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## CLINICAL CHARACTERIZATION OF MYOPATHY IN A RARE AUTOSOMAL DISEASE: HEREDITARY BONE DYSPLASIA/OSTEOSARCOMA AND LIMB GIRDLE MYOPATHY IN A UNIQUE FAMILY

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### ABSTRACT

Autosomal-dominant myopathic disorder associated with diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH) is characterized by myopathy, bone fragility, and osteosarcoma. DMS-MFH was recently associated with mutations in the methylthioadenosine phosphorylase gene (*MTAP*). *MTAP* is a ubiquitously expressed enzyme crucial for polyamine biosynthesis. Two disease-causing mutations have been identified in *MTAP*: c.813-2A>G and c.885A>G, both of which result in dysregulated alternative splicing of *MTAP* isoforms. Here, we report on myopathy in two cousins with the c.813-2A>G mutation. Both developed a progressive limb-girdle type myopathy at age 30 years. To our knowledge, we are the first group to characterize the myopathy associated with DMS-MFH, discovering varied muscle fiber size, degeneration, and increased centralized nuclei. In this report, expression levels of transactive response DNA-binding protein (TDP)-43, light chain (LC)3-I/II, and p62/sequestome 1 (*SQSTM1*) in the muscle fibers were increased, suggesting a possible dysregulation of autophagy. Elucidation of the pathologic mechanism(s) in DMS-MFH offers the potential to uncover key molecular signaling pathways and the promise of novel future treatments.

### INTRODUCTION

Diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH) (MIM 112250) is an autosomal-dominant syndrome characterized by myopathy, bone fragility, and osteosarcoma.<sup>1-5</sup> Patients with this disorder experience limb-girdle myopathy, fractures, defective healing of long bones, cortical growth abnormalities, presenile cataracts, potential coronary artery disease, and osteosarcoma.<sup>1-8</sup> The bone pathology begins in childhood and affects ~90% of DMS-MFH individuals.<sup>7,8</sup> It has a unique bone-dysplasia phenotype and is characterized by cortical growth abnormalities, including diffuse diaphyseal medullary stenosis with overlying endosteal cortical thickening and metaphyseal striations, and scattered bone infarctions.<sup>8</sup> Osteosarcomas/malignant fibrous histiocytomas develop in ~35% of individuals with DMS.<sup>1,3-5</sup> Progressive muscle weakness affects ~70% of members of families with DMS-MFH, with onset in the 20s or 30s.<sup>7,8</sup> The myopathic phenotype affects a significant portion of the DMS-MFH population and is characterized in this report.

The cause of this disorder was mapped, by Martignetti et al<sup>1</sup> in 1999, to chromosomal region 9p21-22, establishing

a disease gene 2.9-Mb critical region between markers D9S736 and D9S171.<sup>3</sup> A number of DMS-MFH candidate genes were originally screened, including the methylthioadenosine phosphorylase gene (*MTAP*; MIM 156540). Initially, *MTAP* had been thought to consist of eight exons and seven introns<sup>9</sup>; however, Camacho-Vanegas et al. identified mutations in the previously unknown terminal exons of the *MTAP* gene. They found that all affected members of five unrelated DMS-MFH families possessed one of two synonymous mutations, one located within exon 9, c.885A>G, and the other upstream of exon 9 in the intron splicing boundary, c.813-2A>G. These mutations result in exon skipping and subsequent loss of exon 9 in alternatively spliced, biologically active isoforms.<sup>10</sup> *MTAP* is a ubiquitously expressed enzyme that plays a crucial role in the salvage pathway for adenine and methionine in all tissues.<sup>11,12</sup> This dysregulated expression of the *MTAP* splice variants has an effect on overall *MTAP* enzymatic activity because increased levels of MTA have been found in the serum of affected DMS-MFH patients<sup>10</sup>; however, how this dysregulation affects the pathophysiology remains unclear. In this report, we examine myopathy, *MTAP*

splice variant expression, and pathologic mechanism(s) in two cousins with a c.813-2A>G mutation affected by limb-girdle myopathy caused by DMS-MFH.

