Inflammatory Changes in Limb Girdle Muscular Dystrophy Type 2I

H.J. McMillan, J. Michaud

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Muscular dystrophies can show clinical and muscle biopsy features that mimic or overlap the changes seen in a primary myositis, particularly in the early stages of disease or when the clinical onset is abrupt. We present a child who was eventually diagnosed with limb girdle muscular dystrophy type 2I (LGMD2I). She presented with mild hip-girdle weakness and post-infectious myalgia. Although her clinical symptoms favoured a muscular dystrophy, her muscle biopsy showed inflammatory infiltrates within muscle fibres and around blood vessels that were more typical of juvenile polymyositis. Additional immunocytochemical antibody tests and genetic sequencing were key to obtaining an accurate diagnosis and avoiding immunosuppressant therapy.

Limb girdle muscular dystrophy type 2I is an autosomal recessive disorder resulting from mutations within the fukutin-related protein (FKRP) gene. The age of onset varies from early childhood to mid-adulthood[1]. The pattern of weakness is reminiscent of dystrophinopathy patients; initial pelvic-girdle weakness and frequent calf pseudohypertrophy followed by progressive proximal weakness and a high probability of cardiac and respiratory dysfunction. We provide a clinical summary and review the literature surrounding the clinical and diagnostic overlap that exists between patients with a muscular dystrophy versus polymyositis.

Case

A 10-year-old girl was hospitalized with cervical lymphadenitis requiring intravenous antibiotics. High serum transaminase levels (AST 359 U/L, ALT 146 U/L) were noted during her admission prompting an outpatient gastroenterology referral. The source of her elevated serum transaminases were identified as muscle when a very high serum creatine kinase (CK) level was found, ranging from 9,209 to 30,377 U/L (normal <175 U/L). The patient was evaluated by neurology. She reported long-standing disinterest in athletics and poor exercise tolerance. She estimated that she could walk one to two kilometres (km) before becoming fatigued. She climbed stairs in a non-alternating manner (2-feet-per-step). Her early milestones were appropriate; she sat at six to seven months old; her first independent steps were at 12-months-old. She rode a tricycle at three-years-old and a bicycle without training wheels at five to six-years-old. She had been ice skating as a recreational activity but had not participated the most recent winter as the family had been busy moving into a new home. She reported no exercise-induced myalgia or cramping and no pigmenturia. She had noted mild myalgia of both gastrocnemius each evening since her lymphadenitis. She reported no upper extremity weakness nor functional difficulty, no oculobulbar weakness and no sensory symptoms. Her medical history was otherwise unremarkable with no symptoms of a connective tissue disorder. She did very well academically. Family medical history was unremarkable; her non-consanguineous parents and older brother were healthy and of Chinese descent. Physical examination revealed appropriate height (25-50th percentile), weight (50-75th percentile) and head circumference (98th percentile). Cranial nerve examination was normal. Symmetric weakness was noted in the following muscles: neck flexors 4, biceps 4+, gluteus medius 4+, gluteus maximus 4, hip flexors 4-, quadriceps 4. All other muscles were normal. Deep tendon reflexes were normal. Her gastrocnemius muscles appeared mildly prominent and her heel cords were tight, with passive ankle dorsiflexion possible just beyond neutral position. No contractures were noted elsewhere. No scapular winging, spine rigidity nor scoliosis was noted. Trendelenberg gait was noted and a Gowers' sign was present. Her general medical examination was unremarkable. Muscular dystrophy was suspected upon clinical grounds. DMD genetic testing was normal. Her left quadriceps muscle biopsy (Figure 1) revealed moderate variation in the calibre of skeletal muscle fibres with occasional hypertrophic and atrophic fibres seen (Figure A). Perifascicular atrophy was absent. Clusters of degenerating and regenerating fibres were noted. (Figure B). Inflammatory cells were present within these fibres, in the endomysium surrounding normal fibres (Figure A) and in the interstitium surrounding a blood vessel (Figure C). There were no examples of partially-invaded non-necrotic fibres. The inflammatory infiltrate was dominated by CD68 positive macrophages, T-lymphocytes (T4>T8) and rare eosinophils. CD20 staining was negative reflecting the absence of B-lymphocytes. No split fibres, internal nuclei or abnormal storage deposition was noted. Very minimal endomysial fibrosis was seen. Standard histochemical and histoenzymological stains were unrevealing. Immunohistochemical analysis was normal for; spectrin, dystrophin (rod domain, C- and N- domains), adhalin, β, γ, and δ sarcoglycans, β-dystroglycan, merosin, dysferlin, and actinin. Utrophin showed a moderate to strong positivity in the sarcolemma of several muscle fibres. Electron microscopy did not reveal any evidence of tubuloreticular

From the Division of Neurology (HJM), Department of Pathology and Laboratory Medicine (JM), University of Ottawa, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

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Correspondence to: H.J. McMillan, Division of Neurology, Children's Hospital of Eastern Ontario, 401 Smyth Road, Ottawa, Ontario, K1H 8L1, Canada.
Email: hmcmillan@cheo.on.ca.

