Case Report

Myofibrillar Myopathy with Limb-Girdle Phenotype in a Thai Patient

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Myofibrillar myopathy (MFM) encompasses a genetically and clinically heterogeneous group of inherited or sporadic skeletal muscle disorders characterized pathologically by the presence of myofibrillar dissolution associated with accumulation of myofibrillar degradation products and ectopic expression of multiple proteins especially Z-disk related proteins. Patients with MFM initially present with muscle weakness and commonly developed cardiomypathy in the advanced stage. To date, mutations of genes encoding Z-disk proteins or proteins maintaining myofibrillar integrity including ZASP, MYOT, DES, FLNC and CRYAB underlie MFM. The authors herein report a 29-year-old Thai woman with a clinical diagnosis of autosomal dominant limb-girdle muscular dystrophy (LGMD1) who has one affected grandmother. The patient was subsequently found to have MFM based on her myopathological findings. Analyses of all MFM-genes known to date revealed no mutations. The current case emphasizes the importance of muscle biopsy in LGMD1 patients and a wide range of phenotypic variations among patients with MFM. The causative genes underlying the majority of MFM remain uncovered. Close monitoring of the cardiac function is crucial to prevent mortality among these patients.

Keywords: *αB-crystallin, Desmin, Filamin C, Limb-girdle muscular dystrophy, LGMD, Myofibrillar myopathy, Myotilin, ZASP*

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The term "myofibrillar myopathy (MFM)" was originally coined by Nakano et al to cover a variety of myopathies sharing a common finding of abnormal desmin accumulation. It has recently been accepted worldwide to cover a group of clinically and genetically heterogeneous muscle disorders. Those disorders share the pathological findings of initial myofibrillar degradation commencing at the Z-disk leading to disintegration of the sarcomeres and subsequent abnormal ectopic accumulation of Z-disk related proteins including desmin and other products of degradation[1, 2].

Patients with MFM develop slowly progressive muscle weakness that can involve both proximal and distal muscles at the age of 7 to 77 years (mean 54 years)[3]. The majority of patients have predominant proximal muscle involvement, although about 25% of affected individuals could mimic a feature of distal myopathies[3]. Cardiomyopathy is present in 17% and is the leading cause of death among these patients[4]. MFM could be either sporadic or autosomal dominant inheritance. The serum creatine kinase (CK) level is

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Table 1. Summary of myofibrillar myopathies (MFM) with identified mutations

Causative gene	Mode of inheritance	Age at onset (years old)	Proportion of MFM caused by this mutation	Extramuscular presentations	CK level	Allelic disorders
ZASP (*LDB3*)	AD	44-73	16%	Polyneuropathy (45%), cardiomyopathy (27%)	Normal-6x	Markesbery distal myopathy , dilated cardiomyopathy, late onset multiminicore disease with conduction block
Myotilin (*MYOT*)	AD	44-77	10%	Polyneuropathy (100%), cardiomyopathy (50%)	Normal-2x	LGMD1A, spheroid body myopathy, dilated cardiomyopathy, late onset autosomal dominant distal myopathy
Desmin (*DES*)	AD, AR	20's-30's	9%	Cardiomyopathy (60%)	Mildly elevated	Dilated cardiomyopathy
Filamin C (*FLNC*)	AD	37-57	3%	Polyneuropathy (40%), respiratory compromise (50%), cardiac conduction defect (10%)	2-8x	-
alphaB-crystallin (*CRYAB*)	AD	Early-middle adulthood	3%	Cataract , dilated cardiomyopathy, respiratory compromise	Normal-7x	AD congenital posterior polar cataract, dilated cardiomyopathy

AD, autosomal dominant; AR, autosomal recessive; CK, creatine kinase; LGMD, limb girdle muscular dystrophy; MFM, myofibrillar myopathy

normal or mildly to moderately elevated up to 7-fold above the normal limit[3]. Electromyography shows myopathic changes in the vast majority of the patients and neuropathic changes in 20% of affected individuals[3].