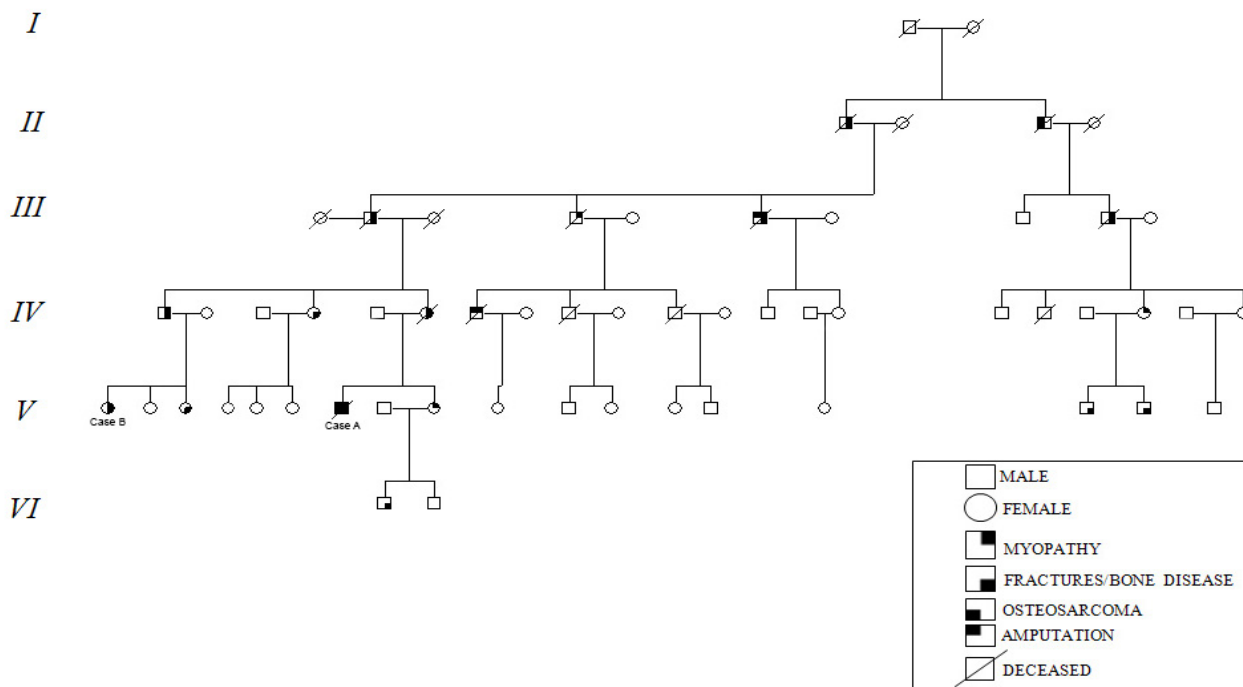
### CASE REPORT

#### Clinical History: Case A, V:7

We report a 45-year-old white man with a deteriorating course of muscle strength, bone fragility, and osteosarcoma, leading to early death (Figure 1, individual V:7). The patient had had 22 fractures throughout his life, including in the tibia, fibula, wrists, hands, shoulder, and femur. He experienced chronic pain disorder largely due to these fractures. At the age of 31 years, he was found to have myopathy, with weakness and atrophy in his biceps, brachioradialis, wrist extensors, and forearm muscle. His motor strength, as graded using the Medical Research Council (MRC) scale, was 4 or 4+/5 in most muscle groups: neck flexors, deltoids, triceps, wrist extensors, hip flexors, knee extensors, and ankle dorsiflexors. His reflexes were graded as 1 to 2+ in the knees and upper extremities, and he had normal

coordination. His creatine phosphokinase concentration was 498 U/L (normal, 20–220 U/L), and nerve conduction studies were normal.

