Published in final edited form as:

Neuromuscul Disord. 2013 December ; 23(12): 955–961. doi:10.1016/j.nmd.2013.08.003.

‘Double Trouble’: Diagnostic Challenges in Duchenne Muscular Dystrophy in Patients with an Additional Hereditary Skeletal Dysplasia

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Abstract

Duchenne muscular dystrophy (DMD) is caused by mutations *Dystrophin* and affects 1 in 3600-6000 males. It is characterized by progressive weakness, leading to loss of ambulation, respiratory insufficiency, cardiomyopathy, and scoliosis. We describe the unusual phenotype of 3 patients with skeletal dysplasias in whom an additional diagnosis of DMD was later established. Two unrelated boys presented with osteogenesis imperfecta due to point mutations in *COL1A1* and were both subsequently found to have a 1bp frameshift deletion in the *Dystrophin* gene at age 3 and age 15, respectively. The third patient had a diagnosis of pseudoachondroplasia caused by a mutation in the *COMP* gene and was found to have a deletion of exons 48-50 in *Dystrophin* at age 9. We discuss the atypical presentation caused by the concomitant presence of 2 conditions affecting the musculoskeletal system, emphasizing aspects that may confound the presentation of a well-characterized disease like DMD. Additional series of patients with DMD and a secondary inherited condition are necessary to establish the natural history in this “double trouble” population. The recognition and accurate diagnosis of patients with two independent genetic disease processes is essential for management, prognosis, genetic risk assessment, and discussion regarding potential therapeutic interventions.

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Keywords

Double trouble; Duchenne muscular dystrophy; Genetic counseling; Osteogenesis imperfecta

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) (OMIM#310200) is an X-linked disorder, caused by mutations in the *Dystrophin* gene, and affects 1 in 3600-6000 live male births [1] [2]. DMD was first described in the early 19th century, and the causative gene was only identified in 1987 [3] [4]. It is characterized by progressive muscle weakness leading to loss of ambulation, respiratory insufficiency, cardiomyopathy, and scoliosis. Boys typically present at age 3 to 5 years with evidence of proximal muscle weakness and calf hypertrophy. Diagnosis is made on the clinical presentation, elevated serum creatine kinase (CK) levels and confirmatory genetic testing of the *Dystrophin* gene. In a small number of patients with intronic splice site mutations interfering with normal splicing, confirmatory muscle biopsy staining or cDNA studies may be necessary. Immunohistochemistry on muscle biopsy tissue shows an absence of *dystrophin*, a large membrane-associated protein essential for muscle cell stabilization [5]. Frameshifting or terminating deletions and mutations are typically associated with the severe Duchenne phenotype, while frame-preserving deletions are typically associated with the milder Becker muscular dystrophy phenotype.

The clinical presentation of a well characterized disorder such as DMD may be masked and initially overlooked by the presence of another disorder of the musculoskeletal system. We present three patients with the rare combination of a hereditary skeletal dysplasia whose DMD diagnosis was delayed, illustrating the diagnostic challenges of their confounding phenotype.

PATIENTS

Patient 1

A now 16-year-old boy presented at 4 months of age with a hairline fracture of the femur. After a second femoral fracture at 8 months of age, concerns arose about the possibility of osteogenesis imperfecta (OI). Further examination identified pale blue sclerae. The family history was negative for OI, neuromuscular, or neurological disease. Targeted genetic testing confirmed a mutation in *COL1A1* (3532-2 AG substitution), associated with OI type IV at age 1 year. He started receiving Pamidronate at age 8 years. After a period of prolonged immobility while recovering from surgery for a right tibia and left femur fracture, he became non-ambulatory at age 8 years. He lost the ability to stand independently at age 10 years. At age 12 years he sustained another fracture and he started complaining of a worsening of weakness in both upper and lower extremities, proximally more than distal and lower extremity cramping. His examination was significant for short stature, bilateral blue sclerae, mild macroglossia, and a transverse smile. He also had contractures of jaw, shoulders, elbows, wrists, knees, and ankle dorsiflexors and bilateral distal finger laxity (Fig. 1A&D). Dextroconvex thoracic scoliosis and levoconvex lumbar scoliosis were present. At age 15 years he had generalized weakness, proximal more than distal, and diffuse global areflexia.

