

Exercise induced cramps and myoglobinuria in dystrophinopathy – a report of three Malaysian patients

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Abstract

Dystrophinopathies commonly present as Duchenne or Becker muscular dystrophy but rare, unusual phenotypes have also been described. We have identified three Malaysian boys with an unusual form of dystrophinopathy, presenting with exercise-induced cramps and myoglobinuria, but with no apparent muscle weakness. Immunohistochemistry for dystrophin and genetic analysis confirmed the diagnosis. The frequency of this phenotype is unknown but there have been several case reports. Consistent with these reports, we also found that two of our patients had deletions in the rod domain of dystrophin, which has been suggested to be associated with this unusual manifestation.

INTRODUCTION

Dystrophinopathy is an X-linked recessive form of muscular dystrophy in which there is total or partial deficiency of dystrophin. Dystrophin is a sarcolemmal protein which forms part of the protein complex which links intracellular cytoskeletal proteins with the extracellular matrix. Dystrophinopathies result from mutations in the *DMD* gene and is the most common cause of muscular dystrophy in males. Globally, every 1 in 3,500 newborn males are inflicted with the disease.¹

The major dystrophinopathy phenotypes are Duchenne muscular dystrophy (DMD), if there is an out-of-frame mutation and Becker muscular dystrophy (BMD), if there is an in-frame mutation of the dystrophin. However, dystrophinopathy can also present atypically with isolated cardiomyopathy and asymptomatic raised creatine kinase. The variability in phenotype can even result in the disease presenting differently in identical twins sharing the same mutation.²

A rare phenotype is that of exercise-induced rhabdomyolysis with myoglobinuria³ which initially usually points to a diagnosis of metabolic myopathy. It has been suggested previously that this syndrome is associated with loss of part of the rod domain of the dystrophin protein.³

We report three boys, with this unusual phenotype in our series of dystrophinopathy patients. All presented with exertional intolerance

with exercise induced muscle cramps (with myoglobinuria in two) but no muscle weakness at rest. We found they had abnormal dystrophin staining with at least one anti-dystrophin antibody and genetic analysis showed deletions in *DMD*.

METHODS

The three patients presented to different hospitals (University of Malaya Medical Centre (UMMC) and National University of Malaysia Medical Centre) but their muscle biopsy samples were all referred to the Department of Pathology, UMMC for further diagnostic evaluation. Clinical data including details of neurological examination, electromyography tests and serum creatine kinase results were obtained from the referring clinicians.

For routine H&E and modified gomori trichrome stains, enzyme histochemistry and immunohistochemistry (IHC), 8µm thick cryostat sections were taken from frozen muscle biopsies. For IHC, a standard protocol was used using three primary monoclonal antibodies: DYS-1 to detect the rod domain of DYSTROPHIN, DYS-2 to detect the Cysteine-rich and C-terminal of DYSTROPHIN and DYS-3 to detect the N-terminal of DYSTROPHIN (Novocastra Laboratories, UK). Briefly, sections were incubated with the monoclonal antibodies, washed with TRIS buffer and the secondary antibody (goat anti-mouse) was added. The IHC was then

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developed using DAB and counterstained with Harris haematoxylin, after which they were dehydrated and mounted using DPX. Stained sections were viewed under a light microscope.

For genetic analysis, DNA was extracted from frozen muscle tissue using the phenol-chloroform method. Multiplex polymerase chain reaction (PCR) was performed on the samples to amplify the common 18 deletion hotspot exons which include exons 1, 3, 4, 6, 8, 13, 19, 42, 43, 44, 45, 47, 48, 50, 51, 52, 53 and 60, as previously described.⁴ PCR products were electrophoresed in a 2% agarose gel and visualised under a UV transilluminator. All PCRs were performed in duplicate.