Also at 31 years, needle electrode examination in several muscle groups revealed fibrillation potentials and short-duration small-amplitude polyphasic motor unit potentials in the biceps and brachioradialis on the left side. Subtle changes were noted in the triceps and deltoid, consistent with a myopathic process. These findings are not suggestive of amyotrophic lateral sclerosis or any neurogenic process, and nerve conduction studies were normal in both studies. A muscle biopsy specimen showed no abnormal muscle fiber type grouping, atrophy, regenerating, or "ragged" red fibers. No increase in fat or connective tissue and no structural abnormality of the fibers were seen. Staining for glycogen, periodic acid Schiff (PAS), desmin, nicotinamide adenine dinucleotide + hydrogen (NADH), adenosine triphosphatase (ATPase), and Gomori trichrome were all normal. However, myopathy was confirmed on electromyography and clinical examination



**Figure 1** Pedigree of a diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH)-affected family. A, DMS-MFH-affected family with hereditary bone dysplasia/osteosarcoma and limb girdle muscular dystrophy. All affected family members have mutation c.813-2A>G located in the intron region on a splicing boundary region, resulting in the dysregulation of methylthioadenosine phosphorylase (MTAP) splice variants. The mutation analysis was performed, with informed consent from all patients and unaffected family members, at the laboratory at Icahn School of Medicine at Mount Sinai, New York, New York.

despite the muscle biopsy specimen showing no pathology. It is likely that the biopsy specimen was taken too early in the onset of myopathy. The myopathy was concluded to have been most likely facioscapulohumeral dystrophy, or limb-girdle muscular dystrophy.

At the age of 44 years, the patient's MRC scale grades of strength of his neck flexors were 4/5; deltoids were graded at 4–/5; he had very little strength in his biceps, which were graded at 2/5; and his triceps were graded at 4+/5. His cardiovascular examinations by echocardiography and electrocardiography were normal. Also at age 44 years, the patient developed acute pain in his right tibia, which was incorrectly attributed to his fractures; magnetic resonance imaging revealed a large lesion present in his right proximal tibia that measured 11 cm by up to 6 cm (**Figure 2A**). A biopsy revealed a high-grade Stage III osteosarcoma. The patient underwent three cycles of chemotherapy with cisplatin and doxorubicin. The osteosarcomal mass in the proximal right tibia, however, progressed despite chemotherapy. Subsequently, follow-up imaging showed a new lesion on the thoracic spine at T8. The patient's right leg was amputated just above the knee, and a biopsy specimen of the T8 lesion revealed that it was a metastatic osteosarcoma. The patient died a year later, at the age of 45 years, from complications due to the metastasis of this osteosarcoma.

#### *Clinical History: Case B, V:1*

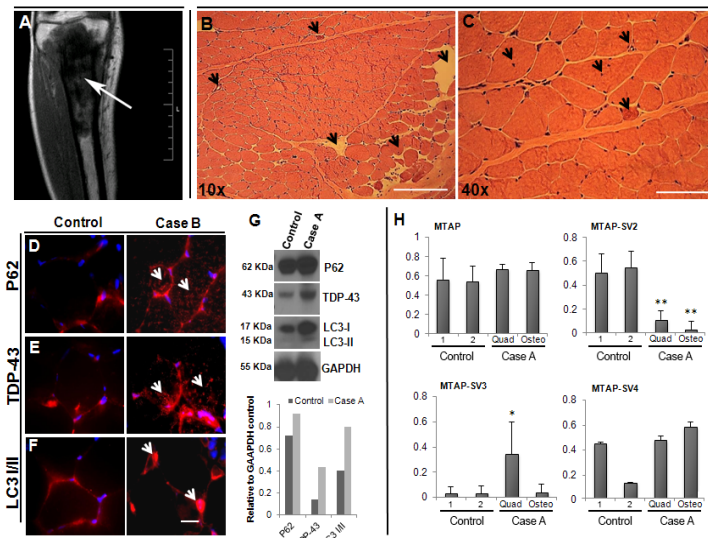
We report a 53-year-old white female patient (**Figure 1**, individual V:1), the cousin of case A with the familial disease. As a child, she had had bruising but seemed to have no problems with the healing of superficial cuts. At age 18 years, investigation of her bruising led to a diagnosis of type 1 von Willebrand disease, based on a low von Willebrand antigen level of 0.26 U (normal, >0.50 U). By age 30 years, the patient had developed graying hair and was experiencing proximal muscle weakness, back pain, and numbness in her hands. At age 35 years, she found climbing stairs to be difficult from the muscle weakness. A physical examination demonstrated bilateral muscle weakness in her legs; however, she demonstrated no weakness in her shoulder girdle or arms. She had asymmetric weakness in the hip flexors, with an MRC grade of 2 on the right and 4 on the left, and most of the other muscle groups demonstrated an MRC grade of 4– or 4. Her reflexes were all normal. The patient's creatine phosphokinase concentration was 75 U/L (normal, 20–220 U/L).

