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Unclassified polysaccharidosis of the heart and skeletal muscle in siblings

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Abstract

We describe a 15-year-old boy and his 19-year-old sister with progressive dilated cardiomyopathy and mild non-progressive proximal lower limb myopathy, secondary to the accumulation of amylopectin-like fibrillar glycogen, (polyglucosan) bodies, in heart and skeletal muscle.

Evidence of idiopathic amylopectinosis or polysaccharidosis was demonstrated in heart and skeletal muscle tissue by histology, electron microscopy, biochemical, and genetic analysis. In both siblings the heart muscle stored PAS-positive, proteinase-k resistant and partly diastase resistant granulo-filamentous material, simulating polyglucosan bodies. Glycogen branching enzyme activity, and phosphofructokinase enzyme activity, measured in skeletal muscle tissue and explanted heart tissue were all within the normal limits, however glycogen content was elevated. Furthermore, *GBE1*, *PRKAG2*, *desmin*, *αB-crystallin*, *ZASP*, *myotilin*, and *LAMP-2* gene sequencing revealed no mutation, excluding e.g. glycogen storage disease type 4 and desmin-related myofibrillar cardiomyopathies. In both patients the diagnosis of an idiopathic polysaccharidosis with progressive dilated cardiomyopathy was made, requiring heart transplantation at age 13 and 14 respectively. Both patients belong to an autosomal recessive group of biochemically and genetically unclassified severe vacuolar glycogen storage disease of the heart and skeletal muscle. Up to now unidentified glycogen synthesis or glycogen degradation pathways are supposed to contribute to this idiopathic glycogen storage disease.

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Keywords

glycogenosis type 4; idiopathic polysaccharide storage disease; polyglucosan body; cardiomyopathy; heart transplantation

Introduction

Glycogen storage diseases (GSDs) encompass a group of at least thirteen autosomal recessive diseases [1]. The classification of each is based on the known enzyme deficiency and time point of discovery. Most of these diseases reveal a dynamic type of impairment, such as exercise induced myalgia, cramps or myoglobinuria. These types of GSD, for example McArdle disease (GSD5, MIM:232600) or phosphofructokinase deficiency (GSD7, MIM:232800), are rarely linked to excessive glycogen storage in tissue. In contrast, the so called static variant of GSD, e.g. Pompe disease (GSD2, MIM: 232300) shows glycogen storage combined with muscle atrophy and weakness at rest. Cardiac muscle may also be involved, basically in glycogen storage diseases with a permanent muscle weakness as acid maltase, brancher and debrancher deficiencies.

Glycogen storage disease type 4 (GSD4) is a clinically heterogeneous disorder (MIM: 232500). The 'classic' hepatic presentation is progressive liver disease in childhood, succeeding to lethal cirrhosis. The neuromuscular presentation of GSD4 is distinguished by age at onset into 4 groups: i) perinatal, presenting as fetal akinesia deformation sequence with perinatal death; ii) congenital, with hypotonia, neuronal involvement, and death in early infancy; iii) childhood, with myopathy or cardiomyopathy; and iv) adult-onset, with isolated myopathy communally so called adult polyglucosan body disease [2–7]. The enzyme deficiency results in tissue accumulation of abnormal glycogen with fewer branching points and longer outer branches, resembling an amylopectin-like structure, also known as polyglucosan body (MIM:263570) [8–10]. Besides, mutations in *PRKAG2* (MIM:602743), the gene encoding the $\gamma 2$ subunit of AMP-activated protein kinase (AMPK), were found to cause ventricular pre-excitation and progressive atrioventricular conduction block, and hypertrophic cardiomyopathy. Histopathology showed vacuolar changes and periodic acid-Schiff (PAS)-positive inclusions, suggestive for accumulation of glycogen-related material, in the hearts of affected individuals and in the myocardium of transgenic mice overexpressing mutant *PRKAG2* [11–14]. Rarely, phosphofructokinase deficiency (GSD7) can mimic GSD4 and has to be biochemically excluded [1–7]. Additionally, few patients were reported with biochemically unclassified idiopathic amylopectinosis or polysaccharidosis [3,5,11,15–17]. Clinicopathologic syndrome encompassed in all these idiopathic cases morphologic, histochemical, and ultrastructural similarity to GSD4, however without enzymatic concordance [3,5,11,15–17].