Exam was limited due to his skeletal dysplasia and his history of fractures. His serum CK level was elevated at 1374 U/L (0-249 U/L). He had diffuse osteopenia and scoliosis on spine radiographs (Fig. 1F&G). Electrocardiogram showed right ventricular conduction delay; echocardiogram results were normal. Force vital capacity (FVC) was 50% of predicted value. Muscle ultrasound demonstrated moderately to severely increased echogenicity in all muscle, consistent with a dystrophic or myopathic process. Results of muscle biopsy at age 12 was consistent with muscular dystrophy showing extensive replacement of muscle with fatty tissue and the presence of only few muscle fibers. Tissue was stained for alpha-dystroglycan, beta-dystroglycan, merosin, collagen VI, perlecan and embryonic myosin heavy chain (Fig. 2A&D). Dystrophin staining was requested at age 15 when the suspicion of DMD was raised. Immunohistochemical staining revealed absent staining for the dystrophin in the majority of muscle fibers compared to a control sample (Fig. 2 B-F). *Dystrophin* gene sequencing revealed a *de novo* out-of-frame 1 base pair deletion at c.1574 in exon 13.

Patient 2

The second patient is a now 12-year-old boy who was diagnosed with pseudoachondroplasia at age 1 year. His maternal family history was positive for pseudoachondroplasia, including the patient's mother, sister, grandmother and several first cousins. The family history was otherwise negative for neuromuscular or neurological disease. In 2012, molecular genetic testing identified a heterozygous c.1540T>G mutation in exon 13 of the cartilage oligomeric matrix protein (*COMP*) gene resulting in a change of Cysteine to Glycine at position 484 (p.Cys484Gly). He was enrolled in Early Intervention services at age 2 years for global developmental delays. He sat independently at age 1 year and walked at 18 months. He also had speech and learning difficulties. Following myringotomy at age 2 years, his verbal skills improved. He had difficulties rising from the floor or from a seated position, and required support climbing stairs. He also had difficulties with balance, excessive falling and was noted to lag behind his peers. At this time, his weakness was attributed to his underlying skeletal dysplasia. His medical history is significant for vitamin D deficiency, asthma, and at least 14 documented ear infections. The patient was referred for neuromuscular evaluation at age 8 years due to progressive weakness. He had a slight positional tremor in his fingers, hypotonia and weakness: grip was 4/5, deltoids 3+ to 4-/5, quadriceps 3/5, peroneals 3/5 bilaterally, and anterior tibialis 3/5 (MRC grading scale). He had a positive Gowers' sign and calf hypertrophy (Fig. 1E). Deep tendon reflexes were diminished with flexor plantar responses. Vibration and proprioception were intact. His feet exhibited *planus valgus* bilaterally and he showed an exaggerated lumbar lordosis (Fig. 1B-C). His CK level was markedly elevated at approximately 16,000 U/L. Muscle biopsy was not performed. Molecular genetic testing identified an out-of-frame deletion of exons 48-50 in the *Dystrophin* gene consistent with a diagnosis of DMD.

Patient 3

A now 3-year-old boy presented at age 15 months for concerns for external rotation of his left lower extremity and abnormal gait. Due to presence of blue sclerae noted at a few months of age and a paternal family history of autosomal dominant osteogenesis imperfecta (OI), the patient was referred to genetic evaluation. Examination showed macrocephaly,

normal dentition, no known hearing loss, and no fractures or dislocations at that time. The paternal family history was positive for a genetically confirmed diagnosis of OI in his father and a clinical diagnosis of OI in his paternal aunts and uncles (Fig. 1H). Targeted genetic testing confirmed a c.2390delG mutation in the *COL1A1* gene, consistent with a diagnosis of OI type 1.

At just under age 2 years he sustained a fracture of his femur. At age 2.5 years, concerns were raised by his physical therapist for slowly progressive lower extremity weakness so he was referred for neurologic evaluation. At that time he had inability to stoop-and-recover, was not pushing up to a 4-point position when prone with inability to transition in or out of sitting and inability to kneel. His neurological examination was significant for macrocephaly, blue sclerae, generalized hypotonia, lower extremity proximal weakness, mild prominence of the calves, and normal reflexes. The strength portion of neuromuscular exam was limited due to his diagnosis of skeletal dysplasia. His CK level was >30,000 U/L (30-220 U/L). Cardiac evaluation including echocardiogram was normal. Muscle biopsy was not performed.