At this time, the patient was also diagnosed with type 2 diabetes mellitus. Due to the continued muscle weakness, at age 36 years, the patient had a muscle biopsy specimen obtained from her right quadriceps, and histology revealed normal variability in muscle fiber size and shape, with no muscle fiber type grouping, atrophy, or ragged red fibers seen, and normal staining for glycogen, ATPase, and Gomori trichrome. There was prominent lipid staining in many muscle fibers, which was dismissed as possibly related to the diabetes. Immunohistochemical studies for dystrophin showed normal sarcolemmal localization of the three dystrophin domains.

At age 40 years, the patient continued to experience progressive muscle weakness and had stopped all exercise activities. At the age of 44 years, a second muscle biopsy specimen obtained from the right quadriceps revealed extensive areas of fat replacement of muscle and groups of atrophic fibers. Staining for glycogen, PAS, desmin, NADH, ATPase, and Gomori trichrome were all normal. At age 47 years, the patient had developed chronic osteomyelitis of the right femur due to methicillin-sensitive *Staphylococcus aureus* infection. Her progressive muscle weakness was exacerbated by her inactivity related to the fracture. At this point, she was at 40% weight-bearing with a walker and crutches. At age 51 years, she experienced another subtrochanteric fracture of her right femur, and during surgery a muscle biopsy specimen from her right quadriceps was obtained (**Figure 2**).

#### *Characterization of Muscle Pathology*

In case B, muscle pathology was characterized by histopathology, and in case A, by biochemical analysis (western blot). The muscle biopsy was obtained from the right quadriceps in case B at age 51 years, at the time of the right subtrochanteric fracture. H&E staining of the muscle demonstrated fiber size variability, increased centrally located nuclei, degeneration and regeneration of muscle fibers, large-scale atrophy, and extensive areas of fat replacement. No areas of inflammation were observed (**Figure 2, B and C**). Trichrome staining analysis did not reveal any evidence of ragged red fibers (data not shown). A muscle biopsy specimen revealed normal glycogen staining, normal checkerboard-type distribution with ATPase staining, and normal sarcolemmal immunolocalization of the three dystrophin domains (data not shown). To further explore disease mechanisms, we examined the ubiquitin proteasome and autophagy pathways, as these pathways have been shown to be



**Figure 2.** Analysis of quadriceps and osteosarcoma tissues in two diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH)-affected cousins. A, Magnetic resonance image of a large (11 × 6-cm) osteosarcoma (arrow) in the right proximal tibia (case A). B and C, Quadriceps muscles from case B (V:1), stained with H&E. Scale bars = 250 μm (B) and 100 μm (C). Control and case B (V:1) were stained with autophagy markers anti-p62/sequestome 1 (*SQSTM1*) (D), transactive response DNA-binding protein (TDP)-43 (E), and light chain (LC)3-I/II-specific antibodies (F). Scale bar = 10 μm. G, Upper panel, Densitometry confirming Western blot levels in case A (V:7); lower panel, western blot analysis of p62/*SQSTM1*, TDP-43, and LC3-I/II in case A (V:7) were increased compared with those in the healthy control. H, *MTAP* splice variant expression of *MTAP*, *MTAP\_v2*, *MTAP\_v3*, and *MTAP\_v4* in quadriceps and osteosarcoma tissues from case A (V:7). Data are mean (SE). \**P* < 0.05; \*\**P* < 0.005.

dysregulated in valosin-containing protein-associated diseases (eg, inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia [IBMPFD]) with several similarities to DMS-MFH.<sup>10,11</sup> Immunohistochemistry of the quadriceps muscles in case B revealed increased autophagy marker expression levels of p62/*SQSTM1*, TDP-43, and LC3-I/II compared with those in healthy control muscle (Figure 2, D–F). This was also shown in case A by western blot analysis and was confirmed by densitometry (Figure 2G).

