Novel insights into the phenotype and long-term D-gal treatment in PGM1-CDG: a case series

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Abstract: Phosphoglucomutase-1-congenital disorder of glycosylation (PGM1-CDG) (OMIM: 614921) is a rare autosomal recessive inherited metabolic disease caused by the deficiency of the PGM1 enzyme. Like other CDGs, PGM1-CDG has a multisystemic presentation. The most common clinical findings include liver involvement, rhabdomyolysis, hypoglycemia, and cardiac involvement. Phenotypic severity can vary, though cardiac presentation is usually part of the most severe phenotype, often resulting in early death. Unlike the majority of CDGs, PGM1-CDG has a treatment: oral D-galactose (D-gal) supplementation, which significantly improves many aspects of the disorder. Here, we describe five PGM1-CDG patients treated with D-gal and report both on novel clinical symptoms in PGM1-CDG as well as the effects of the D-gal treatment. D-gal resulted in notable clinical improvement in four patients, though the efficacy of treatment varied between the patients. Furthermore, there was a significant improvement or normalization in transferrin glycosylation, liver transaminases and coagulation factors in three patients, creatine kinase (CK) levels in two, while hypoglycemia resolved in two patients. One patient discontinued the treatment due to urinary frequency and lack of clinical improvement. Furthermore, one patient experienced recurrent episodes of rhabdomyolysis and tachycardia even on higher doses of therapy. D-gal also failed to improve the cardiac function, which was initially abnormal in three patients, and remains the biggest challenge in treating PGM1-CDG. Together, our findings expand the phenotype of PGM1-CDG and underline the importance of developing novel therapies that would specifically treat the cardiac phenotype in PGM1-CDG.

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Plain Language Summary

An update on benefits and challenges of treating PGM1-CDG with galactose

PGM1-CDG is a rare genetic disorder that affects glycosylation, an important biochemical process happening in every cell of the body. Because glycosylation is essential for correct functioning of the cells and happens in every tissue and organ, patients with PGM1-CDG can have a variety of symptoms affecting many different organs. Main symptoms include low blood glucose levels, hyperinsulinism, bleeding disorder, liver, muscle, heart problems, and so on. This disorder is usually diagnosed based on the genetic testing, patient's symptoms, and transferrin glycosylation test, which detects abnormalities in glycosylation in blood. So far, more than 60 patients have been reported. Unlike many genetic disorders, PGM1-CDG has a treatment in the form of a sugar called

galactose, which naturally occurs in milk, and can treat many symptoms of the disorder. The patients are advised to take it every day by mouth in the form of powder. Here, we describe five more patients with PGM1-CDG, who were treated with galactose. Each of the patients had novel symptoms and they responded to the treatment differently, which helps us to better understand the disorder and the effects of therapy better. We found that many symptoms improved or normalized; however, some patients experienced persistent symptoms and even adverse events that made them stop treatment. Unfortunately, we did not observe any improvement of heart-related issues. Given that heart issues are the most severe aspect of PGM1-CDG and can result in early death, therapies that target heart issues in PGM1-CDG are still necessary. In conclusion, we describe novel aspects of PGM1-CDG, which will help understand and diagnose the disorder better, and highlight the importance of developing new therapies for this disorder that would specifically treat the heart.

Introduction

Congenital disorders of glycosylation (CDGs) are a group of inherited metabolic disorders that affect glycosylation, one of the most important post-translational modifications.[1] The majority of CDGs present as multisystem disease and with varying phenotypic severity.[1] Currently, effective treatments have been described for about 10% of CDGs.[2] One of these CDGs is phosphogluco-mutase-1-(PGM1)-CDG (OMIM: 614921), which is treatable with oral D-galactose supplementation (D-gal).[3–5] PGM1-CDG is an autosomal recessive inherited metabolic disorder caused by biallelic pathogenic variants in the *PGM1* gene,[3] which encodes the PGM1 enzyme. PGM1 interconverts glucose-6-P and glucose-1-P and hence plays an important role in multiple biochemical pathways such as glycolysis, glycogen synthesis, and glycogenolysis.[6,7] Due to its involvement in glycogen metabolism, PGM1 deficiency was first characterized as a glycogen storage disorder (GSD XIV).[3,8–10] However, abnormal glycosylation with both a endoplasmic reticulum (ER) (CDG type I) and Golgi apparatus (GA) glycosylation defect (CDG type II) was later observed in the blood of the patients, and the disorder was then reclassified as a congenital disorder of glycosylation (PGM1-CDG).[3] This 'mixed CDG type', which is characterized by both CDG type I and CDG type II glycosylation pattern, has so far only been described in PGM1-CDG[3] and was not understood at the time the disorder was first described. A recent study, however, has shown that PGM1 deficiency results in a depletion of UDP-glucose and UDP-galactose, which are individually crucial for ER and GA glycosylation, respectively, explaining the mixed CDG phenotype.[11]

