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ORIGINAL ARTICLE

Magnetic Resonance Imaging in Mild Recessive *RYR1* Gene–related Congenital Myopathies: Genetic and Histopathological Correlation

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ABSTRACT

Objective: *To review and evaluate the pattern of muscle involvement evidenced on magnetic resonance imaging (MRI) in patients with mild recessive mutations in ryanodine receptor type 1 (*RYR1*) gene–related congenital myopathies, and to compare the consistency with those previously reported, and correlate with genetic and histological analysis.*

Methods: *The thigh and calf MRI (axial T1-weighted and short-tau inversion recovery / T2-weighted) of three Chinese patients who presented with early-onset congenital myopathy and were subsequently confirmed to have a heterozygous autosomal recessive* RYR1 *gene mutation were retrospectively reviewed by two paediatric radiologists. Pattern of muscle involvement was documented, with correlation of focused mutational genetic analysis and histological analysis of muscle biopsies.*

Results: *All three patients showed selective involvement of the thigh muscles with relative sparing of the rectus femoris, gracilis, semitendinosus and adductor longus, consistent with a previously reported pattern. A more diverse pattern was noted within calf muscles, although the soleus still showed a generally more severe degree of fat infiltration than the gastrocnemius. One muscle biopsy confirmed rod bodies on Gomori trichrome staining and nemaline rods on electron microscopy. Mutational analysis of the* RYR1 *gene revealed three novel mutations (c.1675dupA, c.10615delC, and c.11956dupG), and two known missense mutations (c.3523G>A, c.3800C>G). The missense mutation c.3523G>A (p.Glu1175Lys) was identified in two patients, suggesting that this variant is probably a hotspot mutation in the Chinese population.*

Conclusion: *With characteristic and consistent patterns of muscle involvement and fat infiltration, muscle MRI of the thigh and calf is useful in establishing the diagnosis of* RYR1*-related congenital mypopathy in a Chinese population, thereby guiding more focused mutational analysis and management.*

Key Words: *Magnetic resonance imaging; Muscular diseases; Mutation, missense; Myopathies, nemaline; Ryanodine receptor calcium release channel*

中文摘要

輕度隱性*RYR1*基因相關的先天性肌病的磁力共振成像：基因及組織病理學

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目的：回顧和評估輕度隱性*RYR1*基因突變的相關先天性肌病患者的磁力共振成像（MRI）的表現，

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並與已經出版的文獻比較，及分析基因和組織病理學的相關性。

方法：兩名兒科放射科醫生回顧分析3名華裔患者的大腿和小腿MRI（橫斷面T1W和STIR / T2W圖像）肌肉病變模式。患者早期出現先天性肌病，後來證實有異合常染色體隱性*RYR1*基因突變。基因突變和與肌肉活檢組織學的相關性進行了分析。

結果：3名患者均顯示其大腿肌肉有不同程度的涉及，而股直肌、股薄肌、半腱肌和內收肌長肌影響相對較少。這些特點與以前報告一致。小腿肌肉有更多樣的表現，儘管比目魚肌比腓腸肌有更嚴重的脂肪滲透。一名患者的肌肉活檢在電子顯微鏡下確認有Gomori三色染色桿狀體的和肌纖維線形小體。*RYR1*基因的突變分析揭示三個新的突變（c.1675dupA，c.10615delC和c.11956dupG）和兩個已知的錯義突變（c.3523G> A，c.3800C> G）。兩名患者都有c.3523G> A（p.Glu1175Lys）的錯義突變，表明該變體可能是中國人群中的突變熱點。

結論：大腿和小腿肌肉的涉及模式有助建立中國人群中*RYR1*相關先天性輕度病變的診斷，從而指導基因突變分析和處理。

INTRODUCTION

Congenital myopathies represent a group of neuromuscular diseases that usually present in childhood and are associated with distinct histopathological features in skeletal muscles. Mutation in the ryanodine receptor type 1 (*RYR1*) gene represents one of the most common groups of congenital myopathies known to be associated with central core, minicore, and centronuclear diseases. Both autosomal dominant and recessive inheritance have been documented.

