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Amylopectinosis disease isolated to the heart with normal glycogen branching enzyme activity and gene sequence

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Abstract: We report a 17-month-old female patient with a rare cause of cardiomyopathy secondary to accumulation of amylopectin-like material (fibrillar glycogen) isolated to the heart. Evidence of amylopectinosis isolated to cardiac myocytes in this patient was demonstrated by histology and electron microscopy. Glycogen content, glycogen branching enzyme (GBE) activity, as well as phosphofructokinase enzyme activities measured in liver, skeletal muscle, fibroblasts and ex-transplanted heart tissue were all in the normal to lower normal ranges. Normal skeletal muscle and liver tissue histology and GBE activity, normal GBE activity in skin fibroblasts, plus normal GBE gene sequence in this patient exclude the classical branching enzyme deficiency (type IV GSD). We believe that this is an as yet uncharacterized and novel phenotype of GSD associated with cardiomyopathy, in which there is an imbalance in the regulation of glycogen metabolism limited to the heart.

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Epidemiologic studies in infant cardiomyopathies indicate that as many as 30% may have a genetic basis (1). The common causes of pediatric cardiomyopathy include idiopathic, myocarditis, familial dilated cardiomyopathy, endocardial fibroelastosis, Pompe disease (GSD type 2), mitochondrial disease, infants of diabetic mother, and Noonan syndrome. Type IV GSD is an unusual cause of cardiomyopathy presenting with isolated dilated cardiomyopathy without any apparent physical or laboratory abnormality, and can only be diagnosed by pathological analysis of endomyocardial biopsy (2). Cardiac tissue from endomyocardial biopsy samples and explanted hearts provides an opportunity to study the pathophysiology of cardiomyopathy at the level of the organ, cell, and gene.

Type IV, also known as Andersen disease or amylopectinosis, is a rare autosomal recessive disorder caused by deficiency of GBE activity (α-1, 4-glucan 6-glycosyl-transferase). GSD type IV is a heterogeneous disorder with remarkable variability in its clinical presentation (4). The most common and classic form is characterized by progressive hepatic fibrosis in the first 18 months of life, resulting in hepatosplenomegaly, failure to thrive, end-stage liver disease and death by 5 yr of age. Some patients with a rare and milder non-progressive variant do not develop cirrhosis and survive to adulthood without liver transplantation. Patients with the neuromuscular form of the disease may present in late childhood with fatal myopathy or cardiomyopathy or in adults with neurologic manifestations only (polyglucosan body disease) (5, 6). Several patients with features suggestive

Abbreviations: CK, creatinine kinase; GSD, glycogen storage disease; GBE, glycogen branching enzyme; EM, electron microscopy; LV, left ventricle; PFK, phosphofructokinase.

of GSD type IV (PAS positive, diastase-resistant material having appearance of fibrillar aggregates under electron microscopy) have been reported with severe progressive cardiomyopathy and skeletal myopathy, but normal or near-normal GBE activity in liver, muscle, and cultured skin fibroblasts (7, 8).

We report a rare case of isolated severe glycogen storage cardiomyopathy resembling GSD type IV but without any other organ involvement, and with normal glycogen content, phosphofructokinase (PFK), and low-to-normal GBE activity in skeletal muscle, cardiac muscle, liver and skin fibroblasts, and normal GBE gene sequence. The patient underwent a successful orthotopic cardiac transplantation.

Case report

History

A 17-month-old previously healthy female child presented with a 3-day history of URI symptoms and subsequently developed increased respiratory distress. A chest X-ray revealed cardiomegaly and pulmonary edema. An echocardiogram showed poor LV function with shortening fraction 10%, ejection fraction 26%, increased LV size (LV end diastolic diameter 44 mm), and LV outflow tract velocity of only 40 cm/s, suggesting low cardiac output. She was intubated for progressive heart failure and started inotropic medications.