RESULTS

Clinical presentation

Patient 1

A 13 year-old ethnic Chinese boy presented with recurrent muscle pain on exercise since he started school. He had two episodes of passing dark-coloured urine, after physical education class and has not been able to participate in sports. There was no apparent muscle weakness at rest. He had no family history of muscle weakness or similar complaints. On clinical examination, there was bilateral calf hypertrophy but muscle power was normal. Creatine kinase at presentation was 10,793 IU/L and electromyography showed myopathic motor unit potentials. Forearm exercise test did not show significant rise in serum lactate after exercise. Immunohistochemistry in the muscle biopsy was negative for DYS-1 and DYS-3 but positive for DYS-2 (Figure 1). *DMD* gene analysis showed a large deletion from exons 10 to 30.

Patient 2

A 9 year-old ethnic Chinese boy presented with recurrent leg cramps on running and carrying objects since 4 years old. There were no episodes of passing dark urine. An older male sibling died in the neonatal period but the diagnosis is unknown. Clinical examination revealed normal muscle power but he had bilateral calf hypertrophy. Creatine kinase was >70,000 IU/L and electromyography was normal. IHC showed DYS-1 to be negative but DYS-2 and DYS-3 were positive. *DMD* gene analysis showed a large deletion from exons 13 to 37.

Patient 3

A 10 year-old ethnic Malay boy presented with a 3 year history of calf pain on walking associated with passing dark-coloured urine afterwards. Three of his maternal uncles had BMD. Clinically, he had calf hypertrophy but normal muscle power. The serum creatine kinase was 120,000 IU/L. No electromyography was performed. IHC showed DYS-1 to be negative while DYS-2 and DYS-3 were positive with a 'faint and patchy' pattern. *DMD* gene analysis showed deletions in exons 45 and 47.

Biopsy results

For all three patients, the muscle biopsy showed similar mild to moderate dystrophic features which included the presence of atrophic and hypertrophic fibres accompanied by degenerating, necrotic and regenerating fibres. There was also focal inflammation and increased perimysial and endomysial fibrosis.

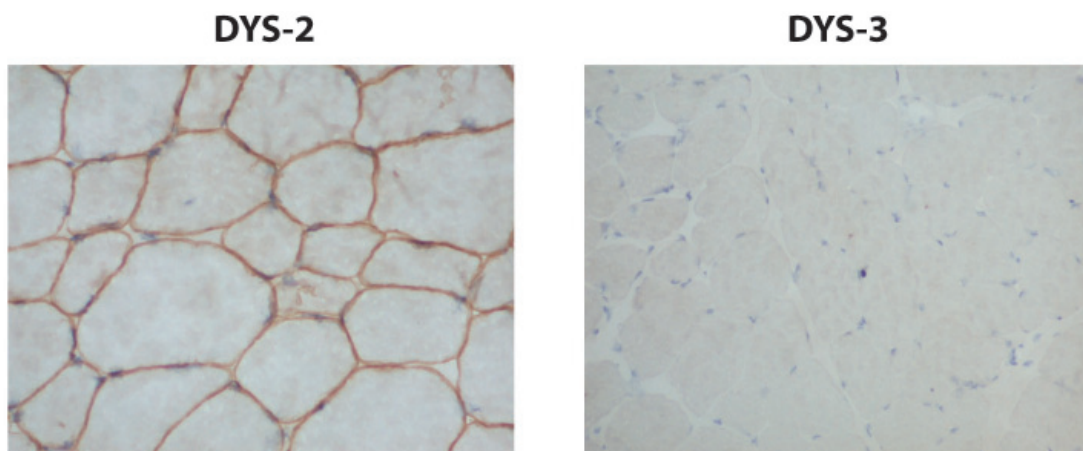


Figure 1: IHC of biceps muscle in patient 1 with antibodies for DYS-2 (positive staining) and DYS-3 (negative staining)

DISCUSSION

We report three unrelated dystrophinopathy patients who presented with a rare phenotype of exercise induced cramps and myalgia. Two of the three had myoglobinuria which typically occurred after strenuous activity. All three had normal muscle strength at rest with bilateral mild calf hypertrophy. In all three patients, serum creatine kinase levels were elevated ranging from 10,000 - 120,000 IU/L. Histologically, the muscle biopsy showed muscular dystrophy with abnormal IHC to DYSTROPHIN.