Other significant family history included a maternal aunt and uncle with a clinical diagnosis of limb girdle muscular dystrophy (LGMD) (Fig. 1). Maternal aunt became symptomatic at age 6 years and was wheelchair bound by age 12 years. She had a muscle biopsy at age 12 years (results unknown) and again at age 17 years. Tissue of the second biopsy was initially read as “neuropathic” and subsequently changed to “dystrophic” following comparison with the maternal uncle's biopsy results. At age 30 years she has severe generalized weakness, contractures, dysarthria, areflexia, tracheostomy and was ventilator dependent. Chromosome analysis was reported as normal. Maternal uncle had difficulty walking and ascending steps at age 6 years. CK level was approximately 10,000 U/L. He was reported to have very prominent calves. He was wheelchair-bound by age 9 years. An electromyography (EMG) was “myopathic” and muscle biopsy consistent with a muscular dystrophy. Genetic testing via deletion/duplication testing of the *Dystrophin* gene in 1997 was normal on both PCR and southern blot. Genetic testing for mutations in *FKRP*, *SGCA* and *SGCB* were negative. He is alive at age 33 years but is severely affected, non-ambulant with contractures, and has a tracheostomy. Consanguinity was denied.

Results of extensive genetic testing of our index patient 3 including *CAPN3*, *DYSF*, *SGCA*, *B*, *G* and *D*, *LMNA*, *LAMA2*, *POMT1*, *POMT2*, *POMGNT1*, *FKRP*, *FKTN*, which were normal. *CAV3* gene sequencing identified a c.233 C>T variant that was maternally inherited. *COL6A2* gene sequencing showed two intronic variants of unknown clinical significance. *Dystrophin* gene sequencing showed a novel out-of-frame 1 base pair deletion at c.2388 predicted to be disease causing and consistent with DMD. When tested, the patient's mother was found to be a carrier. She is clinically unaffected with a normal CK level. Both the maternal aunt and uncle were subsequently tested and found to carry the DMD mutation (Fig. 1E).

DISCUSSION

The frequency of DMD is estimated to be 1 in 3600-6000 boys; the prevalence of OI is 1 in 15,000–20,000, while firm data on the prevalence of pseudoachondroplasia have not been established [6] [7]. The possibility of inheriting two relatively common genetic conditions, albeit rare, should be expected based on chance and considered when examining a patient with findings inconsistent with the primary diagnosis. Although the genetic etiology is different, a “DMD plus” phenotype has been characterized as the Xp21 contiguous gene syndrome, in which patients may be diagnosed with adrenal insufficiency, glycerol kinase deficiency in addition to DMD, due to a large deletion encompassing contiguous genes located on Xp21[8, 9]. Additionally, digenic inheritance has previously been recognized in neuromuscular disease as a contributor to unusually severe presentation such as in patients with Emery Dreifuss with mutations in both emerin and desmin proteins [10]. With genetic testing becoming the gold standard of diagnosis, recognition of clinical phenotypes and possible atypical presentations remain essential to appropriate patient evaluation, diagnosis, prognosis, genetic counseling and long-term care.

The family history in DMD is oftentimes negative as mutations may be inherited through an unaffected carrier mother or as a *de novo* mutation, which is seen in approximately 1/3 of patients. Approximately 65% of DMD patients have a large deletion, [11, 12], 5% of patients have a duplication, [13], while approximately 1/3 of patients have a single base pair deletion detectable through sequencing as identified in two of our patients [14-16]. Mutations in *COL1A1* may be inherited through an autosomal dominant pattern or, more likely in severe cases, caused by *de novo* mutations [17]. Patient 1 was found to have two *de novo* single base pair deletions, in the *Dystrophin* and *COL1A1* gene. Advanced paternal age is associated with an increased risk for *de novo* mutations [18] however; this is not applicable to DMD, an X-linked recessive disorder. Additionally, the patient's father was 36 years old at conception, which does not meet the criteria of advanced paternal age. The possibility of maternal germline mosaicism was not ruled out in this family and this should be addressed as part of genetic counseling [19, 20].