She was born at full term without any complications and her maternal perinatal history was unremarkable for infections. There was no history of jaundice, liver disease, abnormal body odor and no particular food aversion. She had normal growth and development. Family history was unremarkable for cardiac diseases, liver diseases, consanguinity, metabolic disorders, muscle weakness, or infant deaths. She lived with her parents and a 7-yr-old sibling who were healthy.

Physical examination

Her weight was 10 kg (25th percentile), height 82 cm (75th percentile), and head circumference 48 cm (50th percentile). She had tachycardia, tachypneia, and was diaphoretic. Both lung fields had crackles on auscultation. First and second heart sounds were normal but a systolic murmur of grade 2-3/6 with gallop rhythm was present. Her liver was 1 cm below the right costal margin and no splenomegaly was noted. Neuromuscular examination was appropriate for age.

Laboratory tests

Electrolytes, complete blood count, erythrocyte sedimentation rate, liver function tests, CK, and CK-MB fraction were normal. EKG showed sinus rhythm with striking J-point elevation (Fig. 1). Evaluation for infectious etiologies of cardiomyopathy included negative antibody titers for enterovirus, measles, mumps, rubella, cytomegalovirus, Epstein–Barr virus, herpes simplex virus, toxoplasma, hepatitis viruses (A, B, and C), HIV and varicella. Thyroid function tests, urine organic acids and amino acids, serum organic acids and serum carnitine levels were normal. Cytogenetic studies of bone marrow revealed no detectable abnormalities of chromosome number or structure.

Cardiac hemodynamic data

The cardiac catheterization while she was on inotropic support showed a dilated LV. The mixed venous saturation was 59% and LV end diastolic pressure was 14 mmHg. Pulmonary vascular resistance (Rp) was 0.77 units $\times$ m^2 (normal, $\leq$ 2 units $\times$ m^2) and systemic vascular resistance (Rs) 21.94 units $\times$ m^2 (13–18 units $\times$ m^2). The cardiac index by Fick principle was 2.6 L/min/m^2 (normal, $\geq$3.5 L/min/m^2).

Histopathology and ultrastructural analysis

Myocardial biopsy revealed amylopectin deposits within the myofibrils, a storage product observed in GSD type IV (Fig. 2). Amylopectin is diagnosed by the presence of large periodic acid Schiff-positive and diastase resistance cytoplasmic granules under microscopy. Electron micrographs showed that these inclusions were fibrillar in nature and poorly soluble in buffer consistent with the diagnosis of amylopectinosis. Histology of liver, skeletal muscle, bone marrow, thymus and skin biopsies was normal without any evidence of storage material associated with GSD IV. GBE enzymatic activity of each tissue was measured as previously described (9) and all were in the normal-to-low normal ranges (Table 1). In addition, the entire coding region of the GBE gene was sequenced (6) and no mutations or nucleotide changes were detected.

Management and follow-up

Her cardiac function did not improve and she continued to require high inotropic support. She developed a ventricular arrhythmia. Medications included dobutamine, dopamine, milrinone,