We here present two siblings with a severe glycogen storage disease of up to now genetically and biochemically unclassified origin mimicking, GSD4/polyglucosan body myopathy and *PRKAG2* associated disorders.

Family report

In three generations of a Germany-Kazakh family, 2 individuals have been affected by childhood onset progressive dilated cardiomyopathy (DCM) and mild proximal lower limb myopathy. The grandparents (I.1 and I.2) of the father (II.2) were first degree cousins. Five family members could be examined recently: the mother (II.1), father (II.2), and their three children (III.1 index patient, III.2, III.3) (figure 1A). All individual from generation I and II were reported as normal. Medical history of 11 brothers and sisters of the parents (II.1 and II.2) was unremarkable. The youngest child in generation III (III.3) had normal motor mile stones and examinations till his recent age of 6 years. In both parents (II.1, II.2), and the youngest

child (III.3) repeated heart echocardiography, electrocardiograms, and laboratory analysis, including blood count with differential, creatine kinase, liver enzymes, lactate levels, carnitine, acylcarnitine levels, and BNP measurements were within the normal limits.

Patient 1 (figure 1A: III.1)

This currently 19-years old female had normal motor mile stone but mild growth retardation in development. At the age of 6 years she developed increasingly symptoms of mild proximal muscle weakness, e.g. while climbing a staircase. Later, moderate stamina and myalgia were present. Signs and symptoms of a dilated cardiomyopathy with dyspnea and exhaustion appeared at age 13 (NYHA class 2–3). At her first presentation at age 14, she had severe signs and symptoms of dilated cardiomyopathy including dyspnea and peripheral edema. The neurological exam revealed mild ptosis and proximal pareses (gluteal and iliopsoas muscles MCR grade 4). While walking, she showed bilaterally typical Trendelenburg's sign, but getting up from the floor was without using Gowers maneuver. There was no obvious muscle atrophy or contractures. Cardiac examination revealed incomplete right bundle block, without any further signs of heart rhythm alterations. Echocardiography showed predominant left ventricular dilatation, but global dilatation of the heart. The cardiac ejection fraction was compromised below 20%. At this time point the child referred to NYHA class 4. Cardiac MRI was not performed. She underwent heart transplantation at age 14. 5-year follow-up examination revealed persistent proximal pareses without obvious muscle atrophy and exercise intolerance, but evident improvement of heart and lung function. Creatine kinase levels ranged from normal to 765 U/L (normal < 150 U/L), and serum myoglobin levels were elevated, though liver enzyme activities and troponin were at all times within normal limits prior to heart transplantation. A myoglobinuria was not at all found and there were no signs of hemolytic anemia.

Patient 2 (figure 1A: III.2)

This presently 15-years old boy had unremarkable motor mile stones, but moderate growth retardation. Since age 5, he could not run fast. Since age 8 he developed signs and symptoms of dilated cardiomyopathy with dyspnea and decreased stamina. At his first presentation at age 10, he only presented with decrease in stamina and fatigue, e.g. presenting with some slowness while running. His growth retardation was evident (height 134cm, body weight 27.6 kg at age 10) and serum levels of insulin growth factor 1 were decrease to 48µg/l (normal 137–522). Because of progressive dilated cardiomyopathy (Fig 1B), he had difficulties climbing a staircase, and mild limb muscle atrophy was present. The last month prior to heart transplantation he developed a left bundle block on ECG recordings, but no other cardiac rhythm alterations. Echocardiography before heart transplantation showed spongiose dilated global cardiomyopathy with a reduction of the ejection fraction to 18%. A cardiac MRI investigation was not done. At this time point the child referred to NYHA class 4. He underwent heart transplantation at age 13. Thereafter, in a two year follow-up period, he had unchanged mild proximal paresis of the legs with mild limb muscle atrophy. While getting up from the floor he is now using more frequently Gowers maneuver. Creatine kinase levels ranged from normal to 1065 U/L (normal < 180 U/L), and serum myoglobin levels were elevated, whereas liver enzyme activities and troponin were always within normal limits before heart transplantation. An exercise-induced myoglobinuria or hemolytic anemia with hyperuricemia was never found.

