much higher mutation detection rate than previously reported. Most mutations outside the usual 'hot-spots' are localised in exons 47 and 48 that neighbour the central region (exon 39-46). By screening the C-terminal region alone and the three 'hot-spots' together, the reported mutation detection rates are about 59% and 67%, respectively. However, by including exons 47 and 48, the mutation detection rate could be increased up to 89%.[7] Our analysis followed this approach; one of the mutations we found was reported in Wu et al's study[7] and localised at exon 47. This seems a more practical and efficient genetic screening approach than sequencing the entire *RYR1* gene, and has yielded the first case with an *RYR1* gene mutation identified in our locality.

The genotype-phenotype correlations associated with the *RYR1* gene are complex and may be partly explained by the degree of functional differentiation conferred onto the large protein for which it is responsible.[13] In general, patients with C-terminal mutations usually have classical clinical features[3,7] and a characteristic pattern of selective muscle involvement on MRI imaging.[14] In contrast, patients with non–C-terminal mutations have only mild musculoskeletal abnormalities such as joint contracture and scoliosis, and are more liable to have MHS.[7,12] Notably, MHS is a pharmacogenetic disorder in which seemingly normal individuals are prone to develop serious adverse reactions upon exposure to certain inhalational anaesthetics and depolarising muscle relaxants.[15] The most frequent manifestation is isolated masseter muscle rigidity.[16] Other reactions include fever, tachycardia, hypertension, electrolyte disturbances, and rhabdomyolysis leading to acute renal failure. Malignant hyperthermia is predominantly reported in western populations, and central core disease has been largely reported in patients from western Europe.[17] The majority (96%) of the mutations detected in association with MH and central core disease are missense changes.[17] MHS-related *RYR1* mutations are predominantly located in the N-terminal and central portions of the *RYR1* protein.[13] The association of MHS and central core disease is strong but not invariable. All cases of central core disease, however, should be considered at risk of MHS.[18] Any putative triggering anaesthetic (eg halothane) or succinylcholine should be avoided, in order to avoid possible MH.

In our case, the patient's clinical presentation was compatible with a non–C-terminal mutation resulting in a later onset and milder phenotype disorder. There were also some atypical features however. Firstly, the clinical course of central core disease is usually static,[2] whereas rapid progression from muscle atrophy after trivial knee injury to pes cavus deformity within such a short period is not typical. Secondly, the serum creatine kinase concentration is usually normal or only mildly elevated in congenital myopathies.[13] Thus, the finding of a moderately elevated serum creatine kinase level may suggest an alternative diagnosis such as limb-girdle muscular dystrophy. Limb-girdle muscular dystrophies are a heterogeneous group of disorders characterised by weakness and wasting of the pelvic and shoulder girdle muscles. There are currently 19 subtypes, and patients with subtypes 1B and 1C may have only distal muscle involvement.[19] Both the clinical and biochemical findings may not assist in differentiating muscular dystrophies from central core myopathies. In addition, core formation may also be observed in other clinical conditions such as tenotomy, denervation[20] ("target-fibres") or MHS individuals without other features of congenital myopathy.[16] This highlights the importance of molecular genetic confirmation to make a definitive diagnosis in this patient.

Conclusion

The phenotype of central core myopathy is relatively heterogeneous, with variable age of onset, different degrees of muscle weakness, spinal or limb deformity and joint problems. Similar to other congenital myopathies, biochemical tests including serum creatine kinase is of limited diagnostic value. Targeted molecular study after muscle biopsy is helpful for the confirmation of the diagnosis, defining the phenotype and prediction of MHS.

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