A review of the literature for this phenotype (Table 1), suggests that the incidence is very low, although cases may be erroneously diagnosed as having metabolic disorders, rather than dystrophinopathy. These cases are similar to the three boys we describe here, as they all exhibit exercise-induced cramps and myoglobinuria.

Dystrophinopathy is caused by a number of different mutations in the *DMD* gene, including deletions, duplications and point mutations. Deletions are the most common mutation, affecting between 60-65% of all cases, while point mutations are found in 20-30% and duplications between 5-10%. The severity of the disease is not determined by the type of mutation but instead on whether the reading frame is disrupted and also the location of the mutation.⁴ DMD is associated with mutations which disrupt the reading frame, leading to an absent or non-functional protein being expressed. Meanwhile, BMD is associated with an intact reading frame but a truncated DYSTROPHIN protein. This truncated protein may still be partially functional as long as it retains the crucial domains of DYSTROPHIN.

DYSTROPHIN has several protein domains. Domain I or the N-terminal, binds to the actin cytoskeleton. Domain II (rod domain) is not thought to bind to any protein. Domain III or the cysteine-rich domain binds to dystroglycan at the sarcolemma, while Domain IV or the C-terminal binds to dystrobrevin.¹¹ Mutations in domain I are usually associated with milder forms of dystrophinopathy. Meanwhile, domain II can be subject to very large deletions but yet the patients are less affected. In a reported case, over 46% of the *DMD* gene was deleted in the rod domain but the patient showed very mild dystrophinopathy and was still mobile into his sixties.¹² Mutations in domains III and IV tend to be associated with severe forms of dystrophinopathy, although studies have suggested that the most terminal portion of domain IV is associated with mild, non-

progressive BMD.¹³ Figure 2 shows the domains of *DMD* with the corresponding exons.

Previous studies have explored the clinically variable nature of dystrophinopathy. Deletions or duplications at the rod domain have been associated with asymptomatic individuals, patients with elevated serum CK levels and also tend to present more frequently in patients with severe cramps and myalgia.⁴ In their paper, Figarella-Branger *et al.*³, propose that the absence of the rod domain could be used as a reliable tool in diagnosing this syndrome. Our literature review does not support such a strict association as the N-terminal was also found to be involved (Table 1).

Genetic analysis of the *DMD* gene in our patients revealed exon deletions within the rod domain (Figure 3, Table 2). However, their DYSTROPHIN staining was variable (Table 2), with Patients 1 and 2 being negative for DYS-1, suggesting the absence of the rod domain but Patient 3 showed positive, albeit patchy, staining. This would suggest that some preservation of the rod domain can still result in described symptoms.

Interestingly, all our patients were positive for DYS-2 which stains the cysteine-rich and C-terminals. This is consistent with other reports of patients with myoglobinuria who also did not show any deletions in the cysteine-rich and C-terminals (Table 1). Preservation of this domain which binds to dystroglycan in the sarcolemma may confer greater stability to the muscle cell and a less clinically severe phenotype where muscle necrosis only occurs upon exercise.

Recently, there have been reports linking myoglobinuria in DMD due to corticosteroid treatment.¹⁴ Interestingly, the episodes of myoglobinuria were reported after some physical exertion, so it is unclear whether it was truly associated with corticosteroid treatment or the physical exercise. Our patients were not on corticosteroid treatment at the time of clinical presentation of myoglobinuria, so the presence of myoglobinuria in these boys is likely to be exercise induced rather than due to side-effects from their treatment.