Materials and methods

Muscle and heart morphology

From individual III.1, an open quadriceps femoris muscle biopsy was obtained under local anaesthesia, after written informed parental consent. The heart tissue specimens from individual III.1 and III.2 were taken from both explanted hearts.

Light microscopic analysis

The skeletal muscle and heart tissue specimens were processed using standard histological procedures. One part of the biopsy was embedded in glycol methacrylate, and another part was frozen in liquid nitrogen. Cryosections (8–10 µm) were routinely stained, including haematoxylin & eosin, reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH), adenosine triphosphatase reactions (ATPase) at pH 4.6 and pH 9.4, modified Gomori trichrome, van Gieson, cytochrome-C oxidase, succinic dehydrogenase, AMP-deaminase, phosphorylase, oil-red O, Sudan black B, acid phosphatase, non-specific esterase, and periodic acid-Schiff, with and without pre-treatment with diastase or proteinase K 0.1% for 30 min at room temperature. All sections were evaluated by light and electron microscopy according to standard techniques and methods [15].

Ultrastructural analysis

Specimens for electron microscopy were fixed in ice-cold glutaraldehyde 6.25% buffered with 0.1M Soerensen phosphate pH 7.3. After thorough rinsing in buffer, samples were fixed in 2% osmium tetroxide. After rapid dehydration in graded series of acetone, tissue blocks were embedded in Epon. Thin sections of the embedded blocks were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy (Philips, EM420, Germany).

Immunohistological analysis

In the skeletal muscle biopsy of patient 2, a panel of proteins was examined immunohistochemically, including caveolin-3 (Transduction Laboratories, Lexington, Kentucky, USA) α -dystroglycan (Upstate Biotechnology, Lake Placid, NY, USA), α -sarcoglycan, dystrophin, dysferlin, desmin (Novocastra, Newcastle upon Tyne, UK), anti-CD107b (LAMP-2) (eBioscience, Inc, San Diego, USA) and anti-CD4, anti-CD8, anti-B-cell, anti-macrophages, anti-C5B9 complement. (Zytomed, Berlin, Germany).

Biochemical and mutational analysis

For biochemical analysis, muscle and heart tissue specimens were immediately frozen in liquid nitrogen and stored at –80°C. Furthermore, blood leukocytes from both parents were investigated. Glycogen concentration, phosphofructokinase activity, branching activity were determined by previously described methods.¹ Genomic DNA was isolated from blood leukocytes using the QIAmp Blood Kit (Qiagen, Hilden, Germany), from the remaining patients DNA was not available for analysis. The unaffected child (figure 1A III.3) was not biochemically and molecular genetically investigated. Direct sequencing of the entire coding region of *desmin* gene, *alpha-beta crystallin* gene, *ZASP* gene, *myotilin* gene, *LAMP-2*, *GBE1* and *PRKAG2* as well as flanking intronic sequences of *GBE1* and *PRKAG2* was performed [3,14].

Results

Histology

Macroscopically both hearts of individuals III.1 and III.2 showed biventricular hypertrophy with increase in thickness of both ventricular walls (1.5 cm). The interventricular septum was thickened, up to 1 cm. There was no fibrosis of the endo-and epicardium and no myocardial scars were evident macroscopically.

At light microscopy both heart specimens showed interstitial fibrosis (figure 1G). Most conspicuously round vacuoles were present in cardiomyocytes filled with opaque unreactive material in H&E-stained sections (figure 1C,D). The inert material proved to be PAS-positive (figure 1E). However, PAS stains were at least in one of several tests positive to diastase pretreatment (figure 1F), and resistant to proteinase k treatment. Analogous round shaped PAS positive vacuoles were also present in skeletal muscle specimens (figure 2A,B). The vacuoles were predominantly seen in type 2 myofibers (figure 2C), while staining with acid phosphatase was negative (figure 2D). All immunohistological analyses revealed normal pattern of staining, including C5B9 complement and LAMP-2 (data not shown) with the exception of occasionally positive desmin staining of the opaque granulofilamentous material in heart and muscle tissue (figure 1H, figure 2E). At the ultrastructural level in all investigated tissues granulofilamentous material most conspicuous for polyglucosan bodies was found being surrounded by free glycogen. (figure 2F, 3C).