A broad spectrum of presentation has been reported in the literature, from respiratory distress and feeding difficulties in newborns, to muscle weakness and ophthalmoplegia in children or teenagers. Cases of adult-onset *RYR1*-related myopathies have also been described,[1] with a generally milder degree of clinical symptoms. Patients with *RYR1* gene–related mutations are also at risk of developing malignant hyperthermia, a life-threatening anaesthetic complication that is potentially avoidable if recognised prior to surgery.

The non-specific neuromuscular signs and symptoms often overlap with other congenital myopathies, thereby complicating the clinical diagnosis, direction of histological as well as genetic investigations, and eventually the final diagnosis. Patterns of skeletal muscle involvement on magnetic resonance imaging (MRI) have been described in small western cohorts.[2,3] To date, only one case of *RYR1*-related central core myopathy has been reported in Hong Kong.[4] The use of MRI has attracted attention to direct more focused mutational analysis and guide subsequent management.

This study is the first to review and evaluate the pattern of muscle involvement in MRI of Chinese patients with mild recessive mutations of *RYR1*-related congenital myopathies, and to compare the consistency with those previously reported, and correlate their genetic and histological analysis.

METHODS

Three patients presented to the Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, Hong Kong, between November 2003 and June 2013, with symptoms of congenital myopathies and a subsequent confirmed genetic diagnosis of heterozygous autosomal recessive *RYR1* gene mutations. Informed consent from each patient was obtained.

MRI was performed between July 2013 and November 2014, using GE Healthcare Signa HD at 1.5 or 3.0 Tesla. Axial scans of bilateral pelvic girdles, thighs, and calves in non-contrast enhanced T1-weighted (repetition time [TR] / echo time [TE], 440-840/8-9 ms) and short-tau inversion recovery (STIR) [TR / TE, 5000-6300/38-70 ms] sequences were obtained. An alternative to STIR was fast spin-echo T2-weighted (TR / TE, 4460-6720/81-98 ms) sequence. The sections were 5-mm thick, with a 10-mm gap between sections.

These MRIs were retrospectively and independently reviewed by two specialist radiologists at our centre who had at least 3 years' experience in paediatric radiology. Pattern and degree of fat infiltration of the individual muscles of both thighs and calves were evaluated. The degree of fat infiltration was assessed by examining the proportion of fat in each of the muscles, with reference made to the types of fat infiltration (Table 1) and based

on the classification described by Ozsarlak et al.[5] Nonetheless, the emphasis was more on the cumulative pattern of muscle involvement among these patients than on the score for individual muscles. Comparison of the pattern of involvement with that previously reported in *RYR1*-related myopathies was then performed.

Full gene sequencing of *RYR1* was performed by polymerase chain reaction and direct DNA sequencing on all the coding exons and splice sites (NM_000540.2, NP_000531.2). For novel mutation, bioinformatic study with in-silico analysis using SIFT, PolyPhn2 and Align GVGD was performed.

Muscle biopsies were snap frozen in isopentane cooled in liquid nitrogen. Routine stains included haematoxylin and eosin, modified Gomori trichrome, Oil Red O, Periodic acid-schiff, alkaline phosphatase, acid phosphatase, adenosine triphosphatase at pH 4.2, 4.6, 9.4, nicotinamide adenine dinucleotide dehydrogenase–tetrazolium reductuse, succinate dehydrogenase, cytochrome oxidase, and combination of the latter two. Immunohistochemistry for sarcolemmal proteins were also performed. Ultrastructure study with electron microscopy was also performed in all patients.

Muscle biopsy results and reports of genetic mutational analysis were obtained from the patients' record and subsequently evaluated with reference to the MRI results.