In Mayo Clinic's cohort of 70 unrelated MFM patients, only 40% of affected individuals had mutations identified in *ZASP* (*LDB3*), myotilin (*TTID* or *MYOT*), desmin (*DES*), αB-crystallin (*CRYAB*) and filamin C (*FLNC*) genes[4], which leave the causative genes of the majority of MFM patients remain uncovered. The Table 1 exhibits the summary of MFM with mutations of aforementioned genes[3-13]. Herein, the authors report the first Thai MFM patient with autosomal dominant, limb-girdle muscle weakness. Molecular analysis of the aforementioned genes reveals no mutations.

Case Report

Clinical summary

A 29-year-old right handed, healthy Thai woman developed difficulty in combing her hair during her first pregnancy at the age of 27 years. Over 2 years, weakness progressed to involve lower extremities leading to difficulty in standing up from a squatting position and difficulty in climbing upstairs. Currently, the patient ambulates with a cane. The patient was the offspring of non-consanguineous marriage and had normal developmental milestones. Family history was relevant for affected maternal grandmother of similar symptoms in her middle age. No other relatives were affected. On the clinic visit, the patient appeared oriented and cooperative. No cataract was seen on ophthalmologic evaluation. Cardiovascular examination was unremarkable. Neurologic examination disclosed normal facial and orbicularis oculi muscle function but weakness of both upper and lower extremities predominantly affecting proximal muscles. Neither atrophy nor hypertrophy was observed. Based on medical research council scale of power (MRC), deltoid, biceps, iliopsoas, gluteus maximus, hip abductors and adductors were grade 3/5, while quadriceps, hamstring, tibialis anterior and gastrocnemius were grade 4/5. The rest of the muscles' strengths were grade 5/5. Deep tendon reflexes were normal. No sensory deficit was noted. Neither joint contracture nor kyphoscoliosis was observed. Laboratory investigations revealed moderately elevated serum CK level (1023 IU/L). EMG test of deltoid, abductor pollicis brevis, quadriceps femoris, and tibialis anterior muscles depicted myopathic changes while nerve conduction study of median, ulnar, and peroneal nerves was unremarkable. EKG showed normal sinus rhythm.

A muscle sample taken from the biceps brachii was processed in liquid nitrogen-cooled isopentane, with a panel of histochemical stains (hematoxyline and eosin, modified Gomori trichrome, periodic acid-Schiff, oil red O and Congo red), enzyme histochemistry (reduced nicotinamide adenine dinucleotide-tetrazolium reductase or NADH-TR, succinic dehydrogenase or SDH, cytochrome oxidase or COX, myosin ATPase, non-specific esterase, alkaline phosphatase and acid phosphatase), and immunohistochemistry (desmin) as previously described by the authors[14]. Muscle biopsy (Fig. 1) showed moderate variation in fiber size with sparse perimysial lymphocytes infiltration but no myonecrosis. Rimmed vacuoles and typical cytoplasmic bodies were conspicuous in some muscle fibers. Numerous myofibers contained well-demarcated sarcoplasmic hyaline plaques of variable shape and size. These hyaline plaques depicted blue to purple in color with modified Gomori trichrome staining and positive Congo red staining but were devoid of any oxidative enzyme activity including NADH-TR, SDH, and COX. Immunohistochemical study with desmin antibody strongly highlighted these hyaline structures. Analysis of all five MFM-genes including *ZASP*, *MYOT*, *DES*, *FLNC*, and *CRYAB* by using genomic DNA extracted from frozen muscle sample revealed no mutations. Neither muscle biopsy nor DNA of the patient's grandmother was available for study.

Discussion

Limb-Girdle Muscular Dystrophy (LGMD) is a collective term used to describe patients with a heterogeneous group of autosomal dominant (LGMD1) or autosomal recessive (LGMD2) muscular dystrophies with the onset involving the pelvic or shoulder girdle muscles or both simultaneously[15]. Muscle biopsy displays dystrophic changes including variation in fiber size, necrosis and regeneration, and interstitial fibrosis. LGMD classification has been revolutionized with the advent of molecular genetics. To date, seven LGMD1 and 12 LGMD2 subtypes are reported[15,16].