PGM1-CDG is a multisystemic disorder affecting multiple organ systems and tissues.[3,10,12] Most common biochemical findings are elevated transaminases, rhabdomyolysis, and hypoglycemia, which have been reported in more than 65% of the patients.[12] Dysmorphic features such as cleft palate, bifid uvula, and Pierre Robin Sequence (PRS) are present in more than half of the reported patients.[12] Cardiac involvement is also common in PGM1-CDG and usually results in the most severe phenotype and has been reported in about half of PGM1-CDG patients.[12–15] The most common cardiac finding is dilated cardiomyopathy (DCM). Electro-cardiography (ECG) abnormalities such as sinus tachycardia, prolonged QT interval, and ventricular enlargement were also reported.[12] Restrictive

cardiomyopathy was reported in one patient.[12,14] Unfortunately, many of the patients with cardiac involvement require cardiac transplantation and premature death due to cardiac complications is not uncommon.[12,13,15] PGM1-CDG also frequently presents with coagulation abnormalities, with antithrombin III (ATIII) deficiency as the most frequent finding.[12] Moreover, thrombotic events were reported.[12] Other observed endocrine abnormalities include hypogonadotropic hypogonadism,[3,16] decreased levels of growth hormone or insulin-like growth factor 1,[3,6,8,9,11,16–21] abnormal thyroid function,[10,19,22] abnormal plasma cortisol levels, adrenocorticotropic hormone (ACTH), and gonadotropins (luteinizing hormone LH, follicle stimulating hormone FSH).[3,8,11,15,17,19,21–27] Furthermore, the liver is usually affected in PGM1-CDG, with findings including hepatomegaly,[11,19,22] steatosis, cholestasis, and fibrosis.[3] Apart from rhabdomyolysis, muscular involvement includes persistently elevated CK, exercise intolerance, fatigability, and muscle weakness.[12] Notably, muscle involvement can also be the only presenting sign of PGM1-CDG, although this has been reported in only a few patients.[3,8,23,27] Interestingly, unlike many CDGs, the central nervous system is generally not affected in PGM1-CDG, and cognitive impairment has been reported in just over 20% of the patients.[12] Intellectual disability (ID) and seizures were originally thought to be the consequence of hyperinsulinemic hypoglycemia (HH), though recently it has been implicated that ID and seizures present regardless of glycemic status.[22,28] Respiratory system is not commonly affected in PGM1-CDG and the symptoms are usually associated with facial congenital malformations, exercise intolerance, and/or cardiac disease.[3,9,15,20,26,27] Gastrointestinal findings are likewise uncommon and usually include feeding difficulties due to facial congenital malformations.[12] Malignant hyperthermia was reported in two patients who have undergone surgery and presented with rhabdomyolysis.[3]

The prevalence of PGM1-CDG is unknown and there are currently more than 60 reported patients with a confirmed molecular diagnosis.[3,5,10,12–16,19–27,29–32] PGM1-CDG usually presents at birth,[12] and the majority of PGM1-CDG patients are diagnosed in the first years of life. The eldest reported patient up-to-date was 53 years old at the age of diagnosis.[33] Given that effective therapy in the form of D-gal is available

for PGM1-CDG, timely diagnosis is crucial. Biochemical methods based on serum transferrin analysis (transferrin isoelectric focusing, or MS based methods) are usually used to detect abnormal glycosylation and help diagnose and monitor CDGs.[31,34–37] Based on the transferrin profile, CDGs are allocated into two groups – CDG type I, which affects the ER part of glycosylation, and CDG type II, which affects the GA glycosylation. PGM1-CDG is unique among CDGs as it is so far the only CDG which presents with a mixed CDG type.[3] Although the majority of PGM1-CDG patients present with the mixed CDG type, there are reports of patients with a CDG type I or CDG type II.[3,12,31] The appearance of a mixed CDG type is strongly indicative of PGM1-CDG; however, this phenomenon has also been observed in ARCN1 deficient patients during acute illness episodes.[38] Therefore, the final PGM1-CDG diagnosis typically requires molecular genetic testing and can be further confirmed with PGM1 enzymatic activity assay,[12] which usually ranges between 0% and 20 %[3,10,11,12] of normal functionality in PGM1-CDG individuals. Unlike some other inborn errors of metabolism, no correlation has been established between the severity of phenotype and the degree of PGM1 enzymatic activity.[10]