Table 1. Types of muscle fat infiltration on magnetic resonance imaging.[5]

Type	Characteristics
1	Central fat infiltration within the muscles
2	Peripheral fatty rim around normal-looking muscle
3	Tiny fatty areas within the muscle
4	Patchy fat infiltration within the muscles (4a: a few; 4b generalised; 4c diffuse)
5	Diffuse fat infiltration (5a: diffuse fat infiltration surrounded by a rim of residual muscles; 5b: a few small residual muscle islands within diffuse fat infiltration)
6	Completely fatty area (6a: peripheral fascia is preserved; 6b: muscle fascia is not visible)
7	Mixture of two or more patterns
8	Irregular border of muscles

Table 2. Patient clinical details.

Clinical detail	Patient 1	Patient 2	Patient 3
Age at imaging assessment (years)	4	10	20
Age at first concern	14 months	At birth	16 months
Family history of myopathies	No	No	No
Ophthalmoplegia	No	Yes	No
Facial weakness	Mild	Yes	Mild
Proximal girdle weakness (Medical Research Council grading)	3/5	3-4/5	3/5
Functional ability — walk	Yes	Yes	Yes
Feeding difficulty	No	Tube feeding at birth, resume full oral feeding at 4 months	No
Learning difficulty	No	No	No

RESULTS

The three patients were aged 4, 10, and 20 years at the time of MRI. All had normal intelligence. They were independent walkers and presented to the clinic with symptoms of mild proximal girdle, axial, and facial weakness. One of the patients experienced ptosis with ophthalmoplegia. Their clinical details are summarised in Table 2.

Imaging Findings

Patterns of involvement, in terms of relative gradient with adjacent muscle groups, are described in Table 3. Figure 1 is a schematic diagram of the typical pattern of muscle involvement in *RYR1* gene–related myopathies. Figures 2 and 3 show MRI of the thigh and calf muscles of our patients, respectively.

In the thigh, all three patients manifested consistent patterns of involvement, with selective or relative sparing of the rectus femoris, gracilis, semitendinosus and adductor longus muscles. The results are coherent with the typical pattern of *RYR1*-related mypopathies.

A more diverse pattern was noted within the calf muscles, with different degrees of fat infiltration and distribution observed. Nonetheless, the soleus still showed a generally more severe degree of fat infiltration compared with the gastrocnemius, and was compatible with the previously reported pattern.

There was agreement between the two radiologists over the pattern of muscle involvement in all three cases. Slight discrepancies were noted when evaluating the degree of fat infiltration within individual muscles, particularly in the calves. These cases were then further reviewed together and a consensus was reached after discussion.

Table 3. Magnetic resonance imaging results.

Relative gradient of involvement in muscle groups	Patient 1	Patient 2	Patient 3	Consistency
Adductor magnus > Adductor longus	Yes	Yes	Yes	100%
Vastus lateralis > Rectus femoris	Yes	Yes	Yes	100%
Sartorius > Gracilis	Yes	Yes	Yes	100%
Soleus > Gastrocnemius medialis	Yes	Yes	Yes	100%
Peroneus > Tibialis anterior	No	Same	No	33%
Gastrocnemius (lateral) > Gastrocnemius (medial)	Same	Same	Same	0%