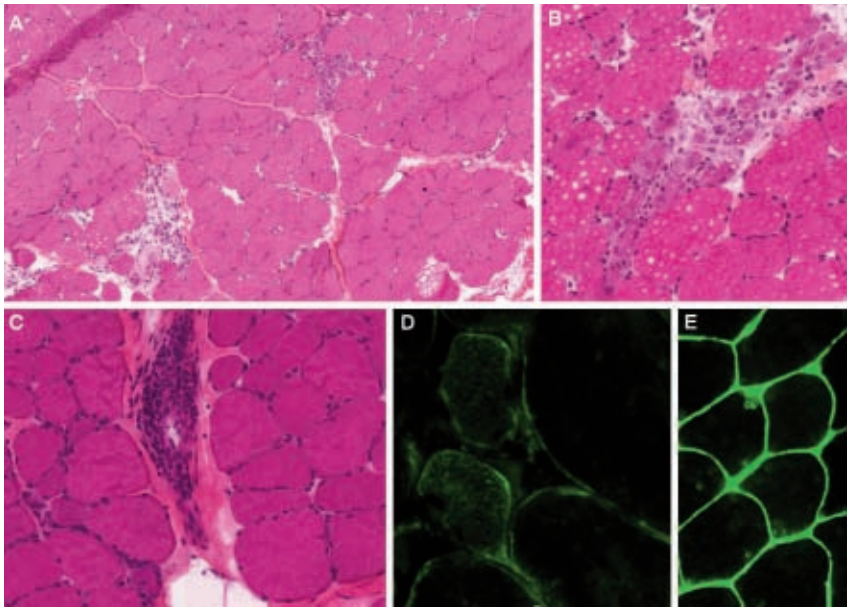

Figure: *Left quadriceps biopsy reveals hematoxylin and eosin staining on low (Figure A) and high power (Figure B, C). Moderate variability is seen in the calibre of the skeletal muscle fibres with some hypertrophic and atrophic fibres seen. Foci of inflammation (Figure A) comprised of CD68 positive macrophages, T-lymphocytes (T4 > T8) and rare eosinophils and clusters of fibre degeneration and regeneration (Figure B) are seen within the endomysium. Interstitial perivascular inflammation was also noted (Figure C). Standard histochemical and histoenzymological stains were unremarkable (not shown). Immunofluorescence testing against α-dystroglycan showed attenuation and focal absence of reaction in our patient (Figure D) compared to a control (Figure E).*

inclusions. In light of the inflammatory infiltrates an inflammatory myopathy was proposed. Blood testing for C-reactive protein, erythrocyte sedimentation rate, anti-nuclear antibody, rheumatoid factor, serum complement and von Willebrand factor antigen were normal. Her echocardiogram was normal. In light of the normal immunohistochemistry, sequencing of the FKRP gene was performed (Prevention Genetics, WI). She was found to possess sequence variants affecting each FKRP allele. The first variant; c.586G>C (p.Gly196Arg) occurred in a highly conserved region and has been reported in other patients with LGMD2I with different heterozygous FKRP mutations (http://www.dmd.nl). The second variant; c.914C>T (p.Pro305Leu) has not been previously reported although an adjacent missense mutation affecting the same amino acid (c.913C>T; p.Pro305Ser) has been found to be disease-causing[1]. The patient's asymptomatic parents were tested, with each possessing a normal serum CK and one FKRP mutation. Further evaluation of the muscle biopsy was performed with α-dystroglycan immunofluorescence (clone VIA4-1; Millipore) showing marked attenuation and focal absence (Figure D) compared to the control (Figure E). Based upon the clinical examination (pelvic-girdle weakness, calf pseudohypertrophy), biochemical testing, α-dystroglycan immunofluorescence and genetic test results the patient was diagnosed with LGMD2I.