PGM1-CDG is treatable by D-gal supplementation.[3,5,11] The mechanism by which D-gal is able to improve glycosylation is by increasing depleted galactose-1-P and nucleotide sugars (UDP-glucose and UDP-galactose).[11] So far, D-gal has been trialed in more than 20 PGM1-CDG patients, with significant clinical improvement observed in the majority of the treated patients.[3,5,11,14,20,21,26,29] Reported D-gal dose ranged between 0.3 and 3 g/kg/day, with the tolerated dose not exceeding 50 g/day.[3,5,11,12,14] The biggest improvement in laboratory findings was seen in coagulation parameters and liver transaminases, which normalized in the majority of patients treated with D-gal.[3,5,11] D-gal treatment improves transferrin glycosylation, and strategies for transferrin biomarker monitoring indices have been proposed to monitor patient compliance.[29,31] Furthermore, D-gal was shown to improve exercise intolerance, hypogonadism, delayed puberty, rhabdomyolysis, and fatigability in some patients.[3,5,11,20,21] Hypoglycemia and CK also improved in some patients;[11] however, fluctuating glucose and CK levels have also been observed on D-gal.[3,5,11,21,26] Hypoglycemia is usually further

managed by frequent feedings and cornstarch,[12] especially in infancy. Nevertheless, D-gal has so far not been successful in treating cardiac presentation in PGM1-CDG. Considering that the cardiac presentation defines the most severe PGM1-CDG phenotype, the development of better therapies that would also improve heart function in PGM1-CDG is still a major unmet clinical need.

Here, we present detailed case reports of five PGM1-CDG patients treated long term with D-gal, each presenting with novel clinical findings and discuss the persisting challenges of treating PGM1-CDG.

Case reports

Five patients with PGM1-CDG were enrolled in the CDG Natural History Study at Mayo Clinic (Mayo Clinic IRB: 19-005187; ClinTrial. gov NCT04199000). In addition, patients were enrolled in the monosaccharide therapy in CDG study (Mayo Clinic IRB: 18-007276, ClinTrial. gov NCT04198987). Written informed consent was obtained from all the patients or their legal guardians. Three of the patients (P1–P3) were previously reported; however, detailed clinical presentation was not provided.[29] Major clinical and biochemical features of all the patients (P1–P5) are described in Table 1 and Table 2.

Patient 1 is a 30-year-old female who has been diagnosed with PGM1-CDG at age of 26. She has been receiving D-gal treatment for more than 3 years. At birth, she was diagnosed with a small posterior midline cleft. Her development was normal apart from a significant speech delay. She had several hypoglycemic episodes in childhood, and she developed exercise intolerance marked by elevated CK and fatigue at the age of 5 years. In addition, she presented with recurrent ear infections and was diagnosed with polycystic ovary syndrome (PCOS) in puberty. She had mild hypothyroidism, a history of migraines and experienced frequent palpitations. Cardiac investigations revealed negative T-waves and a dilated mid-ascending aorta. From 18 years of age, she has been experiencing recurrent episodes of rhabdomyolysis, some of them requiring hospitalization. Liver investigations revealed hepatic steatosis. Laboratory investigations revealed elevated liver enzymes and CK levels while transferrin analysis revealed a mixed CDG type. Genetic

testing was positive for two variants in *PGM1* (c.313A > T; c.200 T > G, see Table 1), which confirmed PGM1-CDG. Upon diagnosis, D-gal was started at 1 g/kg/day, after which transaminases, coagulation factors and blood glycosylation significantly improved (Figure 1). However, she still experienced episodes of rhabdomyolysis (with CK values as high as > 53,000 U). Furthermore, elevated CK levels and cardiac symptoms persisted on therapy. At age of 28, a loop recorder was implanted after an episode of wide-complex tachycardia. Upon implantation, patient had several episodes of rhabdomyolysis, prolonged muscle pain, nausea, and decreased food intake. Cardiac device has registered two separate episodes of tachycardia since implantation. Recently, a continuous glucose monitor was placed to monitor hypoglycemia for 3 months which revealed morning hypoglycemia. Due to her persisting symptoms, her D-gal dose was increased to 50 g/day.