There have also been reports of female patients with this phenotype. Tunteeratum and colleagues found rhabdomyolysis and acute renal failure in a manifesting female carrier of Duchenne muscular dystrophy, who harbours a duplication in *DMD*.⁹ Another female patient with a homozygous deletion of exons 45-55 presenting with exercise intolerance and recurrent myoglobinuria, but

Table 1: Retrospective literature study on previously reported cases of exercise- induced cramps and myoglobinuria in BMD

References	Clinical diagnosis	Protein and DNA analysis
Gold R <i>et al.</i> , 1992 ⁵	Two Greek brothers (33 and 28 years old) presented with exertional cramps and 'pigmenturia', which dated from their 20s. Serum CK levels were between 800-2000 U/L.	<u>Immunohistochemistry</u> Faint staining with dystrophin antibodies. <u>Immunoblotting</u> Truncated protein with reduced expression <u>Genetic analysis of DMD</u> Deletions in exons 2-7 (reported for one of the brothers)
Doriguzzi <i>et al.</i> , 1993 ⁶	9-year-old boy presented with exertional myalgias and severe muscle pain after running and myoglobinuria on two occasions. No muscle weakness. Serum CK level was 8200 units/l (1 week after one of the episodes)	<u>Immunoblotting</u> Truncated protein with a reduced expression <u>Genetic analysis of DMD</u> Deletions in exons 45-48.
Minetti <i>et al.</i> , 1993 ⁷	Two boys presented with elevated serum levels of creatine kinase, cramps and pigmenturia.	<u>Immunohistochemistry</u> Reduced staining intensity with patchy and discontinuous pattern in the muscle fibres. <u>Immunoblotting</u> No DYSTROPHIN protein expression in either boy. <u>Genetic analysis of DMD</u> In frame deletion of exons 3-6 (reported for one patient)
Figarella-Branger <i>et al.</i> , 1997 ³	Three patients presented with exertional rhabdomyolysis with myoglobinuria Serum CK levels were elevated. Unclear from the paper whether there was any muscle weakness.	<u>Immunohistochemistry</u> No or irregular Dys1 staining while Dys2 and 3 were reduced. One of the patients had a mosaic pattern of expression with all the dystrophin antibodies. <u>Genetic analysis of DMD</u> No deletions in DMD in the common hotspot exons. Potentially other types of mutations in <i>DMD</i> (duplication or point mutation).
Löfberg M <i>et al.</i> , 1998 ⁸	Screened 22 patients with recurrent rhabdomyolysis and 26 other patients who had an episode of rhabdomyolysis	Found one patient with Becker's muscle dystrophy.

References	Clinical diagnosis	Protein and DNA analysis
Tunteeratum <i>et al.</i> , 2009 ⁹	A manifesting female carrier presented with rhabdomyolysis and acute renal failure.	<i>Immunohistochemistry</i> Mosaic patterns of dystrophin staining with antibodies against amino-terminal, carboxy-terminal, and rod domains. <i>Genetic analysis of DMD</i> Heterozygous duplication of exons 1-6 in <i>DMD</i> .
Fuji <i>et al.</i> , 2009 ¹⁰	14 year old female BMD presenting with exercise intolerance and recurrent myoglobinuria	<i>Immunohistochemistry</i> Minimal dystrophic staining and faint dystrophin staining pattern <i>Genetic analysis of DMD</i> Homozygous deletion of exons 43-45 in <i>DMD</i> .

