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Case study

A novel *GBE1* gene variant in a child with glycogen storage disease type IV☆

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Summary Glycogen storage disease type IV is an autosomal recessive disorder of carbohydrates caused by deficiency of amylo-1-4-glycanoglycosyltransferase, which leads to accumulation of amylopectin-like polysaccharides in tissues including liver, heart and neuromuscular system. More than 40 different mutations in the glycogen branching enzyme gene (*GBE1*) have been described. In this study, we report a 2-year-old boy who presented with developmental delay and muscle weakness. He subsequently was diagnosed with glycogen storage disease type IV based on a liver biopsy histology and electron microscopy. Glycogen branching enzyme activity was in the low range. Genetic analysis demonstrated a novel heterozygous variant (c.760A>G; p.Thr254Ala) in exon 6 of the *GBE1* gene, which is believed to be pathogenic. This variant was inherited from the patient's mother who was asymptomatic with normal glycogen branching enzyme activity. Whole-exome sequencing failed to reveal additional variations in the *GBE1* gene.

1. Introduction

Glycogen storage disease type IV, also known as Andersen disease, is an autosomal recessive disorder of carbohydrates caused by deficiency of amylo-1-4-glycanoglycosyltransferase, a glycogen branching enzyme (GBE) whose gene (*GBE1*) is located on chromosome 3 (3p12.2). This enzyme is a 702-amino acid protein that catalyzes the transfer of α-1,4-linked glucosyl units from the outer end of a glycogen chain to an α-1,6 position on the same or neighboring glycogen chain. Branching of the chains increases the solubility of the glycogen molecule and reduces the osmotic pressure within the cells. Loss or reduced GBE activity leads to abnormal glycogen structure (amylopectin-like polysaccharides known as polyglucosan) with few branch points and long unbranched outer chains resulting in reduced solubility and accumulation in various tissues including liver, heart and neuromuscular system. This causes osmotic swelling and ultimately cell death.

Glycogen storage disease type IV shows significant variation in clinical presentation, organ involvement, severity and GBE activity level [1]. The genotype/phenotype correlation is not perfect, and the fact that most patients are compound heterozygotes complicates the attempts to study this correlation. However, some data suggest that the severity of the disease correlates with the severity of the molecular mutation [1,2,3,4]. The disease has hepatic and neuromuscular forms but overlap occurs. Clinical manifestations vary and include

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liver dysfunction, hepatomegaly, cardiomyopathy, myopathy, hypotonia, and fetal akinesia deformation sequence. The classic (progressive) hepatic subtype is characterized by hepatosplenomegaly, failure to thrive, end-stage liver disease and death by 5 years of age if not treated with liver transplantation. Patients with the non-progressive hepatic subtype do not develop liver cirrhosis or liver failure [2,5,6]. There is usually a reduction to less than 25% of the GBE activity in symptomatic patients compared to control subjects.

Histologic examination of liver biopsy in patients with glycogen storage disease type IV shows intracellular ground glass–type inclusions that stain positive with periodic acid–Schiff (PAS) and are partially digested with diastase. In addition, varying degrees of fibrosis are usually present depending on the time of the biopsy and stage of the disease. Similar inclusions in other tissues such as the nervous system and skeletal muscle in the neuromuscular form are called polyglucosan bodies.

Multiple different, mostly missense, mutations in the *GBE1* gene have been reported in patients with glycogen storage disease type IV [4,7-9], most of them are located within exon 12 and 13 and cause a change in the catalytic domain of the protein impairing its enzymatic function. The present study describes a new *GBE1* gene variant that is believed to be pathogenic, causing glycogen storage disease type IV.

2. Results

2.1. Patient

The patient is a 2-year-old white boy who presented when he was 5 month old with developmental delay and muscle weakness. The parents however, stated that they had concerns about growth and development since birth. He was born at 37 weeks and 1 day via scheduled cesarean section due to his breech presentation, weighing 6 pounds and 1 ounce. He had transient tachypnea and jaundice as neonate, which did not require treatment. His past medical history was significant for congenital torticollis, plagiocephaly, sleep apnea, tonsillectomy, adenoidectomy, ear infections with ear tubes placed at age 2, multiple methicillin-resistant *Staphylococcus aureus* skin abscesses, hand, foot, and mouth disease and speech delay. He did not have any episode of hypoglycemia. At age 23 months, he was diagnosed with Epstein-Barr virus infection (mononucleosis). This was associated with hepatomegaly and elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) that failed to normalize. As a result, a liver biopsy was performed.

2.2. Family history

The patient had a normal developing fraternal twin brother. His older sister who was also part of twin pregnancy following in vitro fertilization in which the twin sister died at 33 weeks' gestation had normal development and normal liver function tests. The family history was negative for liver diseases, known genetic disorders or birth defects. There was no history of consanguinity.

2.3. Laboratory results

The newborn screen and hearing screen were both negative. AST and ALT were elevated in the range of 200 s and 500 s U/L. γ-Glutamyltransferase was also elevated and ranged between 72–180 U/L. Bilirubin, albumin, platelet count, prothrombin time, α1 fetoprotein, creatinine, and ammonia were within normal range. The following tests were performed and were normal or negative: antinuclear antibodies, anti–smooth muscle antibodies, liver/kidney microsome antibodies, ceruloplasmin, ferritin, α-1-antitrypsin, IgA tissue transglutaminase for celiac disease, lactate, bile acids, creatine kinase, aldolase, pyruvate kinase in red blood cells, sterols profile, oligosaccharidoses screen, β-glucosidase in leukocytes for Gaucher disease, purine/pyrimidine panel, peroxisomal fatty acid profile, acylcarnitine profile, amino acid profile, affinity chromatography–mass spectrometry for congenital disorders of glycosylation, Smith Lemi Optiz screen, urine organic acids, urine amino acid panel, and serology for human immunodeficiency virus, herpes simplex virus types 1 and 2, cytomegalovirus, Epstein-Barr virus, human T-lymphotropic virusI/II, and hepatitis A, B, and C. The patient had a low free carnitine fraction with a relative elevation of the acylcarnitine/free carnitine ratio, which was thought to be due to dietary artifact from fasting. He had MM A1 antitrypsin phenotype. No abnormalities of the chromosome number or structure were detected by karyotype and chromosomal microarray. Genetic testing for fragile X was negative. The following genes were tested at another hospital and reported to be normal: *ABCB4*, *ABCB11*, *ATP8B1*, *JAG1*, and *SERPINA1*.

2.4. Imaging studies

Abdomen ultrasound shows liver and spleen enlargement with normal echotexture. Chest x-ray, echocardiogram and electrocardiogram revealed no abnormalities. Brain magnetic resonance imaging with and without intravenous contrast showed developmental venous anomaly within the right frontal lobe but otherwise unremarkable.

2.5. Liver biopsy pathology

Tissue examination showed features of glycogen storage disease type IV. The first liver biopsy obtained at age 2 showed swollen hepatocytes with eccentric nuclei and intracellular cytoplasmic inclusions that were positive for PAS and partially digested by diastase (Fig. 1). No inclusions were seen in the Kupffer cells or macrophages (confirmed with a CD68 immunostain). Trichrome stain showed mild portal and periportal fibrosis. Electron microscopy demonstrated centrally placed fibrillary inclusions with abnormal large glycogen rosettes in the cytoplasm of hepatocytes (Fig. 2). An increase in fibrosis was seen in the second biopsy that was performed at age 3 and showed periportal and focal bridging fibrosis.