Patient 2 is a 27-year-old female. She was diagnosed with PGM1-CDG at 24 years of age. At birth she presented with macrocephaly, jaundice, and breathing difficulties due to the PRS and cleft palate. In the first months of life, she had undergone a lip-adhesion surgery, during which she had a cardiac arrest for 7 minutes and required hospitalization for 1.5 months. Later, the patient underwent cleft palate repair without complications. She had notable developmental and growth delay. Vision and hearing were also impaired. Neurologic symptoms included balance issues, memory loss, and encephalopathy. Moreover, the patient exhibited behavior abnormalities and has been taking medication for depression. She also had ID, although no HH was ever reported. Furthermore, fatigability, exercise intolerance, progressive muscle weakness, myalgia, and mild myopathy were present. Mitral valve insufficiency and DCM were also present. Gastrointestinal manifestations included acid reflux, swallowing difficulty and constipation. There were no liver abnormalities. She had acute pancreatitis and deep vein thrombosis. Laboratory investigations showed decreased coagulation factors, and transferrin isoelectric focusing revealed a CDG type I pattern. Whole genome sequencing revealed one known pathogenic and one likely pathogenic variant in *PGM1* (c.787G > T; c.988G > C, see Table 1). She has complained of progressive weakness in her hands and worsening balance issues. A single 25 g/day of D-gal has recently

Table 1. Biochemical and clinical features of five newly diagnosed PGM1-CDG patients prior to galactose treatment.

Trait/patient	P1[a]	P2[a]	P3[a]	P4	P5
Age[b]/sex	30/F	27/F	5/M	3/F	2.5/F
Self-reported race	White	White	White	White	White
Consanguinity	NR	NR	NR	NR	NR
Affected family members	NR	NR	NR	Yes	NR
Age of onset	At birth	At birth	At birth	At birth	At birth
PGM1 cDNA change	NM_002633.3 c.313A > T NM_002633.3 c.200T > G	NM_002633.2 c.787G > T NM_002633.2 c.988G > C	NM_002633.2 c.265G > A NM_002633.2 c.988G > C	NM_002633.2 c.871G > A NM_002633.2 c.689duplG	NM_002633.2 c.696_699del NM_002633.2 c.125G > A
PGM1 Amino acid change	p.K105X p.M67R	p.D263Y p.G330R	p.G89R p.G330R	p.G291R p.231Tfsx9	p.Y212* p.S42N
Prenatal/ perinatal complications	-	Breathing issues, jaundice	Decreased fetal movements, two vessel cord	Feeding difficulties	Gestational diabetes, preeclampsia, maternal HTN; hypoglycemia
Congenital malformations	Bifid uvula, posterior small midline cleft palate	PRS, cleft palate; macrocephaly	PRS, cleft palate; macrocephaly	Cleft soft palate, inverted nipples	Cleft palate, micrognathia
Growth delay	-	+	+	+	+
Neuro findings	Migraines	Balance issues, encephalopathy; depression	Abnormal involuntary movement, suspected seizures	Suspected seizures	Abnormal signal splenium of the corpus callosum
Developmental delay	-	+	+	+	-
Speech delay	+	+	+	-	+
Intellectual disability	-	+	-	-	+
Behavioral issues	-	+	-	-	NA
Vision	-	+	-	-	-
Hearing	-	+	+	+	-
Cardiovascular	Dilated mid-ascending aorta, wide complex tachycardia	Mitral valve insufficiency, DCM	Mildly dilated mitral valve annulus, LA dilation, mild mitral valve insufficiency	VSD (closed)	Patent foramen ovale

(Continued)

Table 1. (Continued)