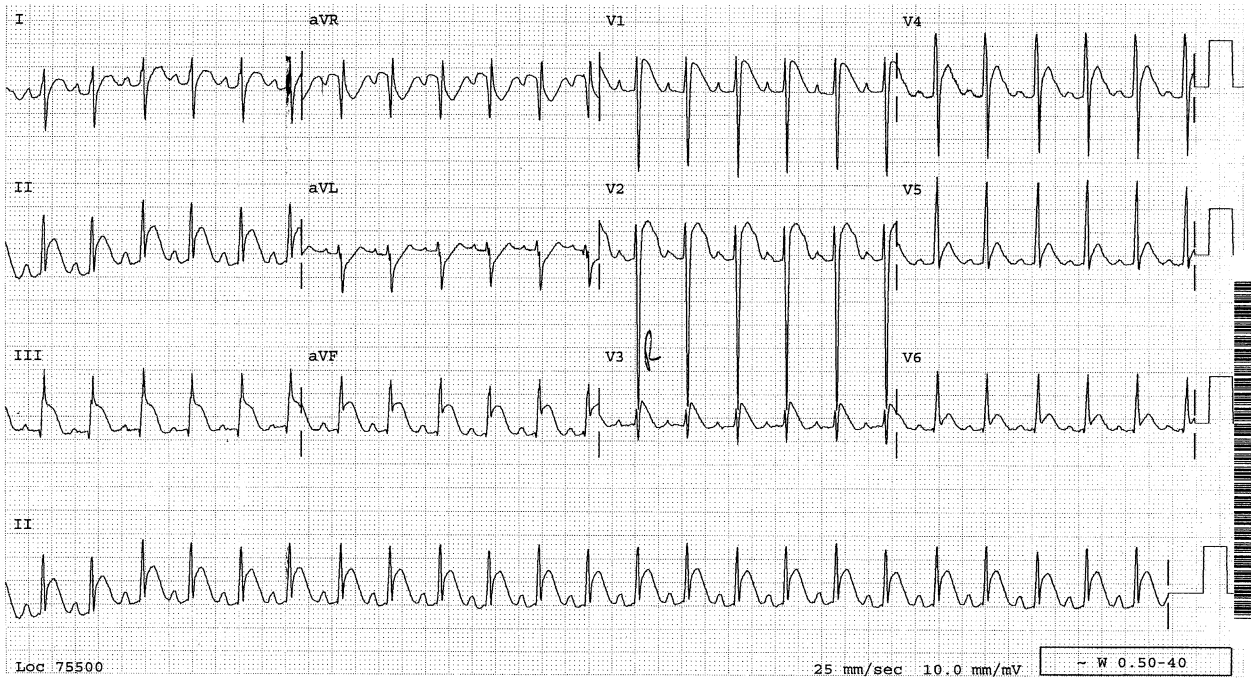

Fig. 1. Electrocardiogram showing sinus rhythm, normal PR interval, but with diffuse ST changes.

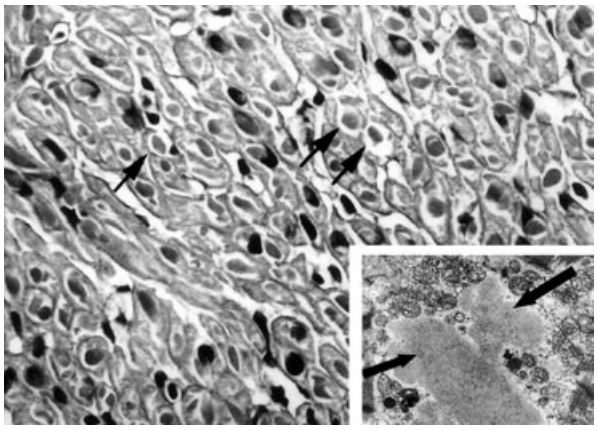

Fig. 2. Cardiac myocytes containing large cytoplasmic inclusions (arrows) stained by PAS reaction for glycogen. ×360. Inset, electron microscopy demonstrates the inclusions to exhibit the fibrillar substructure characteristics of amylopectin. ×8800.

lidocaine, digoxin, captopril, furosemide and metolazone. The patient underwent a successful urgent orthotopic heart transplant after two and half months of her initial presentation. Under cyclosporine-based immunosuppression, the patient remains in good condition 5 yr after heart transplantation. A recent endomyocardial biopsy did not show any evidence of deposition of storage product in the cardiac allograft. Liver function tests, liver size, and muscle strength all remain normal. Repeat cardiac, liver or muscle biopsies are not available to reassess glycogen content or GBE enzyme activities after transplant. General growth and development of this young girl are within normal ranges.