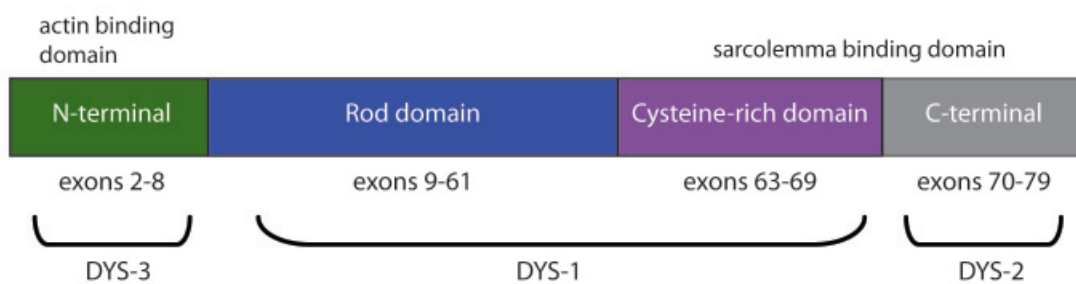


Figure 2: Schematic diagram of DMD domains, the exons within those domains and the binding sites for the three DYSTROPHIN antibodies (adapted from www.dmd.nl)

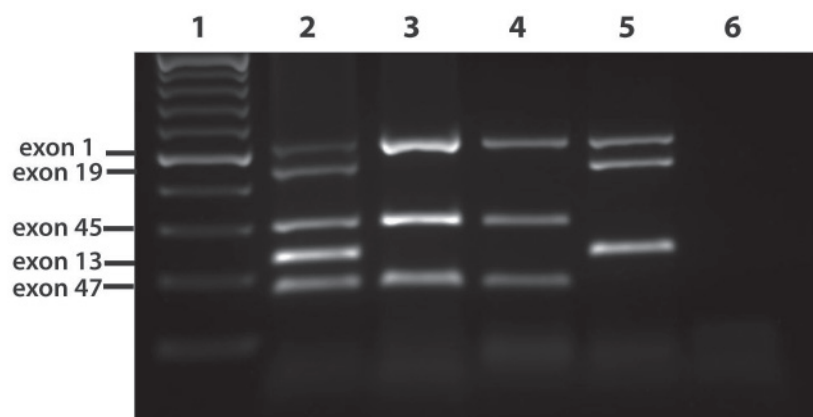


Figure 3: Multiplex PCR analysis showing deletions in all three cases. Representative exons were selected for this figure.

Lane 1: 100bp DNA ladder; lane 2: Normal male; lane 3: Case 1 with deletions in exons 13 and 19;

Lane 4: Case 2 with deletions in exons 13 and 19; lane 5: Case 3 with deletions in exons 45 and 47;

Lane 6: non-template control.

Each PCR for the cases was performed with three internal control exons which were exons known not be deleted in the particular case.

Table 2: Clinicopathological features and genetic analysis of patients

Patient	Age at diagnosis	Presenting symptoms	Clinical status	Serum creatine kinase	Biopsy	Immunohistochemistry	Genetic analysis of <i>DMD</i>
1	13 years	Recurrent muscle cramps upon exercise Myoglobinuria	Calf hypertrophy	10,793 IU/L	Moderate dystrophic features	Dys1 : negative Dys2 : positive Dys3 : negative	Deletions in exons 13 and 19
2	9 years	Recurrent leg cramps on running and carrying objects	No weakness. Normal muscle power. Mild calf hypertrophy	> 70,000 IU/L	Moderate dystrophic features	Dys1 : negative Dys2 : positive Dys3 : positive	Deletions in exons 13 and 19
3	10 years	Recurrent calf pain after exercise for the last 2-3 years. Myoglobinuria	No weakness. Normal muscle power. Calf hypertrophy. Has 3 maternal uncles with BMD and similar symptoms	> 120,000 IU/L	Mild to moderate dystrophic features	Dys1 : positive Dys2 : faint and patchy Dys3 : faint and patchy	Deletions in exons 45 and 47

no muscle weakness, has also recently been reported.¹⁰

In summary, exercise-induced cramps and myoglobinuria can be seen in patients with dystrophinopathy, and is usually associated with deletions in the rod domain. Preservation of the cysteine-rich domain possibly contributes to a less severe phenotype. Full clinical, histological and genetic analysis is recommended in cases of suspected dystrophinopathy when presenting with the features described.

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