Biochemistry and molecular genetics

Pathobiochemical data are presented in Table 1. In sum, all measured enzyme activity levels were repeatedly in all investigated samples within normal limits. Molecular genetics analysis excluded genomic gene mutations in *desmin*, *alpha-B crystallin*, *ZASP*, *myotilin*, *LAMP-2*; *GBE1*, and *PRKAG2*.

Discussion

Our siblings exhibited symptoms of progressive dilated cardiomyopathy with late atrioventricular conduction blockings during their early teens and consecutively were heart transplanted at age 13 and 14, respectively. Muscle symptoms encompassed early exercise intolerance with an onset around age 6, and during a follow-up period of 10 years a non-progressive muscle weakness of the proximal lower limb girdle muscles only. Histopathology of heart and muscle tissue showed vacuolar myopathy with storage of PAS-positive, proteinase-k resistant and almost diastase resistant granulo-filamentous material conspicuous for polyglucosan bodies and surrounded by free glycogen [9]. Regarding accumulated structurally abnormal polysaccharide (polyglucosan) bodies in skeletal and heart muscles, it is mandatory to examine muscle tissue for brancher enzyme activity since this enzyme activity might be normal in leukocytes in affected patients [6]. However, biochemically, all measured glycolytic enzymes including phosphofructokinase activity, another rationale for polyglucosan bodies, in our siblings were within normal limits. Moreover, *GBE1* gene sequencing did not uncover any gene mutation. In addition, mutations in *PRKAG2* were found to cause similar pheno- and morphotypes [11–14]. Particularly, neuromuscular symptoms encompassed cardiomyopathy and muscle weakness, and histopathology of skeletal muscle showed minimal to severe vacuolar-bound glycogen accumulation. Though, muscle sections stained with PAS after diastase digestion were partially resistant, and biochemical analysis of muscle tissue revealed normal values of acid maltase, phosphorylase, phosphorylase kinase, debranching, and branching enzyme [11–14]. Thus, we were further prompted to look for mutations in *PRKAG2* gene, but failed again to detect any gene mutation. Other known causes of cardiomyopathy, conduction system defects and muscle disease were also excludable by

pattern of inheritance and histology, or unremarkable gene sequences, e.g. desmin-related myopathies, X-chromosomal myopathy with excessive autophagy, Danon's disease, and other rare glycogen storage disorders like phosphorylase or phosphoglycerate kinase deficiency, lactate dehydrogenase deficiency, and glycogen storage disease type 2. Moreover, the large cytosolic vacuoles in cardiomyocytes we saw contained inhomogeneous granular material that stained robustly with PAS and was not proteinase k digestible excluding therefore a proteinaceous origin. Altogether these findings are similar to that of those rare cases previously reported with biochemically unclassified idiopathic amylopectinosis or polysaccharidosis [3, 5, 11, 15–17]. A deficiency in the *GBE1* gene in GSD4 and its variant APBD would obviously create an imbalance between glycogen synthase and branching enzyme activities [10]. In phosphofructokinase deficiency (GSD7), the metabolic block results in an increased concentration of glucose 6-phosphate, which activates glycogen synthase [18]. The abnormal increase of glycogen synthase activity in GSD7, or an abnormal decrease of the GBE activity in GSD4, and at least some cases of APBD, will result in polyglucosan formation as proven in a transgenic mice model [10]. Polyglucosan production in skeletal muscle was completely an effect of the increased glycogen synthase activity, establishing a causative link between activation and accumulation [10]. Human muscle glycogen synthase (GYS1) encodes a protein of 737 amino acids and is identical expressed in both fetal and adult heart and skeletal muscle tissue. GYS2, the liver glycogen synthase is distinct [19]. Hepatic glycogen synthase deficiency (also termed GSD0) leads to hereditary infantile hypoglycemia (MIM:240600). The control of glycogen deposition is different in muscle and liver tissue. In muscle, glucose is imported chiefly by GLUT-4, a high-affinity and low-capacity glucose transporter and therefore mainly insulin dependent. Muscle expresses two isoforms of hexokinase I (HK1, MIM: 142600) and its major form II (HK2, MIM:601125), both lead by dephosphorylation and their inactivation to dephosphorylation and therefore activation of glycogen synthase. Muscle glycogen synthase concentrates in the nucleus at low glucose and translocates to the cytosol when the glucose concentration increases. Glycogen synthase distribution closely resembles that of glycogen [20]. Therefore, the synthesis of the polysaccharide and accumulation is near the plasma membrane, as seen in GSD5 and 7. In our situation, glycogen deposits are mainly centrally in the cytosol of the myofibers, thus other factors have to be involved, disturbing the ordered deposition and degradation of non-membrane-bound glycogen in the metabolism of the polysaccharide.