Trait/patient	P1[a]	P2[a]	P3[a]	P4	P5
Musculoskeletal	Exercise intolerance; rhabdomyolysis, mild myopathy; persistently elevated CK	Muscle weakness, progressive weakness; mild myopathy; myalgia	Low core tone, elevated CK	Muscle weakness	Mild myopathy, elevated CK
Gastrointestinal	Loss of appetite, feeding difficulties	GERD, constipation, difficulty swallowing hard food	Stenosis of anus and rectum	-	Feeding difficulties, recurrent vomiting, constipation, GERD
Liver	Hepatic steatosis, elevated transaminases	Acute pancreatitis	Mildly elevated transaminases	Mildly elevated transaminases	Elevated transaminases
Endocrine	Hypothyroidism, hypoglycemia	DVT, coagulation factor abnormalities	Hyperinsulinism, hypoglycemia, coagulation factor abnormalities, short stature	Short stature, hypoglycemia, coagulation factor abnormalities	Hyperinsulinism, hypoglycemia, coagulation factor abnormalities, extremely low IGF-1 and IGFBP-3
Other	Recurrent ear infections, PCOS	Chronic rhinitis	Underwent tonsillectomy and adenoidectomy; recurrent acute suppurative otitis media	Skeletal findings including mild anterior wedging involving the L2 vertebral body and mild inferior beaking of the L1 vertebral body, and mild flaring of the ribs posterior laterally with narrowing at the posterior aspect creating an oar shaped rib appearance	Low ferritin levels, chronic rhinitis
Serum carbohydrate deficient transferrin	Mixed CDG type	CDG type I	Mixed CDG type	Type II	Mixed CDG type
Age at the start of D-gal therapy	27 years	24 years, stopped due to adverse events and no clinical improvement	2 years	1 year	1 year
Effects of D-gal	Improvement in transaminases, coagulation, and glycosylation	Higher dose resulted in urinary frequency, no clinical improvement was observed	Significant improvement in glycosylation, liver transaminases, coagulation factors, CK levels, hypoglycemia resolved	Blood glucose fluctuated, coagulation factors and liver transaminases fluctuated on treatment; one reported episode of high CK on treatment	All laboratory findings normalized, except for transaminases which remain marginally elevated

CDG, congenital disorder of glycosylation; CK, creatine kinase; DCM, dilated cardiomyopathy; DVT, deep vein thrombosis; GERD: gastroesophageal reflux disease; HTN: hypertension; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3; LA: left atrial; NR, not reported; PCOS, polycystic ovary syndrome; PGM, phosphoglucomutase; PRS, Pierre Robin sequence; VSD, ventricular septal defect.
[a]First reported in Perales-Clemente *et al*.[29]
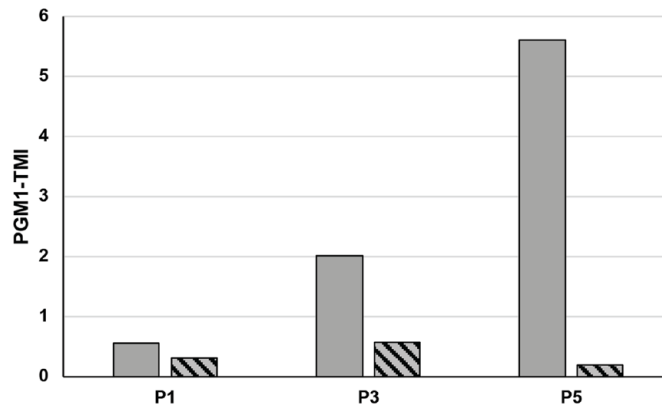[b]At the time of publication.

Figure 1. PGM1 monitoring index (PGM1-TMI)[29] of transferrin biomarkers for select patients in the case series.
For patients 1, 3, and 5, the PGM1-TMI pre-galactose treatment (gray bars) and at last clinical follow-up (patterned bars) is shown demonstrating improvement in transferrin glycosylation. Patients 2 and 4 were excluded. Patient 2 discontinued galactose treatment. Patient 4 did not have a pretreatment sample available for analysis. Refer to Supplementary Information for more details.

been proposed to the patient and D-gal treatment restarted, recently increased to 50 g/day. The patient is currently taking several medications to help manage her symptoms, including mometasone, medroxyprogesterone, betamethasone valerate, digoxin, dulotexine, enalapril, pantoprazole, furosemide, gabapentin, rivaroxaban, sertraline, spironolactone, codeine, Pepcid, and Ativan.

