Dysferlinopathy and Very-Long-Chain Acyl Coenzyme A Dehydrogenase Deficiency Segregating in the Same Family

Sharon Aharoni MD[1,4], Menachem Sadeh MD[2,4], Esther Leshinsky Silver MD[3,4] and Rachel Straussberg MD[1,4]

[1]Institute of Pediatric Neurology, Schneider Children's Medical Center of Israel, Petah Tikva, Israel
[2]Department of Neurology and [3]Molecular Genetics Laboratory, Wolfson Medical Center, Holon, Israel
[4]Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

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Limb girdle muscular dystrophy is the collective name for a diverse group of disorders characterized by progressive weakness of the shoulder and pelvic girdle. It is caused by mutations in multiple genes encoding proteins involved in all aspects of muscle cell biology. To date, 21 cases of LGMD, classified by mode of inheritance, have been identified.

Very-long-chain acyl coenzyme A dehydrogenase deficiency is a disorder of fatty acid oxidation and ketogenesis. It is one of eight inherited defects within the mitochondrial beta-oxidation pathway. When fatty acid oxidation is impaired, fatty acid-dependent organs such as cardiac and skeletal muscle, liver and brain undergo decompensation. Milder variants of the disease may manifest as chronic weakness, pain or recurrent rhabdomyolysis in adolescence or early adulthood.

In the present report we describe a patient with a family history and manifestations compatible with VLCAD deficiency who was, however, ultimately diagnosed with limb girdle muscular dystrophy type 2B (LGMD2B). These findings emphasize the clinical and genetic distinction between these two disorders and the importance of the correct diagnosis.

LGMD = limb girdle muscular dystrophy

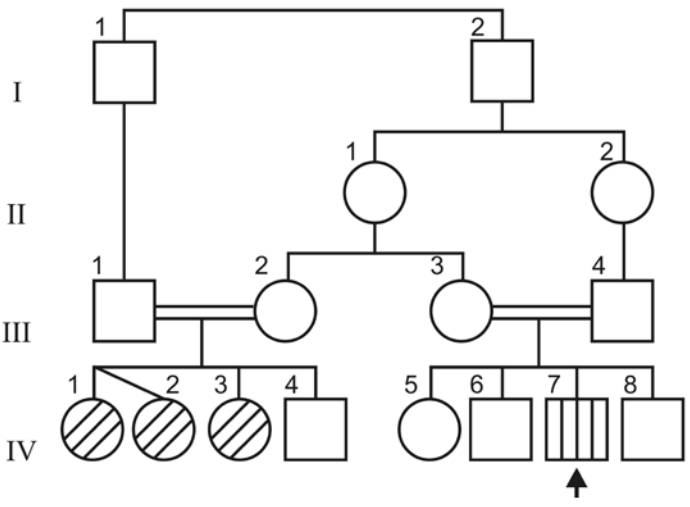
PATIENT DESCRIPTION

An 18 year old man presented with persistent exercise-induced muscle cramps of 6 months duration. He reported no change in the color of his urine.

The patient was the third of four children born to consanguineous parents (first-degree cousins) of Jewish Iraqi origin. His early development was entirely normal. Family history revealed that three siblings who were first cousins to the patient and whose parents were also blood related had had similar complaints of paroxysmal exercise intolerance and were diagnosed with VLCAD deficiency. They were found to be homozygous for a novel mutation, G637A, in which alanine 173 is replaced by threonine [1,2]. The family pedigree is shown in the Figure.

Physical and neurological examination on admission revealed no abnormalities. Laboratory tests showed a creatine phosphokinase level of 30,000 IU/L. Given

Genogram of family showing the index patient with limb girdle muscular dystrophy and his three cousins with VLCAD deficiency



the family history of consanguinity and VLCAD deficiency, we tested for blood level of acylcarnitine, which participates in the oxidation of very-long-chain fatty acids. Surprisingly, the result was within normal range. Genetic testing for the specific VLCAD mutation found in the cousins was negative. These findings prompted a muscle biopsy study which disclosed an inflammatory and degenerative process. On immunohistochemical investigation, staining was negative for dysferlin and normal for dystrophin, merosin, and sarcoglycan alpha and gamma. On Western blot analysis, complete absence of the dysferlin signal was noted. These results were consistent with dysferlin-deficient muscular dystrophy. Molecular genetic testing was performed for the most common mutation in Jewish patients with dysferlinopathy originating from Libya [3] and the Caucasus region [4]. The results were negative.

On follow-up 3 years later, the patient had difficulty walking, climbing stairs and standing on his toes. When he stood with his knees slightly bent, we observed the "diamond on quadriceps" sign.