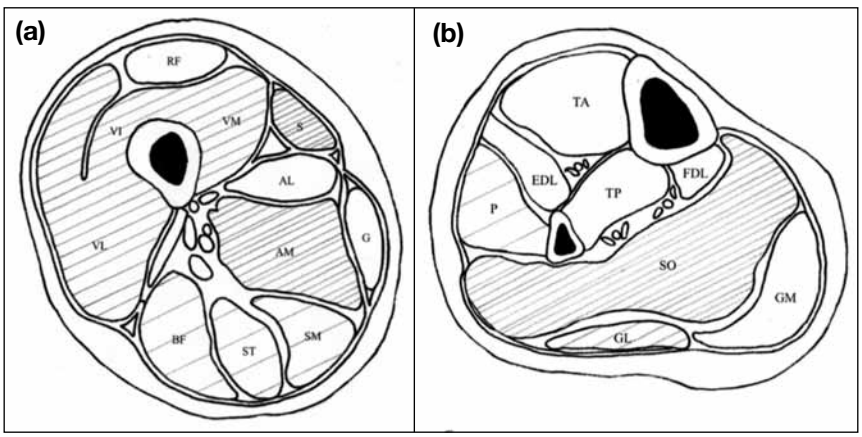

Figure 1. Schematic illustration of typical pattern of *RYR1* gene–related myopathy. Density of lines indicates the relative degree of fat infiltration across different muscles. (a) Thigh: the adductor magnus (AM), sartorius (S), vastus medialis (VM), vastus intermedius (VI), and vastus lateralis (VL) are affected, with relative sparing of the rectus femoris (RF), adductor longus (AL), and gracilis (G). (b) Calf: the posterior compartment showing an overall greater degree of involvement than the anterior compartment, most significantly affecting the soleus (SO).
Abbreviations: BF = biceps femoris; EDL = extensor digitorum longus; FDL = flexor digitorum longus; GL = gastrocnemius lateralis; GM = gastrocnemius medialis; P = peroneal group;SM = semimebranosus; ST = semitendinosus; TA = tibialis anterior; TP = tibialis posterior.

Genetic Analysis

Genetic analysis of patients is shown in Table 4. Results showed compound heterozygous mutations in all. Three mutations were novel (c.1675dupA, c.11956dupG and c.10615delC) and two were known missense mutations (c.3523G>A, c.3800C>G). Among the three patients, two had the missense mutation of c.3523G>A (p.Glu1175Lys).

Muscle Biopsy Results

One of three patients had clinical manifestations of ptosis and ophthalmoplegia, and her muscle biopsy confirmed rod bodies on Gomori trichrome staining and nemaline rods on electron microscopy. The other two patients showed non-specific myopathic changes with unremarkable immunohistochemical studies, although electron microscopy revealed focal disruption of myofibrillar architecture and minicores, respectively.

DISCUSSION

Congenital myopathies include a group of neuromuscular diseases with a wide spectrum and non-specific presentations that frequently overlap with other disease entities. Clinical phenotypes of patients with *RYR1* gene–related congenital myopathies can show a similar distribution of muscle weakness to some limb girdle muscular dystrophies, Becker muscular dystrophy, milder forms of spinal muscular atrophy or other congenital myopathies. Diagnosing patients with a specific type of myopathy can therefore be difficult and complicated. Patterns of skeletal muscle involvement on MR images have been described in the literature.

RYR1 gene–related congenital myopathies express consistent patterns of muscle involvement: selective or relatively sparing of the rectus femoris, gracilis, semitendinosus and adductor longus muscles compared with other thigh muscles.[2,3] Anterior thigh muscles also generally show a greater degree of involvement than the posterior muscle group. In the lower legs, the peroneus is more frequently involved than the tibialis anterior, the soleus is often more affected than other muscles in the posterior compartment, and the lateral head of gastrocnemius more affected than the medial head.

With reference to previously published studies, analysis of the relative extent of fat infiltration between muscles is more conclusive than assessment

