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Serial case report: Becker's muscular dystrophy phenotype with dilated cardiomyopathy in patients with out-of-frame deletion involving exons 38-43 of *DMD* gene

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Abstract

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive allelic muscular dystrophies due to mutations of the *DMD* gene, showing more severe and milder phenotype, respectively. Here we present, two brothers, ages 23 years old (case 1) and 19 years old (case 2) who presented with clinical symptoms typically associated with BMD, including dilated cardiomyopathy in the first case and mild cardiac enlargement in the second. Muscle symptoms were moderate enabling independent ambulation of both patients until the present. Dystrophin protein was patchy on immunohistochemistry staining confirming the diagnosis of BMD. However, genetic analysis using Multiple Ligation-dependent Probe Amplification (MLPA) identified out-of-frame deletions involving exons 38-43 of the DMD gene in both cases. Here, we report a case series with an exception to the reading frame rule due to mutations affecting the central rod domain of the *DMD* gene.

Keywords: Becker muscular dystrophy, Duchenne muscular dystrophy, cardiomyopathy, out-of-frame deletion, reading frame rule

INTRODUCTION

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive allelic muscular dystrophies due to mutations of the *DMD* gene.[1,2] A meta-analysis study on the epidemiology of BMD reported pooled prevalence of DMD and BMD were 4.78 and 1.53 per 100,000 males, respectively.[1] BMD is characterized by less severe clinical phenotypes than DMD with the onset mostly during school-age, between 5-15 years old, even though there are several reported cases of late onset BMD in the third and fourth decades of life.[2,3] In DMD, the majority of cases have onset between 3-5 years of age and the children usually lose their independent ambulation before reaching 12 years old.[3,6] Clinical phenotypes of BMD are varied, ranging from mild symptoms, such as muscle cramps, myalgia, myoglobinuria and mild myopathy, to severe myopathy that can interfere with patients' ambulatory ability.[2,4] Cardiac involvement is reported in 70% of BMD cases. The most common form of cardiac abnormality is dilated cardiomyopathy which is associated with congestive heart failure and premature death.[5]

The *DMD* gene was first identified in 1982. The gene is located in the short arm of the X chromosome (Xp21).[6] Mutation of the gene results in defects in dystrophin, a sarcolemma membrane protein, that is essential for stabilizing the membrane during muscle contractions.[6,7] The protein is not only located in the skeletal muscle membrane but also in the brain, retina and myocardium.[8] Unlike DMD, the clinical presentation of BMD is milder because a partially functional dystrophin is still produced.[4]

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The definitive diagnoses of DMD and BMD are made based on medical history, physical examination, muscle biopsy examination and genetic testing.[9] The examination of the serum creatinine kinase (CK) level may be performed as an early detection before muscle biopsy. The CK serum levels are markedly increased even in mild BMD cases with subtle clinical manifestations or in the early phase of severe cases before the appearance of muscle weakness.[10] To evaluate dystrophin expression, immunohistochemistry staining of muscle biopsy specimens remains the gold standard for diagnosis of any dystrophinopathies.[4] At the present, genetic testing is essential not only for diagnostic purposes but also to provide prognostic information and facilitate genetic counseling. The mutational status is also important for novel therapies such as exon skipping, gene transfer, and nonsense suppression for DMD cases.[4]

The reading frame rule differentiates BMD and DMD based on the underlying genetic features and their correlated phenotypes. The mutation that disrupts the results of the open reading frame causes the pathogenesis of the DMD phenotype while the one that maintains the open reading frame allowing a shortened but functional dystrophin expression results in the milder BMD phenotype. However, the rule is not absolute as confirmed by various studies that reported exceptions to the reading frame rule which may be caused by several molecular mechanisms. At present, there is no exact explanation for the connection between the genetic mutation process and the phenotypes of dystrophinopathies. Recent findings of genetic studies suggest some relation between the clinical phenotypes' variability in BMD with the location of mutation in the *DMD* gene even though the exact association has not been proved.[11-15]