Patient 3 is a 5-year-old male that presented with a two-vessel cord, PRS, cleft palate, and short femurs at birth. He failed the initial hearing test, and he spent the first 40 days of his life in the hospital for mandibular distraction. Mild developmental delay was noted, which was characterized mostly by speech delay, and articulation problems were present. Patient had HH and required frequent overnight feeding through a G-tube. At 2 years of age, the patient experienced an episode of a suspected seizure. However, his electroencephalogram (EEG) was unremarkable. Abnormal involuntary movements and central hypotonia were present. A transthoracic echocardiogram showed mild dilated mitral valve annulus, mild mitral valve insufficiency, severe left atrial dilation, and severe left atrial enlargement. He underwent tonsillectomy and adenoidectomy due to recurrent acute suppurative otitis media. Notable laboratory findings included recurrent abnormal coagulation parameters, elevated liver transaminases, increased CK levels and abnormal glycosylation in blood suggestive of CDG mixed type. Genetic testing revealed two pathogenic

variants in *PGM1* (c.265G > A; c.988G > C, see Table 1) confirming the diagnosis of PGM1-CDG. At 3 years of age, D-gal has been started (current dose 1.8 g/kg/day in two doses). In addition, cornstarch (2x 1 tbs; approximately 30 g) has been prescribed to further manage hypoglycemia, which has resolved since starting the treatment. CK levels likewise normalized. Moreover, D-gal significantly improved serum transferrin glycosylation and coagulation parameters (Figure 1). Liver transaminases improved but did not normalize. Cardiac presentation did not improve.

Patient 4 is a 3-year-old female, who presented at birth with cleft soft palate, inverted nipples, and breathing and feeding difficulties. She did not pass the newborn hearing screening, and had ear tubes inserted at the time of her cleft palate surgery. Short stature, developmental delay, mild hypotonia, and a (closed) ventricular septal defect (VSD) were present. Skeletal findings included mild anterior wedging involving the L2 vertebral body, mild inferior beaking of the L1 vertebral body, and mild flaring of the ribs posterior laterally with narrowing at the posterior aspect creating an oar-shaped rib appearance. The patient also developed hypoglycemia. In addition, she experienced episodes of non-responsiveness and fixed staring that were suspected to be seizures; however, the EEG was not performed. She had mildly elevated liver transaminases, low coagulation factor activities, and abnormal serum transferrin glycosylation indicative of CDG type II.

Table 2. Detailed biochemical changes recorded before D-gal therapy and at the most recent visit during D-gal treatment.

Trait/patient	P1[a]		P2[a]		P3[a]		P4		P5	
Time point	Before	On therapy	Before	On therapy	Before	On therapy	Before	On therapy	Before	On therapy
Liver transaminases (ALT-7–45 U/L; AST 8–43 U/L)	ALT 56 AST 124	ALT 38 AST 107	ALT 44 AST 86	N/A	ALT 63 AST 284	ALT 37 AST 100	ALT 27 AST 64	ALT 33 AST 74	ALT 965 AST 1108	ALT 55 AST 87
Coagulation factors (ATII 80–130%) (aPTT 24–39 s)	ATIII 51%	ATIIII 61 %	aPTT 47	N/A	ATIII 27.2%	ATIII 67%	ATIII 65%	ATIII 46%	ATIII 23%	ATIII 92%
CK (26–192 IU/L)	4911	5897	NR	N/A	858	170	535	92	482	119
Blood glucose (70–140 mg/dL)	81	86	3.8 (reference range 3.3–11.0 mmol/L)	N/A	25	99	3.3	3.1	42	79
Carbohydrate deficient transferrin (CDT) (CLIA certified test for CDG, serum, MALDI-TOF)										
Mono-oligo/Di-oligo Ratio ⩽ 0.06	0.22	0.18	NR	N/A	0.7	0.26	NR	NR	1.26	0.11
A-oligo/Di-oligo Ratio ⩽ 0.011	0.021	0.02	NR	N/A	0.388	0.031	NR	NR	0.799	0.007
Tri-sialo/Di-oligo ratio ⩽ 0.05	0.08	0.09	NR	N/A	0.17	0.05	NR	NR	0.16	0.04

ALT, alanine transaminase; AST, aspartate transaminase; ATIII, antithrombin III; ATIIII,; CDG, congenital disorder of glycosylation; CDT, carbohydrate deficient transferrin; CK, creatine kinase; N/A, not applicable; NR, not reported.
[a]First reported in Perales-Clemente *et al.*[29]

Genetic testing showed two variants in *PGM1* (c.871G > A; c.689duplG, see Table 1). D-gal was started at 1 year of age, and the dose was increased up to 1.5 g/kg/day. During D-gal treatment, patient had an episode of elevated CK. Her blood glucose, coagulation factors, and liver transaminases continued fluctuating on treatment.