Discussion

Limb girdle muscular dystrophy type 2I results from mutations within the fukutin-related protein (FKRP) gene. FKRP is a glycosyltransferase of α-dystroglycan, the latter conferring membrane stability by acting as a link between laminin α2 (merosin) and the extracellular matrix. Fukutin-related protein mutations result in a broad spectrum of clinical phenotypes, ranging from the more severe congenital muscular dystrophies (MCD1C) to less severe LGMD2I phenotypes with pediatric to adult-onset disease. Since FKRP performs an enzymatic rather than a structural function it cannot be directly tested by immunohistochemical or immunoflourescent stains. However, a secondary reduction in α-dystroglycan and laminin α2 can be seen[2]. The reduction of α-dystroglycan noted in our patient was important for confirming the diagnosis of LGMD2I. Given that LGMD2I is one of the more frequent causes of autosomal recessive LGMD[3], α-dystroglycan staining should be considered in any child with limb-girdle weakness and normal membrane immunohistochemistry for dystrophin and sarcoglycans. A clinical correlation has been found between the degree of the reduction of α-dystroglycyan staining and the severity of the clinical phenotype[2].

This case illustrates two important points. First, perimysial or perivascular inflammatory cells on muscle biopsy do not necessarily indicate that a primary inflammatory myopathy is present. Second, specialized immunocytochemical antibody tests are important tools for the complete and accurate interpretation of a muscle biopsy. These tests provide valuable information and compliment the routine microscopic and immunohistochemical studies. This is particularly important in situations such as this when clinicians are faced with diagnostic uncertainty or novel variants on genetic testing.

Controversy exists as to the extent to which polymyositis may be over diagnosed, particularly in children and adolescents. Caution must be taken when inflammatory changes are noted on muscle biopsy in order to avoid the unnecessary use of high dose corticosteroids or other immunosuppressant medications in these children. Inflammatory infiltrates within muscle fibres and vessels have been reported in many types of muscular dystrophies particularly in patients with facioscapulohumeral

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muscular dystrophy, dystrophinopathy, dysferlinopathy (LGMD2B) and laminopathy[4]. An Australian retrospective study found that 12/13 (92%) of children who were initially diagnosed with juvenile polymyositis were ultimately found to have a form of muscular dystrophy that included; caveolinopathy, calpainopathy, dystrophinopathy or a laminopathy[5]. All patients in that study had repeat muscle biopsies performed due to an inadequate response to corticosteroids or other immunosuppressant medications. In each child the initial biopsy findings of perimysial and perivascular inflammation had resolved by the time of repeat biopsy with the second biopsy showing changes consistent with an established dystrophy (i.e. increased number of internal nuclei, increased variation of fibre size, increased number of split and angulated fibers as well as abundant connective tissue).[5] Whether the loss of inflammatory changes was due to the corticosteroid therapy or the emergence of the more predominant dystrophic changes is unclear. Biopsy features can assist the pathologist in distinguishing an early dystrophy from a true myositis including; 1) greater fibre size variability in dystrophy compared to myositis; 2) isolated fibre degeneration that exists independently from the inflammatory infiltrates; 3) predominantly CD68 positive infiltrates in muscular dystrophy (compared to predominantly CD8 or CD20 positive infiltrates in myositis patients) and 4) the presence of subsarcolemmal 'blebbing' in dystrophy, the significance of which remains unclear[5]. The muscle biopsy of our LGMD2I patient possessed all but the last feature. Clinicians cannot use a clinical response to corticosteroids as a means of differentiate a primary myositis from a dystrophy process since the benefit of these drugs is well established in some diseases such as Duchenne muscular dystrophy. Some LGMD2I patients have also reported to show clinical benefit to corticosteroid therapy[6] however, the data is limited and given the long lifespan of patients with this disease caution must be taken to consider long-term complications. Corticosteroids were not used in our patient given the slow insidious nature of her disease.

The use of special immunocytochemical assays such as α-dystroglycan, CD20 and CD68 provide important information that were key to making the definite diagnosis in our patient. Although such studies may be time consuming and add to the cost of muscle biopsy analysis the economic and human costs associated with misdiagnosis, including the expense and potential secondary effects of corticosteroid and immunosuppressant use, is indeed significant. Genetic testing is increasingly being used as an initial diagnostic test and in many cases can obviate the need for more invasive testing when a definite diagnosis can be obtained.

This case illustrates the value of α-dystroglycan immunofluorescent testing and FKRP gene sequencing in children with limb-girdle muscle weakness.

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