Discussion

GSD limited to the heart is a rare condition and can be caused by deficiency of either cardiac

Table 1. Summary of enzyme activities in our patient and respective cumulative control values

Tissues	Glycogen content (% wet weight)*	Glucose 1-phosphate/glucose ratio (% wet weight)	Branching enzyme (μmol/min/g tissue)	Phosphofructokinase (μmol/min/g tissue)
Heart	0.5 (0.475 ± 0.309)	23 (20% ± 10%)	8.0 (69.8 ± 43.76)	5.6 (5.075 ± 1.43)
Liver	2.0 (3.3 ± 1.7)	50 (25–35%)	29 (85 ± 31)	
Skeletal muscle	0.2 (0.94 ± 0.55)	40 (25–35%)	13 (32 ± 10)	17 (25.12 ± 10.3)
Skin fibroblasts			495 (1300 ± 390)	

*Cumulative control reference ranges for heart, liver, skeletal muscle and skin fibroblasts (Glycogen Storage Disease Laboratory, Pediatric Medical Genetics, Duke University Medical Center, Durham, NC).

phosphorylase kinase or the gamma-2 regulatory subunit of AMP-activated protein kinase (10, 11). However, in both conditions the accumulated glycogen has normal structure (glycogen with granular appearance under EM). GSD type IV (branching enzyme deficiency) is a rare cause of amylopectinosis-associated cardiomyopathy with or without involvement of other organs (12, 13). Our case is extremely unusual in that the amylopectin-like accumulated material was only seen in the heart tissue (glycogen with fibrillar appearance under EM), whereas other organs biopsied (liver, skeletal muscle and skin) had no sign of amylopectinosis. Glycogen content measured in all the biopsied tissues (liver, heart, skeletal muscle) is in the normal ranges, and GBE activity measured in all of these organs was in the low normal range. The low GBE activity measured in ex-planted heart tissue, as well as liver and skeletal tissue, could be due to the sample quality or preservation problems, (however, PFK activities in both heart and skeletal muscle were in the normal ranges) but it is definitely not in the deficiency ranges seen in typical GSD IV patients ($< 5\%$).

Sporadic cases of atypical GSD type IV with normal GBE activity have been reported in the literature; however, in virtually all of these cases, there is evidence of other organ involvement with abnormal storage material resembling amylopectin in other tissues besides heart, such as muscle and liver (7, 14).

The normal histologic findings, normal glycogen content and low normal GBE activity in liver and muscle, along with the normal GBE gene sequence in our patient exclude classical GSD type IV. EM, biochemical and enzymatic studies carried out in the explanted heart of our case showed that glycogen was fibrillar in nature (amylopectinosis) but the glycogen content was normal. GBE activity in the myocardium was in the low normal range (25% of control) (Table 1) which is much higher than what is seen in classical type IV GSD. Electron microscopic examination as well as enzymatic analysis of liver and skeletal muscle revealed no pathologic changes at the ultrastructural or biochemical levels in our case. At the present time, we do not know the underlying defect causing this unusual isolated cardiac amylopectinosis in our patient. We hypothesize that our patient may manifest an isolated defect in one of the glycogen synthase regulatory pathway genes, which are expressed in a tissue-specific manner, thus causing an imbalance between glycogen synthase and branching enzyme gene products specifically in heart tissue. The multiple phosphorylation/dephosphorylation sites and kinases involved in the glycogenosis pathway, many of which are expressed in a tissue-specific manner, may be candidate genes causing this disorder.

The fact that our patient was treated successfully with orthotopic heart transplantation and remains asymptomatic for more than 5 yr after heart transplantation, and that a recent endomyocardial biopsy shows no evidence of recurrent cardiac amylopectin deposition on EM, suggests that the original disease was limited to the heart only. This case may represent an underrecognized subtype of GSD with an as yet unknown defect in the glycogen synthetic pathway and with near-normal branching enzyme activity and manifestations limited to isolated severe cardiomyopathy. In young patients presenting with isolated cardiomyopathy, uncommon causes such as amylopectinosis should be considered in the differential diagnosis. This case is an example demonstrating that endomyocardial biopsies provide significant diagnostic information in cases of apparently idiopathic cardiomyopathy and can be performed safely in children. Furthermore, patients with type IV GSD require a complete heart, liver, and muscle evaluation before consideration of transplantation.

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