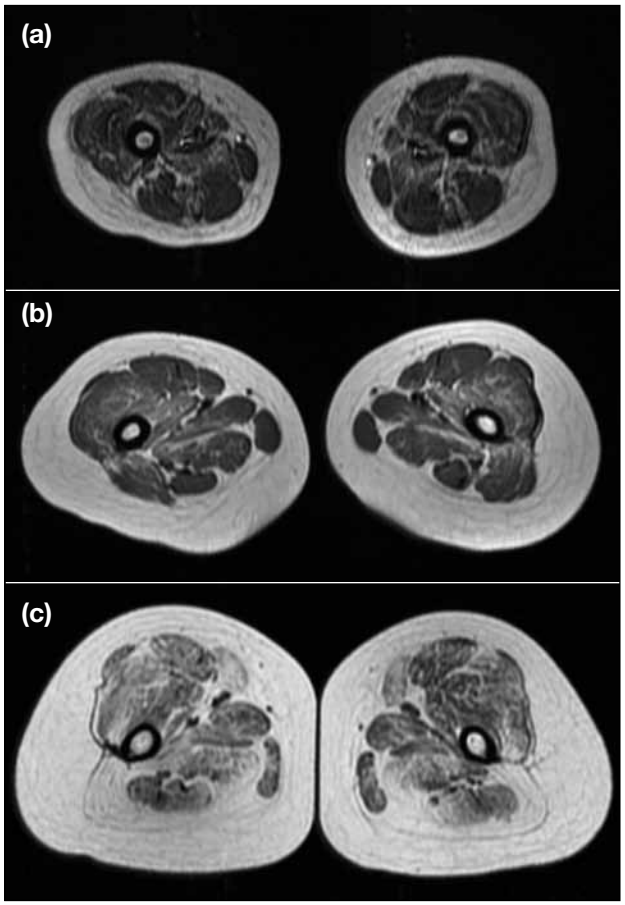

Figure 2. Axial T1-weighted magnetic resonance images of the thigh. (a) Patient 1: there is involvement of vasti and adductor magnus and brevis, with relative sparing of rectus femoris, gracilis, adductor longus, semimembranosus and long head of biceps femoris. (b) Patient 2: moderate fat infiltration at bilateral vastus lateralis, vastus intermedius, vastus medialis and adductor magnus. Mild fat infiltration is also observed at bilateral sartorius and biceps femoris. Bilateral rectus femoris, gracilis, adductor longus and semitendinosus are spared. (c) Patient 3: there is diffuse fat infiltration. Rectus femoris, adductor longus, gracilis and semitendinosus muscles are relatively less severely involved.

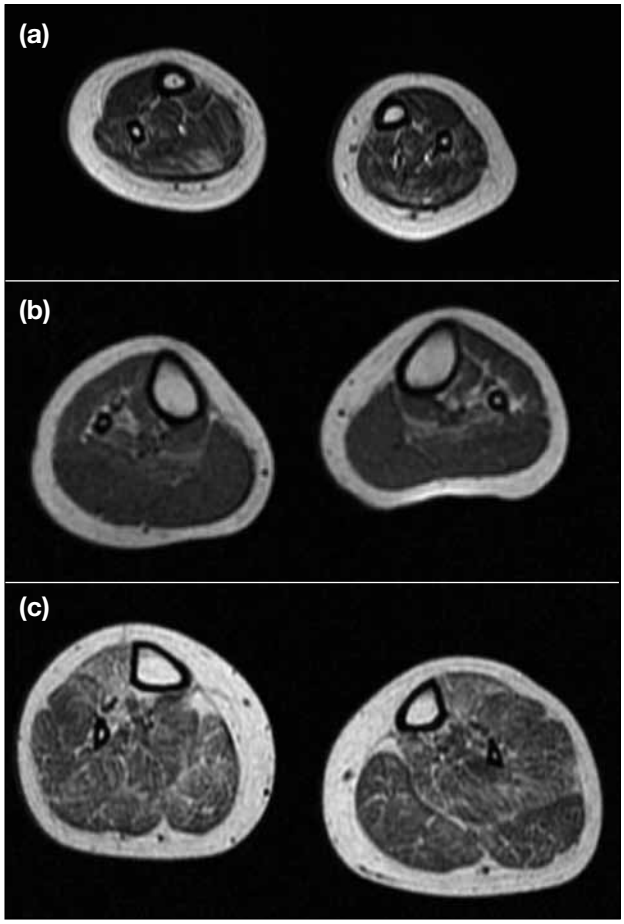

Figure 3. Axial T1-weight magnetic resonance images of the calf. (a) Patient 1: there is selective involvement of soleus and tibialis posterior, with relative sparing of tibialis anterior. (b) Patient 2: there is mild fat infiltration of bilateral soleus, peroneal and tibialis posterior. No definite fat infiltration of the tibialis anterior, gastrocnemius medialis and gastrocnemius lateralis is noted. (c) Patient 3: the tibialis anterior and tibialis posterior are more severely involved than the rest of the calf muscles.