CASE REPORTS

We present two brothers, 23 years old and 19 years old, with BMD. Informed consent for this case report study had been given by both patients. The chief complaint of both patients was weakness in their lower extremities. Both were healthy children until they were 12 years old when they started to feel cramps and myalgia, particularly in their calves and thighs immediately after running. At present, both patients are still managing to walk without any assistance and can get up from the floor. The first patient complained of fatigue and shortness of breath when he walked since the last 5 months before his presentation to our clinic. The condition has made him unable to climb up stairs and he had to sleep with more than one pillow to elevate his head. His younger brother is still able to climb stairs. There were no family members with similar complaints with the brothers (Figure 1). However, their mother usually complained of myalgia in her legs after vigorous activities such as standing or walking for a long time. The first patient spent most of his time at home but he was still able to perform his daily activities and housework without significant difficulty. The second patient is still continuing his college education.

The second patient's general physical examination was normal. Low blood pressure and impression of cardiomegaly were noticed in the first patient. The patient was consulted to a cardiologist and chest x-rays, electrocardiography and echocardiography confirmed dilated cardiomyopathy (Figure 2). The echocardiography showed left ventricle hypertrophy with decreased

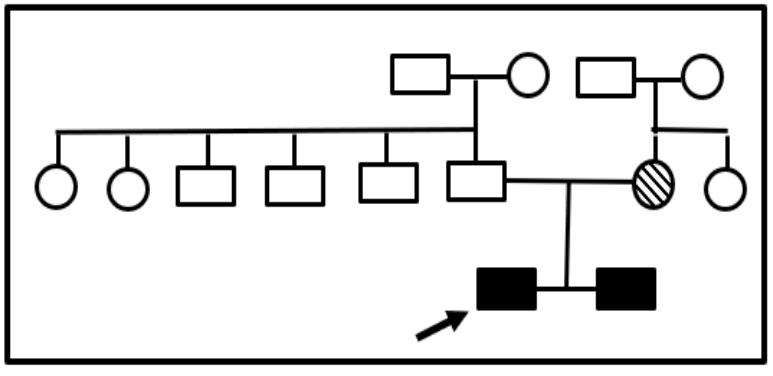

Figure 1. The patients' pedigree shows that there is no history of muscle weakness disease in patient's family. The male siblings (black square) were symptomatic, while their mother was only minimally affected with myalgia on exertion.

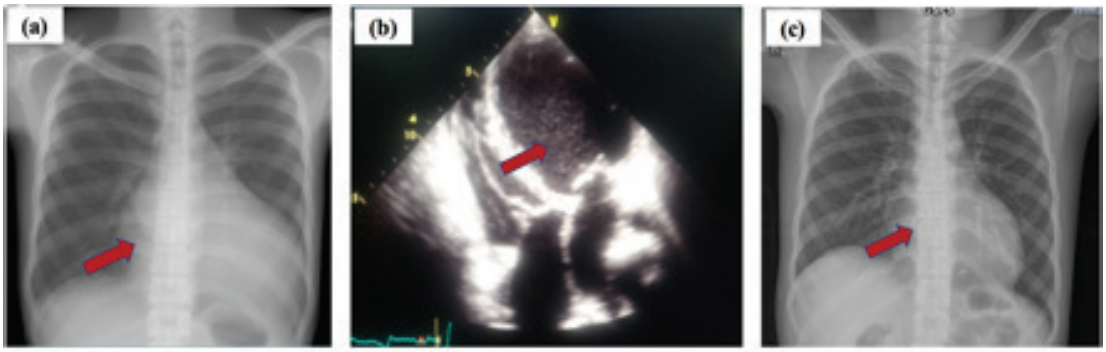

Figure 2. (a). Dilated cardiomyopathy and intramural thrombus (indicated by red arrow) in first case confirmed by chest X-ray. (b). Echocardiography showing a spontaneous echo contrast (indicated by red arrow). Meanwhile in second case, (c) mild cardiomegaly was confirmed by chest X-ray (indicated by red arrow).

systolic and diastolic function. Moderate grade of mitral and tricuspid regurgitation were identified and intramural thrombus was suspected due to the presence of spontaneous echo contrast. There was no sign of heart failure in the second patient but a mild cardiomegaly was noted from the chest x-rays.