The present case developed progressive muscle weakness initially involving shoulder girdle and then pelvic girdle muscles. High serum CK level indicated damage of muscle tissue. Similar symptoms reported in the patient's grandmother hints autosomal dominant inheritance, although her clinicopathological data were not available. Based on this clinical picture, the patient was diagnosed clinically as LGMD1

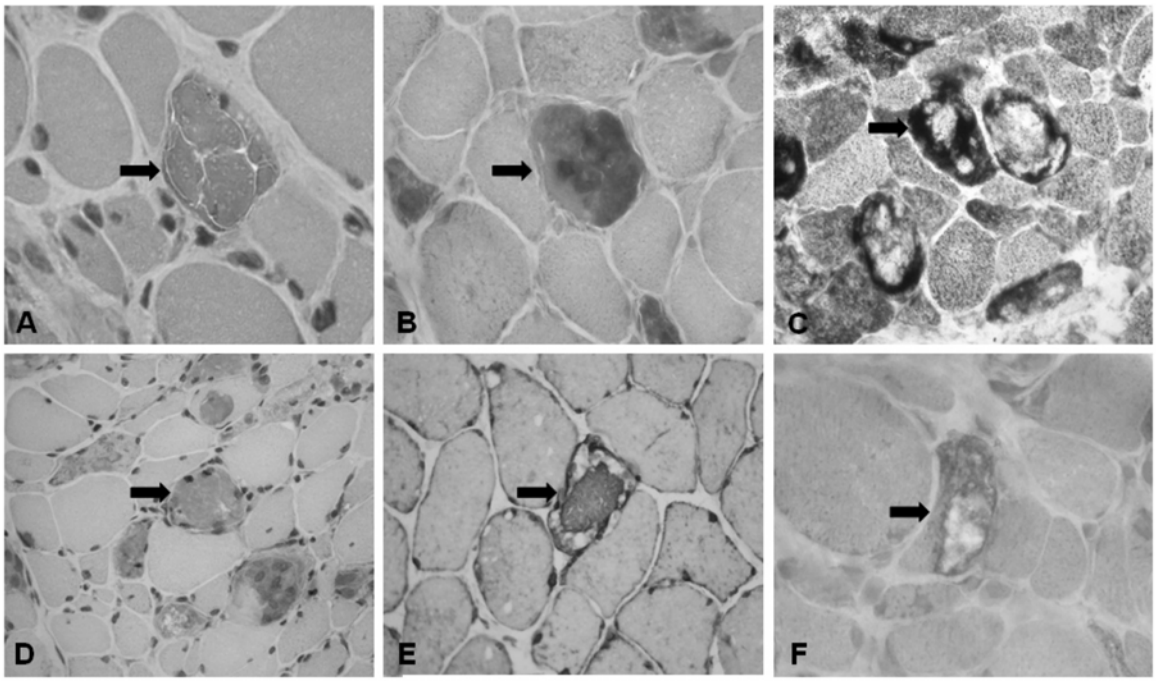

Fig. 1 Biopsy showed moderate variation in fiber size. On hematoxylin and eosin staining, abnormal muscle fibers contained sarcoplasmic hyaline plaques (A, H&E x 400) which appeared blue-green in color on modified Gomori trichrome staining (B, mGT x 400). These hyaline plaques were devoid of oxidative enzyme activities (C, NADH-TR x 400) and revealed positive Congo-red staining (D, Congo-red x 400). Immunohistochemistry study demonstrated strong expression of desmin immunoreactivity in these inclusions (E, Desmin x 400). A few fibers harbored rimmed vacuoles (F, mGT, x 400)

phenotype; however, muscle biopsy subsequently demonstrated a pathognomonic feature of MFM as mentioned earlier. The pathological finding prompts us to re-diagnose the current case as having MFM with limb-girdle phenotype. To the best of the authors' knowledge, the present case is the first Thai patient with MFM. Although currently the patient has no cardiac abnormalities, long-term follow-up of cardiac function is very crucial due to the common association of MFM with cardiomyopathy[2,3].

As mentioned earlier, MFM is a pathologically defined group of hereditary skeletal muscle diseases and its clinical presentation is very variable. Weakness may preferentially affect limb-girdle muscle or distal muscle or may equally involve both muscle groups[3]. Clinical overlap of MFM and LGMD has been clearly demonstrated in patients with *MYOT* and *ZASP* mutations[7,9,17,18]. There is no definite genotype-phenotype correlation in patients with myotilinopathy and ZASPopathy[7,9,16,18].