Table 4. Genetic analysis results.

Patient No.	Genetic analysis	Mutation
1	1. Heterozygous c.3523G>A, GAA>AAA,p.Glu1175Lys	Known missense mutation
	2. Heterozygous c.11956dupG, p.Asp3986Glyfs*89	Novel frameshift mutation
2	1. Heterozygous c.3800C>G, CCC>CGC, p.Pro1267Arg	Known missense mutation
	2. Heterozygous c.1675dupA, p.Ile559Asnfs*11	Novel frameshift mutation
3	1. Heterozygous c.3523G>A, GAA>AAA, p.Glu1175Lys	Known missense mutation
	2. Heterozygous c.10615delC, p.Arg3539Valfs*4	Novel frameshift mutation

of individual muscles.[2] In the present study, all three patients manifested consistent patterns of involvement in the thigh muscles, but a more diverse degree and distribution of fat infiltration in the lower legs. Yet when emphasis was placed on the gradient of involvement other than that of selected muscles, the soleus still showed a generally more severe degree of fat infiltration than the gastrocnemius, in keeping with the previously

reported pattern.

On the contrary, although relative sparing of the adductor longus, gracilis and semitendinosus muscles in Becker muscular dystrophy has been described, the rectus femoris tends to be involved severely and the gastrocnemii are typically more prominently involved than the soleus.[3,6-8] Studies performed on limb girdle muscular dystrophies have revealed various distinct MRI patterns of muscle involvement associated with specific genetic defects.[3,9,10]

Our results are therefore useful in guiding diagnosis of *RYR1*-related myopathies. The advantages of MRI, such as increasing availability, no radiation exposure, and the ability to assess degree as well as progression of disease entity, and recognising this pattern of muscle involvement guides clinicians in conducting more focused genetic testing and analysis. This avoids the need to interpret the large-size *RYR1* gene.

One of the three patients experienced ptosis with ophthalmoplegia. Patients with ophthalmoparesis demonstrate a consistent pattern on MRI, but with a lesser gradient between involved and spared muscles.[2] This distribution was also demonstrated in our patient. We also observed that when correlating with the patient's muscle biopsy report, nemaline rods were identified (but were not present in the remaining two patients). She showed the earliest presentation at birth with hypotonia and poor breathing, the most severe degree of muscle weakness among our three patients. Another case with *RYR1*-related nemaline myopathy and ophthalmoplegia has been described.[11]

When evaluating mutational analysis of the *RYR1* gene, three novel mutations (c.1675dupA, c.10615delC and c.11956dupG) and two missense mutations (c.3523G>A, c.3800C>G) were identified. The missense mutation c.3523G>A (p.Glu1175Lys) was present in two patients; this might imply a hotspot mutation in Chinese patients with *RYR1*-related myopathy.

As congenital myopathies are rare disease entities, the number of local patients affected and subsequently confirmed with *RYR1* mutations is few, thereby limiting the size of our cohort. With increasing awareness of this disease entity, together with increased availability of MRI and genetic testing in diagnosis, we aim to include more patients and identify more specific patterns of muscle involvement and hotspot mutations in Chinese patients.

CONCLUSION

Our study showed consistent patterns of muscle involvement on MRI of the thigh and calf muscles in local patients compared with the previously reported patterns in western populations. Selective sparing of the rectus femoris, gracilis, semitendinosus and adductor longus muscles was demonstrated. Mutational analysis of our patients identified three novel *RYR1* mutations and the missense variant c.3523G>A (p.Glu1175Lys) that may represent a hotspot mutation in Chinese patients. Therefore, MRI of lower limb muscles aids in establishing the diagnosis of *RYR1*-related congenital mypopathy in Chinese patients, and guides more focused mutational analysis and management.

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