Neurological examination showed winged scapula, weakness of pelvic girdles, marked hypotrophy of quadriceps muscles, and slight pseudohypertrophy of the calves in both patients. Gowers' sign and waddling gait were observed in both cases. Swayback postures and scoliosis were spotted in the second patient.

Creatinine kinase enzyme levels were high, with 5,150 U/L in the first case and 4,600 U/L in the other. The mother's serum CK level was moderately elevated (437 U/L) with mild clinical complaints of pain in her calves only after strenuous activity. Electrodiagnostic examination with electroneuromyography (ENMG) captured spontaneous activity of PSW +4, fibrillation +4, MUAP with early recruitment, polyphase, low amplitude, short duration, and complete IP in both cases which confirmed myopathy. The combination of the aforementioned examinations supported the diagnosis of muscular dystrophy.[3]

Based on the ENMG examination, muscle biopsy was performed in the gastrocnemius muscle. Hematoxylin and eosin staining showed variation in muscle fibers size, ranging from small with atrophic fibers to large fibers with increased number of nuclei. Inflammatory infiltrates were minimally noted in the muscle biopsy samples (Figure 3). The immunohistochemistry staining showed partial staining of dystrophin in the muscle membrane, confirming diagnosis of BMD in both cases (Figure 4). Multiple Ligation-dependent Probe Amplification (MLPA) examination was performed to analyze the genetic features of the patients. MLPA analysis was conducted to screen all exons of the *DMD* gene using SALSA MLPA P034 and P035 probe sets (MRC Holland, Netherlands).[16] Out-of-frame deletions of exons 38-43 of the *DMD* gene in both patients were identified. As predicted, the deletions of exon 38-43 were also found in one of the mother`s allele using MLPA method, confirming the mutation inheritance.

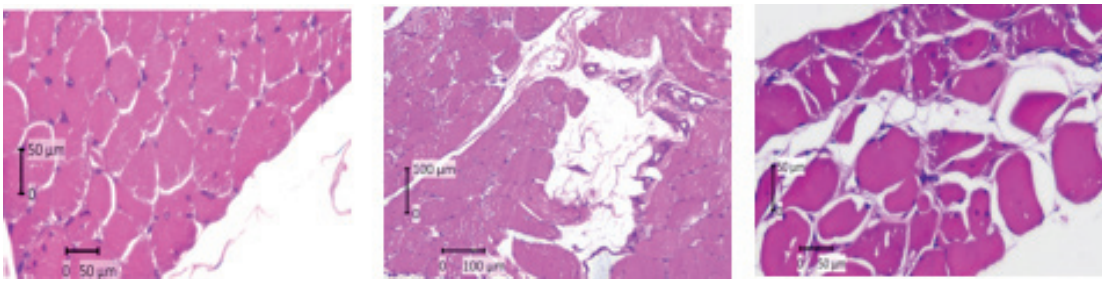

Figure 3. Hematoxylin and eosin (HE) staining of muscle biopsy samples in normal control and affected cases. (a) Normal individual exhibited relatively similar size of muscle fiber without any fat infiltration (200x). In the other hand, both of our cases showed (b) fat infiltration within muscle fibers (100x) and (c) variable sizes of muscle fibres due to muscle degeneration (200x).

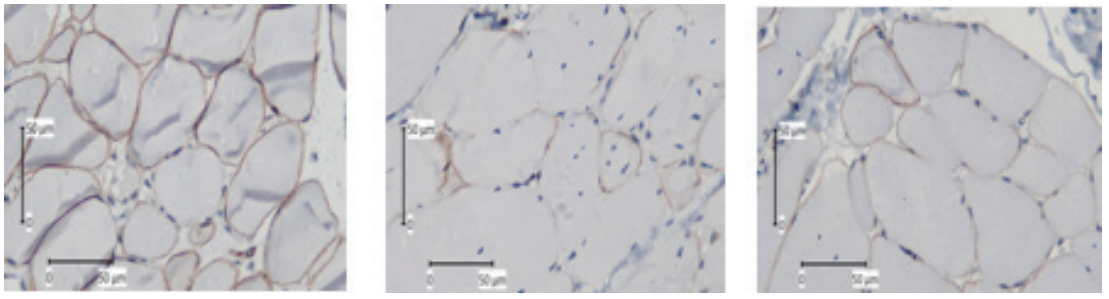

Figure 4. Dystrophin immunostaining of muscle biopsy samples in normal control and affected cases (400x). (a) Normal individual strongly expressed dystrophin in sarcolemal membrane. Meanwhile, case one (b) and case 2 (c), showed dystrophin patchy expression with less intensity in some of their muscle fibers. In both cases, dystrophin is not completely lost.