To date, less than half of MFM patients were found to have mutations in Z-disk related proteins (*ZASP, MYOT, DES, FLNC* and *CRYAB*)[2]. There are no definite clinicopathological findings that could distinguish these mutation-identified MFM patients among each other. However, cataract is rather common in patients with *CRYAB* mutations. Patients harboring *DES* and *CRYAB* mutations may develop weakness since their 20's, while patients carrying *ZASP*, *MYOT* and *FLNC* likely experience weakness first in late adulthood or elderly. Molecular analysis of all five genes in the present case revealed no mutations. D' Amico A et al reported a 5-year-old girl carrying *LMNA* mutations with myopathological features mimicking MFM[19]. Although molecular analyses of *ZASP, MYOT, DES* and *CRYAB* were negative in this particular case, mutations of *FLNC* or other not-yet identified MFM-genes in the presented patient with laminopathy could not be excluded. For this particular reason, molecular analysis of *LMNA* was not conducted in the present case.

In conclusion, MFM is a distinct pathological entity with a wide range of phenotypic variations. The present case is the first Thai MFM patient with

LGMD1 phenotype in whom no mutations were found in all causative MFM-genes known to date. The present case emphasizes the importance of muscle biopsy in patients with LGMD1 and distal myopathy phenotypes to identify myofibrillar disorganization as a diagnostic clue of MFM. Cardiac monitor and prompt intervention is vital in decreasing the mortality rate among these patients. The molecular basis of mutation-negative MFM remains to be further elucidated.

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รายงานผู้ป่วยไทยด้วยโรค myofibrillar myopathy ที่มีอาการกล้ามเนื้อต้นแขนและต้นขาอ่อนแรง

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Myofibrillar myopathy (MFM) เป็นกลุ่มโรคที่ประกอบด้วยโรคที่มีความหลากหลายทางคลินิก และจีนกลายพันธุ์ที่เป็นสาเหตุการเกิดโรค พบทั้งชนิดถ่ายทอดทางพันธุกรรม และชนิดไม่ถ่ายทอดทางพันธุกรรม กลุ่มโรคนี้มีลักษณะพยาธิสภาพที่จำเพาะ พบมีการสะสม myofibrillar degradation product ในเซลล์กล้ามเนื้อลาย และพบการสะสมผิดที่ของโปรตีนหลายชนิดโดยเฉพาะ Z-disk related protein ผู้ป่วยด้วยโรค MFM มีอาการเบื้องต้นของกล้ามเนื้ออ่อนแรง และมีโรคกล้ามเนื้อหัวใจร่วมด้วยในระยะต่อมา ปัจจุบันพบจีนกลายพันธุ์ที่เป็นสาเหตุการเกิดโรคหลายจีน ได้แก่ ZASP, MYOT, DES, FLNC และ CRYAB ผู้นิพนธ์รายงานกรณีศึกษา ผู้ป่วยหญิงไทยอายุ 29 ปี มีลักษณะทางคลินิกแบบ autosomal dominant limb-girdle muscular dystrophy (LGMD1) เนื่องจากย่าของผู้ป่วยมีอาการเช่นเดียวกับผู้ป่วย ภายหลังผู้ป่วยได้รับการวินิจฉัยเป็น MFM โดยลักษณะพยาธิสภาพของกล้ามเนื้อ อย่างไรก็ตามไม่สามารถตรวจพบจีนกลายพันธุ์ชนิดที่เคยมีผู้รายงานไว้ในผู้ป่วยได้ รายงานผู้ป่วยรายนี้แสดงให้เห็นว่าการส่งตรวจ muscle biopsy มีความสำคัญในผู้ป่วยที่มีอาการ ทางคลินิกแบบ LGMD1 และ MFM เป็นโรคที่มีลักษณะหลากหลายทาง phenotype มาก น่าจะมีจีนกลายพันธุ์สาเหตุการเกิดโรคที่ยังไม่สามารถตรวจพบอีกหลายจีน การตรวจการทำงานของหัวใจอย่างใกล้ชิดมีความสำคัญอย่างยิ่ง ในการป้องกันการเสียชีวิตในผู้ป่วย MFM