Although we did not identify any gene mutation in genes predominantly involved in accumulation of polyglucosan body-like material in heart and skeletal muscle tissue of our two heart transplanted siblings, we postulate an autosomal recessive glycogen storage disease of unclassified origin, as a major differential diagnosis for dynamic types of GSD, esp. GSD4/ polyglucosan body myopathy and *PRKAG2* associated disorders. Both, failures in glycogen synthesis or glycogen degradation pathways engaged in glycogen accumulation might contribute to this enigmatic disease.

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Figure 1. Family pedigree and clinico-pathological findings of both siblings

A) Family pedigree, filled boxes are affected family members in generation III. B) Chest X-ray of patient 1 (III.2) before heart transplantation revealed global cardiac enlargement and pulmonary edema. C, E, G) heart tissue sections of patient 1 (III.2), D,F,H) heart tissue sections of patient 2 (III.1). C and D): H&E sections showing hypertrophic vacuolar cardiomyopathy with opaque material inside the vacuoles. E) PAS staining presenting vacuolar-bound glycogen deposits. F) Diastase-pretreated sections reveal minimal reduction of PAS-positive vacuolar deposits. G) Van Gieson's staining reveals endomysial fibrosis H) Anti-desmin immunohistology presents false-positive desmin staining in the vacuoles of some cardiomyocytes. Bars in C–H: 30µm.

Figure 2. Histopathology of skeletal muscle of patient 2 (III.1)

A) H&E: Vacuolar myopathy with opaque material and increase in variation of fiber size, including angulated fibers. B) PAS: PAS-positive glycogen filled vacuoles in small fibers. C) ATPase pH 9.4: The vacuoles are predominantly present in atrophied small type 2 fibers (dark fibers). D) Acid phosphatase: Almost normal histochemistries, especially unreactive vacuoles are seen. E) Anti desmin immunohistology presents marking of small fibers and partly of vacuoles. F) Electron microscopic examination revealed typical polyglucosan bodies surrounded by free dispersed glycogen granules (magnification X3300). Bars in A–E: 50µm.

Figure 3. Electron microscopy of heart tissue

A) Deposition of fibrillary glycogen is noticed (III.2 magnification X3300). B) Dense osmiophilic structure comparable with a polyglucosan body, surrounded by diffuse glycogen (III.1 magnification X3300). C) Higher magnification of a polyglucosan body showing the mixed electron dense granulo-fibrillar nature of the glycogen (III.1, magnification X8800).

Table 1

Pathobiochemical data

	Mother II.1	Father II.2	Patient III.1	Patient III.2
Blood leucocytes				
branching enzyme (normal 0.1–0.5 mol/min/mg protein)	0.24	0.25		
Heart tissue				
glycogen content (normal 0.5–1.0g/100g tissue)			1.7	1.5
branching enzyme (normal 0.2–1.0 μ mol/min/mg protein)			0.2	0.3
phosphorylase a+b (normal 150–300 nmol/min/mg protein)				173
phosphofructokinase activity (normal 5–50 nmol/min/mg protein)				7.9
amyloglucosidase (normal 0.3–1.0 nmol/min/ mg protein)				0.51