DISCUSSION

Most patients with BMD have the symptom-onset between 5-15 years old age, while DMD occurs in early childhood, between the age of 2-5 years old, and then progresses rapidly, and independent ambulation is lost in the early second decade of life, mostly before the age of 12 years.[2,3,4] Only a small proportion of case reports of BMD onset occur in the early phase of life and in the third to fourth decades of life.[2,3,17] The disease progression is also variable among cases with BMD.[2,3,17]

The serum CK level is the first test usually performed in the clinical setting once dystrophinopathy is suspected. It is always elevated 50 to 100 times higher than the normal level in DMD and lower in BMD where it peaks around the age of 10 to 15 years. Our patients' CK levels were increased 23-25 times higher than normal when were they examined at the ages of 19 and 23 years old. Elevated CK level is associated with myofiber necrosis since the muscles are lacking in functional dystrophin to stabilize the membrane each time the muscle contracts.[4]

Muscle biopsy and immunohistochemistry examination with anti-dystrophin antibody remain the gold standard for diagnosing dystrophinopathy. Muscle biopsy in DMD and BMD may show various sizes of muscle fibers, myofiber necrosis, macrophage invasion, and regeneration process of adipose tissue and fibrosis replacement depending on the degree of muscle weakness.[4] In BMD

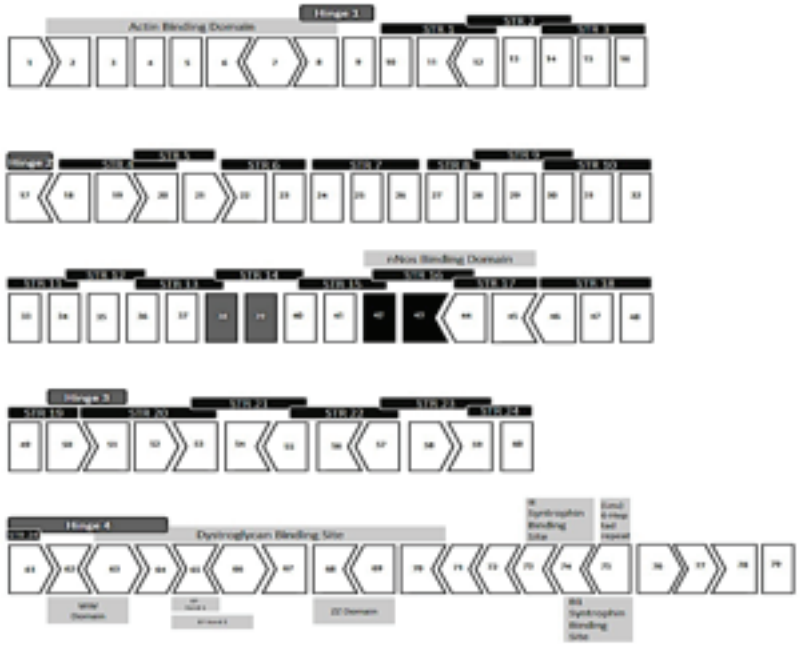

Figure 5. Schematic structure of *DMD* gene's exons, showing its protein binding domains and reading frame.

muscles, partially functioning dystrophin is still produced. Thus, it is still identified partially in the muscle membrane with immunohistochemistry staining. Immunoblot analysis allows more quantitative analysis to differentiate between BMD and DMD if there is any doubt of the qualitative result. In our patients, results showed partial expression of dystrophin in the muscle membrane. The conformity of the results and the clinical presentation lead us to confirm the diagnosis of BMD in both cases.

