Hickam’s Dictum in Genetic Myopathies: When a Proven Pathogenic Mutation does not Explain the Phenotype

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PEARLS

- Exercise intolerance is the most common phenotype in McArdle disease. However, fixed weakness can be present in advanced ages, mainly affecting the shoulder-girdle.
- ANO5 patients may present with a wide spectrum phenotype, including limb-girdle dystrophies, distal myopathies and also asymptomatic or mild symptomatic hyperCKemia. A characteristic radiological pattern, involving the posterior compartment of the thigh, soleus and medial gastrocnemius muscles in the legs, may help to guide genetic diagnosis of this entity.

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- Consider the coexistence of genetic conditions, particularly among patients with a history of consanguinity.
- Although fixed muscle weakness may be present in patients suffering from McArdle disease, in the vast majority of cases, it is mild to moderate and associated or preceded by a history of exercise-related symptoms.
- The presence of subsarcolemmal vacuoles that may contain PAS-positive glycogen and the absence of histochemical staining for myophosphorylase are hallmarks of McArdle disease on muscle biopsy. However, severe dystrophic changes are very rare and should raise the suspicion of an additional concomitant myopathy.
CASE REPORT

A 67-year-old woman, born to consanguineous parents, was referred to our Neuromuscular National Reference Center with the diagnosis of McArdle Disease made at the age of 58. She first noticed difficulties in climbing stairs and standing up from chairs in her forties, which progressed over ten years to where she lost independent ambulation. She denied any history of exercise intolerance or episodes of rhabdomyolysis or myoglobinuria. She did not complain of symptoms of bulbar or extraocular musculature weakness, nor did she report cardiac or respiratory issues.

Initial laboratory tests showed elevated creatine-kinase (CK) levels (range: 1049-1269U/l) and normal lactate levels. EKG showed a first-degree atrioventricular block, with a normal echocardiogram. Given the progressive proximal weakness and hyperCKemia, a muscle biopsy was performed and showed sub-sarcolemmal accumulation of glycogen (PAS-positive vacuoles) and myophosphorylase deficiency, but also striking dystrophic changes (Figure 1a-d). Sanger sequencing of the PYGM gene (NM_005609.3) identified the c.13_14del (p.Leu5ValfsTer22) frameshift variant in homozygosity, previously associated with McArdle disease (HGMD accession number: CD066398; ClinVar Variation ID: 371064).

Neurological exam revealed symmetric proximal muscular weakness and bilateral calf hypertrophy. Manual muscle strength testing revealed the following Medical Research Council (MRC) scores: shoulder abduction 4/5, elbow flexion 4-/5, elbow extension 4+/5, distal upper limbs musculature 5/5, hip abduction, adduction and flexion 2/5, knee flexion and extension 4/5, distal lower limbs musculature 5/5. She was not able to rise from a chair, and independent ambulation was not possible, requiring bilateral support. The rest of the neurological examination was unremarkable, and reflexes were normal. A non-ischemic exercise forearm test showed a flat curve for both venous lactate and ammonia, probably due to insufficient effort during the test.

After the review of clinical and paraclinical features, an overlapping muscle dystrophy was suspected. A muscle MRI was performed (Figure 1e-g) confirming an extensive muscular fatty infiltration, which involved almost the whole pelvic and thigh musculature, sparing solely gracilis and sartorius muscles. At the leg level, it showed a predominant involvement of medial gastrocnemius and soleus.

Subsequently, next-generation sequencing (NGS) of a targeted panel of 202 genes related to
muscular disorders was performed, and an additional homozygous pathogenic mutation was identified in the ANO5 gene (NM_213599.2: c.191dup, p.Asn64Lysfs*15), associated with limb-girdle muscular dystrophy 12 (LGMDR12)\(^1\), formally called LGMD2L. The patient was finally diagnosed with double genetic trouble, harboring proven pathogenic mutations in homozygosis in two different genes. The latter is probably the main cause that explains the clinical phenotype.

**DISCUSSION**

Approximately 1.875 protein-coding genes are known to be linked to autosomal recessive disorders, according to the Clinical Genomic Database of the National Human Genome Research Institute\(^2\). It has been estimated that every individual is a carrier of more than 20 of these disorders or traits\(^3\). The probability of sharing carrier status for a rare autosomal recessive disease is considerably higher for related couples than for unrelated individuals. In this report, the patient, born to consanguineous parents, was found to have two recessive conditions, which we will briefly review below.