Skeletal anomalies such as OI and pseudoachondroplasia may present with limitations in mobility and developmental motor delays, masking the presentation of weakness in DMD and thereby delaying a diagnosis. Muscle weakness may also occur in OI and may in fact be the presenting symptom [21] [22]. Patients, especially males, with delayed motor development and/or weakness will benefit from a CK level as a low-cost and easy screening tool by pediatricians prior to referral to pediatric neurology. Case series like these illustrate the importance of evaluating CK levels in young boys presenting with motor delay as a screening test, until newborn screening is implemented.

Recognition of multiple independent underlying genetic conditions is essential for appropriate diagnosis, genetic risk assessment, genetic counseling, management, prognosis, and identification of possible co-morbidities. Prednisone treatment is the standard of care to slow muscle deterioration in ambulatory boys with DMD [23, 24]. Careful consideration is needed to determine the risk-benefit ratio of administration of prednisone in a patient with brittle bones, given the increased risk for osteopenia. In addition, DMD-related immobility

may also contribute to osteopenia and warrants additional skeletal monitoring. On the other hand, there is growing recognition that heritable disorders of the extracellular matrix frequently include some degree of muscle involvement also [25], in this case potentially aggravating the impact of the *dystrophin* deficiency. A DMD diagnosis warrants additional multidisciplinary management, including cardiorespiratory, nutrition and muscle function monitoring. Delayed diagnosis of DMD may significantly impede life-prolonging surveillance, management and treatments, such as ACE-inhibitor treatment to prevent cardiomyopathy complications and introducing pulmonary assistive devices and techniques to improve and prolong proper respiratory function. The promising results of antisense mediated exon skipping in slowing disease progression emphasize the importance of early diagnosis in the DMD population to increase success of potential therapeutic interventions [26, 27]. Additional indirect promising therapeutic advances include increasing muscle mass by modulating other genes in pathway such as follistatin or myostatin or utrophin (dystrophin homolog), dystrophin restitution: compounds that promote codon misreading during eukaryotic messenger RNA (mRNA) translation and allow read-through of nonsense mutations (i.e. aminoglycosides or Ataluren) [28, 29]. Lastly, an accurate genetic diagnosis allows for female carrier testing followed by pre-conception and/or prenatal testing for those families interested in family planning.

Case series like these reinforce the importance of being aware of the possibility of baseline occurrence risks for unrelated secondary conditions to patients and family members of a genetically confirmed patient population. Additional case series of patients with DMD and secondary genetically confirmed condition are necessary to establish the natural history in this “double trouble” patient population.

ACKNOWLEDGEMENTS

The authors wish to thank the families for participating. The authors thank Elizabeth Hartnett and Keonna Harrison for their coordinating efforts and Vickie Zucher, MD for her clinical expertise

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Figure 1.

Phenotype of patient 1 at age 16 years, non-ambulatory with (A) short stature and (D) distal laxity. Phenotype of patient 2 (B) Short stature, normal face, brachydactyly, and incomplete extension at the elbows; (C) lumbar lordosis; (E) genu valgum and calf hypertrophy. Radiography of patient 1 showing (F) thoracolumbar scoliosis, convex left from T7 and through L4 measured at 64 degrees at age 16 years and (G) severely osteopenic and gracile bones at age 12 years. There is stable bowing deformity of the humerus. An oblique lucency is present within the mid humeral diaphysis compatible with a nondisplaced fracture. This is

near the site of a prior humerus fracture. There is no periosteal reaction. (H) Family history of Patient 3 consistent with autosomal dominant inherited OI and maternally inherited DMD.

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Figure 2.

(A&D) Histomorphology of muscle from the left quadriceps of Patient 1 at age 12 years (A) 10x H&E showing necrotic fibers, split fibers, diffuse variation in fiber size with many small fibers, extensive fatty replacement and endomysial fibrosis. Higher magnification 20X trichrome (D) also shows some basophilic fibers with enlarged nuclei suggestive of regeneration. Confocal images (20x) of frozen control muscle sections stained with spectrin (Novacastra 1:400) and dystrophin (DYS2 Novacastra 1:200) of control (B&E) and normal spectrin with absent dystrophin staining in patient 1 (C&F).