In addition to muscle biopsy examination, molecular technology has been developed to analyze the mutation profile in dystrophinopathy. However, in Indonesia, genetic examinations are not performed regularly.[18] The first study of the molecular profile of DMD and BMD in Indonesia was performed using polymerase chain reaction (PCR) to identify exon 52 mutation, however, it failed to show any mutations in 18 subjects with dystrophinopathy.[18] Another study was performed using Multiplex PCR and succeeded to reveal *DMD* gene deletion in 15 out of 34 cases examined, with the hot-spot region of exon 43 to 52.[19] The MLPA has been proven to be more sensitive and vigorous than other methods for detection of deletions and duplications in the *DMD* gene.[20] Mutation type and the size of mutation in the gene do not directly relate to the phenotype.[21,22] The reading frame rule explains the phenotype differentiation in the two diseases with the similar mutation of the very same gene in the majority of cases (80-90%).[22,23] The mutation that disrupts the open reading frame results in total loss of dystrophin expression and causes the DMD phenotype. A mutation that maintains the open reading frame allows shortened but functional dystrophin expression.[21-23] Approximately 10% of dystrophinopathy cases show some exceptions to the reading frame rule. Mild phenotype patients may have an out-of-frame deletion, meanwhile severe phenotype patients may show an in-frame deletions in the *DMD* gene.[21-23]

The MLPA spotted out-of-frame deletions of *DMD* gene of exons 38-43 in both of our patients. The similar out-of-frame deletion of exons 38-43 has been reported with earlier onset, more severe phenotype and no dystrophin identified in immunohistochemistry staining.[24] Based on the reading frame rule, the mutation is supposed to relate to the DMD phenotype and the absence of dystrophin in immunohistochemistry staining of biopsy specimens. The discordance of the clinical presentation with the molecular finding may be explained by an exception of the reading frame rule.[14,21,24,25] Endogenous exon skipping, post transcriptional-translational processing, occurrence of tandem duplications and other complex alterations in splicing are several mechanisms that may be responsible for maintaining the reading frame and allowing production of a certain amount of dystrophin.[14,21,23] Unfortunately, splicing pattern analysis using mRNA in this case series could not be further performed due to some limitations in our laboratory facility.

The clinical presentation of our patients with out-of-frame deletion of exons 38-43 can be explained by exon skipping mechanism in the mRNA level. Despite the out-of-frame deletion, the clinical presentations which are typically BMD can be the result of endogenous exon skipping leading to an exception of the reading frame rule that still allows the production of functional dystrophin. Deletions of exon 38-43 have been reported previously, resulting in the DMD phenotype.[26] In the case of exon 38-43 deletion in the *DMD* gene, when exon 44 is skipped, the reading frame can be theoretically restored, resulting in functional dystrophin that leads to the milder phenotype of BMD. Splicing therapy is developed based on the idea of transforming DMD to BMD phenotype by skipping the exons with mutations in the pre-mRNA transcripts.

The first case showed dilated cardiomyopathy with symptoms of heart failure, meanwhile the second case showed no cardiac symptoms even though mild enlargement of the heart was observed. Most of the cardiac involvement in BMD is asymptomatic, and only one third of the cases are reported to have symptoms.[3,5,17,27] Most of the BMD cases with cardiomyopathy will develop the symptoms after 18 years of age. The disease can rapidly progress and lead to early death before they reach 30 years old.[5] Some studies reported that the slower the progression of muscle dysfunction, the more risk of the cardiac involvement. It is suggested that mobilization and physical activities of those patients with skeletal muscle weakness can give mechanical burden to cardiac muscles that are already suffering from functional dystrophin deficit.[28-30]

The definitive correlation between the mutation type and cardiac involvement has yet to be identified. Earlier studies suggested that early cardiac involvement mostly occurs in mutations involving exons 48 and 49.[14,31] In a more recent study of 69 patients with DMD/BMD, 31 subjects with dilated cardiomyopathy were reported to have significant association between mutations

involving exons 31-42 and cardiomyopathy.[5] The study also found that protection against cardiac involvement may be related with mutations involving exons 51 and/or 52.

In conclusion, BMD phenotype in patients with out-of-frame deletion of exons 38-43 in the *DMD* gene may be related to endogenous exon skipping leading to an exception of the reading frame rule that still allows the production of functional dystrophin, contributing to the moderate presentation of muscle weakness and dilated cardiomyopathy in patients.

DISCLOSURE

Conflict of interest: None

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