*Disruption of MTAP Splice Variants in Two Cousins with DMS-MFH*

To analyze the expression patterns of the *MTAP* splice variants in these two DMS-MFH-affected cousins, we quantified the mRNA levels in muscle and tumor tissue samples. ANOVA revealed the muscle tissue sample from case A with unaffected levels of wild-type *MTAP*, whereas *MTAP\_v2* was significantly downregulated in both muscle (*P* = 0.002) and osteosarcoma (*P* = 0.001), and *MTAP\_v3* was significantly upregulated in the muscle sample (*P* = 0.012); however, *MTAP\_v3* was not upregulated in the osteosarcoma (Figure 2H). The difference in *MTAP\_v3* levels in case A quadriceps and osteosarcoma could possibly be explained by a loss of heterozygosity, with the *MTAP* gene being deleted in one allele in the osteosarcoma—a common occurrence with tumor suppressor genes in several types of malignant tumors.

**DISCUSSION**

DMS-MFH is an autosomal-dominant syndrome characterized by myopathy, bone fragility, and osteosarcoma, caused by mutations in the *MTAP* gene. Affected family members were first clinically described by Henry et al<sup>2</sup> in 1958 as having histologic evidence of an irregular osteopetrotic process with coarse trabeculation with osteosarcoma, a rare complication of Paget disease of bone. The human *MTAP* locus on chromosome 9p21 is one of the most frequently somatic, hypermethylated, translocated, and/or deleted regions in human cancer. Several types of human tumors are deficient somatically in *MTAP*, including non-small-cell lung cancers<sup>13</sup>; hepatocellular carcinomas<sup>14</sup>; and, highly relevant to this disease, osteosarcomas.<sup>15,16</sup>

In this case study, we investigated the expression of the *MTAP* splice variants within this family with the c.813-2A>G mutation and found upregulation of *MTAP\_v3*. We did not find a difference in expression with *MTAP\_v6*, as was previously reported in a family with the c.885A>G mutation.<sup>10</sup> We characterized the myopathy of DMS-MFH, finding variability in fiber size, increased centrally located nuclei, degeneration of muscle fibers, and fatty replacement of muscle.

Due to the crucial role of *MTAP* in cancer suppression, the relationship between cancer growth and autophagy inhibition,<sup>17</sup> and the pathologic similarities between DMS-MFH and IBMPFD,<sup>10</sup> we decided to investigate the autophagy pathway in DMS-MFH. We discovered increased expression of several autophagy markers in the two affected individuals in this case study, making it plausible that the autophagy cascade is crucial in the



pathophysiology of tissue damage seen in this disease. Several papers have been published demonstrating that there were changes in levels of polyamines and amino acids, which may influence autophagy in normal cells, and that a disruption in the autophagy cascade may cause damage to these cells.<sup>18–20</sup> Autophagy has been postulated as a [pathogenic](#) event in several muscle disorders.<sup>21–25</sup> Potential therapies for [MTAP patients](#) may also include carbamazepine, tamoxifen, and rapamycin compounds, which have been demonstrated to influence autophagy in experimental systems.<sup>26–28</sup> Future translational metabolomics studies using in vitro disease modeling of DMS-MFH disease and investigation of the genomic regulation of the splice variants of *MTAP* will determine their association with myopathy, bone dysplasia, and osteosarcoma.

## CONCLUSIONS

Autosomal-dominant myopathic disorder associated with DMS-MFH is characterized by myopathy, bone fragility, and osteosarcoma associated with mutations in the *MTAP* gene. Here, we report on the myopathy in two cousins with DMS-MFH. They developed a progressive limb-girdle type myopathy at age 30 years. We characterized the myopathy associated with DMS-MFH, discovering varied muscle fiber size, degeneration, and increased centralized nuclei. The expression levels of LC3 -I/II and p62/*SQSTM1* in the muscle fibers were increased, suggesting a possible dysregulation of autophagy. Elucidation of the pathologic mechanism(s) of DMS-MFH offers the potential to uncover key molecular signaling pathways and the promise of novel future treatments.

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