McArdle disease is the most common glycogen storage disease. It is an autosomal recessive condition caused by mutations in the myophosphorylase gene (PYGM) that encodes the skeletal muscle-specific isoform of phosphorylase and leads to blocked muscle glycogenolysis. Age of onset is frequently in the first decade of life, but the diagnosis is often delayed, and many patients are diagnosed after early adulthood\(^4,5\). The usual presentation is exercise intolerance that consists of premature muscle fatigue, contractures, pain and episodes of myoglobinuria, triggered by short intense exercise or by dynamic, ‘aerobic’, exercises involving large muscle mass (e.g. stair-climbing, running or walking very fast). A characteristic feature of the condition is the so-called “second wind phenomenon”, considered pathognomonic for the disease. It is described by patients as relief of symptoms triggered by the onset of exertion, which appears about 10 minutes after starting the exercise, and allows them to resume physical activity. Persistent hyperCKemia is present in the vast majority of patients\(^5\).

Recessive mutations in the ANO5 gene are associated with muscular diseases named anoctaminopathies. Patients may present with proximal or distal fixed weakness or isolated persistent hyperCKemia\(^6\). Calf hypertrophy is a relatively common sign of this condition\(^7\).
Muscle MRI may reveal typical signs, with preferential involvement of adductor and gastrocnemius muscles.\textsuperscript{8}

In the case we report, the patient was initially diagnosed with McArdle disease based on the evidence of myophosphorylase deficiency in the muscle biopsy, which prompted the performance of a targeted PYGM genetic analysis. A muscle biopsy was performed prior to a forearm exercise test because the reported symptoms did not suggest a metabolic muscle disorder. Fixed muscle weakness occurs in approximately 20-30\% of affected individuals with McArdle disease, but it is normally preceded by a history of exercise-related symptoms. It is more likely to involve proximal muscles and is more common in individuals of advanced age\textsuperscript{4,9}. In the literature, there are only a few reports of patients diagnosed in later adulthood with a phenotype of fixed weakness as the sole clinical manifestation\textsuperscript{10}. The cause of the fixed weakness in McArdle disease and its phenotype heterogeneity remains unclear\textsuperscript{4}. To date, no genotype-phenotype correlation has been described\textsuperscript{5,9}.

Regarding complementary tests, muscle pathology may show some degenerating or necrotic fibers, accompanied by regeneration, but the most consistent finding is PAS-positive subsarcolemmal vacuoles. The presence, in this case, of intense dystrophic changes in the muscle biopsy led, therefore, to the suspicion of another concomitant myopathy. Muscle MRI showed extensive fatty infiltration. Little is known about radiological features in McArdle disease, and there are solely very sporadic studies reporting fatty degeneration involving proximal muscles in those patients with fixed weakness or of advanced age\textsuperscript{9}.

Therefore, all the reasons described above made it necessary to rule out an associated limb-girdle muscular dystrophy in this case. A genetic analysis confirmed a concomitant anoctaminopathy, which better correlates with the patient’s clinical picture.

At this point, we would like to highlight the importance of carefully evaluating the entire clinical picture. The clinical features should outweigh the complementary tests to guide the definitive diagnosis. It is also important to remember that the coexistence of genetic conditions is possible, particularly among patients with a history of consanguinity. If complementary findings, including genetic results, do not explain well the patient’s clinical manifestations, the investigation should continue until it leads to a proper answer.
FIGURE LEGEND

Figure 1. Muscle biopsy and MRI

Hematoxylin and eosin (H&E) staining shows changes in fiber size with endomysial fibrosis (*) and fatty replacement (♦) (A). H&E staining also shows subsarcolemmal vacuoles with excess glycogen storage, determined by periodic acid-Schiff (PAS) staining (arrowheads) (A, B). Histochemical staining reveals the absence of myophosphorylase activity (C, patient; D, control). Axial T1-weighted MRI image shows extensive muscular fatty infiltration (♦) at pelvic and thigh levels (E, F), sparing solely gracilis (blue-arrowhead) and sartorius (black-arrowhead) muscles. Axial T1-weighted image at leg level shows a predominant involvement of medial gastrocnemius (black-arrows) and soleus muscles (blue-arrows) (G), a characteristic radiological pattern of LGMDR128.
### Appendix 1: AUTHORS

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REFERENCES


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