COMMENT

Dysferlin is a 237 kDA protein expressed predominantly in skeletal muscle fibers. It includes C2 domains thought to be important for calcium-mediated membrane fusion and membrane repair of skeletal muscle fibers. Mutations in the dysferlin gene on chromosome 2p13 cause four distinct phenotypes of muscular dystrophy, known as dysferlinopathy. The two more common phenotypes, LGMD2B and Miyoshi's myopathy, manifest in the second decade of life. The others are distal anterior compartment myopathy and scapuloperoneal type. Additional phenotypes have also been described.

LGMD2B is characterized by a slow progression of weakness and atrophy of the pelvic and shoulder girdle muscles in young adulthood. Respiratory and cardiac muscles are not involved. Miyoshi's myopathy is an early adult-onset form of distal muscular dystrophy, with involvement predominantly of the calf muscles. Patients have difficulty standing on their toes due to weakness of the soleus and gastrocnemius muscles. It is still unknown why mutations of the same gene cause either proximal or distal myopathy.

The dysferlin gene (*DYSF*) was identified in 1998, and more than 180 pathogenic mutations have since been described in different populations [5]. The prevalence of the dysferlinopathies caused by mutations in the *DYSF* gene in the general population is not known. However, three ethnic clusters have been reported within the Libyan Jewish, Italian and Spanish populations. Recently, Leshinsky-Silver et al. [4] reported ethnic clustering of dysferlinopathy among Jews of Caucasus origin in Israel (who number about 80,000), with a 4% carrier frequency of the 927delG mutation. LGMD2B has also been described in a large inbred pedigree of Jews of Yemenite descent, but no frequency data are available.

Fatty acids serve as a major energy source for the body tissues. Different defects of fatty acid oxidation lead to various neuromyopathic diseases with a wide range of clinical presentations both in adults and children. VLCAD deficiency is an autosomal recessive defect of mitochondrial fatty acid oxidation caused by mutations on chromosome 17p13. It is characterized by a lack of VLCAD, an enzyme responsible for breaking down long and very long carbon chains (C14-C22). The disease presents essentially as recurrent episodes of vomiting and coma induced by fasting. Common biochemical features include hypoketonemia, hypoglycemia, hyperammonemia, secondary carnitine deficiency, and increased urinary excretion of dicarboxylic acids. There are three main clinical forms of VLCAD deficiency: a severe, early-onset form that presents within the first days of life with cardiomyopathy and leads to early death; a milder form that begins by age 4 years, with lesser cardiac involvement and hypoketotic hypoglycemia; and a milder form that begins after age 13 years, with muscle involvement and rhabdomyolysis but no cardiac involvement. The diagnosis is based on biochemical findings of the presence of C12 to C14 dicarboxylic acids in the urine and a distinctive acylcarnitine profile; it is confirmed by demonstration of the enzyme defect in cultured fibroblasts. Similar to the dysferlinopathies, the prevalence rate of VLCAD deficiency in the general population is not known. Treatment with intravenous infusion of 10% glucose is effective during acute attacks. Avoiding fasting, an adequate caloric intake and carnitine supplementation may prevent recurrence. A high carbohydrate diet supplemented with medium chain triglycerides is recommended.

In the present case, the initial manifestations were muscle cramps and elevation of muscle CK. On repeated evaluation of the case history, we found that the patient's cousins – who had a known VLCAD deficiency – also presented with exercise intolerance and paroxysmal myoglobinuria, with CK levels up to 100,000 IU/L. However, in contrast to the cousins in whom the CK levels normalized after 3 weeks (once they were started on a low fat diet supplemented with caritine), in our patient the CK levels remained persistently high. This finding, together with the patient's muscle biopsy results, negative laboratory results for acylcarnitine, and negative genetic tests for the specific mutation found in the cousins, suggested a different diagnosis and prompted further immunohistochemical evaluation. We assume that the patient has LGMD2B, which might be associated with a Jewish Iraqi founder mutation.

It is of interest that two diseases of different etiologies and a distinct chromosome linkage appeared in a single double-consanguineous family. The prevalence of a mutation in the *DYSF* gene among Jews from Libya and the Caucasus region is probably high [4]. Likewise, VLCAD deficiency is relatively frequent in Jews of Iraqi origin. Given that the two myopa-

CK = creatine kinase

thies are caused by mutations in different genes located on different chromosomes, we believe their occurrence in the same family of Iraqi origin was coincidental. This report highlights the importance of careful family history taking and proper laboratory and genetic testing in preventing erroneous diagnostic assumptions. Establishing an unequivocal diagnosis in patients with suspected genetic myopathy is essential to ensure successful therapy.

Corresponding author:
Dr. S. Aharoni
Institute of Pediatric Neurology, Schneider Children's Medical Center of Israel, Petah Tikva 49202, Israel
Phone: (972-3) 925 3615, **Fax:** (972-3) 925 3871
email: aharonis@zahav.net.il

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