Patient 5 is a 2.5-year-old female. The pregnancy was complicated for maternal diabetes, preeclampsia, and hypertension. Emergency C-section was performed at 37 weeks due to poor fetal tone. Cleft palate, micrognathia, and hypoglycemia were present at birth. Right after birth, she was taken to neonatal intensive care unit where she spent 39 days. At 5 weeks of age, a G-tube was placed due to feeding difficulties and poor weight gain. The patient experienced acid reflux, constipation, and recurrent vomiting episodes. At 10 months of age, she was hospitalized for hypoglycemia and started on cornstarch. Abnormal thyroid function, low ferritin levels, chronic rhinitis, elevated transaminases, and coagulation factor abnormalities were also reported. She had a cleft palate repair without complications at 18 months. Cardiac investigations revealed patent foramen ovale. Brain magnetic resonance imaging revealed abnormalities in the splenium of the corpus callosum; EEG and abdominal ultrasound were normal. There was no history of developmental delay except for speech delay which was attributed to cleft palate. Molecular genetic testing revealed two pathogenic variants in *PGM1* (c.696_699del; c.125G > A, see Table 1). Serum transferrin glycoform analysis revealed abnormal glycosylation

pattern suggestive of PGM1-CDG. She was started on iron supplements and received speech and feeding therapy. She has been taking D-gal for 7 months (current dose 2 g/kg/day), and all of her laboratory findings normalized, except for transaminases which remain marginally elevated. Serum transferrin glycosylation improved on D-gal as well (Figure 1).

Discussion and implications for therapy

Here, we offer a detailed report on the clinical manifestation and outcome of D-gal supplementation in five PGM1-CDG patients, three of which have previously been reported in Perales-Clemente *et al.*[29] We observed clinical signs that have not been previously reported in PGM1-CDG, expanding the clinical phenotype of the disorder. Novel clinical findings were noted in the cardiac, gastrointestinal, skeletal, and nervous system. Novel cardiac presentations include dilated mid-ascending aorta (P1) and dilated mitral valve annulus (P3). Novel gastrointestinal findings included constipation (P2, P5) and stenosis of the rectum and anus (P3). Other notable findings included encephalopathy (P2), depression (P2), as well as abnormality in splenium in corpus callosum (P5), which were previously not reported. One patient (P1) suffered from migraines, which was previously reported in one patient.[3,10] Patient 4 presented with inverted nipples, which are frequent in PMM2-CDG,[1,2,39–41] but uncommon in PGM1-CDG.[12] In addition, P4 presented with skeletal abnormalities (including mild anterior wedging involving the L2 vertebral body and mild inferior beaking of the L1 vertebral body, and mild flaring of the ribs posterior laterally with narrowing at the posterior aspect creating an oar shaped rib appearance), which were formerly only reported in one patient.[10] Finally, minimal neurologic involvement was previously considered in PGM1-CDG, with most of the symptoms attributed to be secondary to hypoglycemia.[12] However, recent reports have shown that PGM1-CDG patients can present with neurologic symptoms regardless of hypoglycemia.[21,28] Similarly, we observed intellectual disability without hypoglycemia in P2, suggesting that intellectual disability could also be a direct consequence of PGM1 deficiency and/or abnormal glycosylation, as previously suggested.[28]

Apart from the novel clinical presentation, we also report here on the effects of D-gal

supplementation in our patient cohort. Previously, short term D-gal supplementation has shown positive clinical changes[3,5,11,14,21,26] in transaminases, serum transferrin glycosylation, coagulation factors, reproductive hormones, myopathy, while long-term treatment was reported to further stabilize CK levels.[4] Yet, not all aspects of PGM1-CDG are treatable with D-gal and further therapeutic considerations remain. Crucially, so far, there have been no reports on improvement of cardiac function in PGM1-CDG on D-gal. This is essential, as cardiac complications can lead to early death in PGM1-CDG patients and are usually part of the most severe phenotypes.[12]

We observed clinical improvement in four of five patients treated with D-gal. No bleeding episodes were observed on therapy. However, D-gal did not resolve all symptoms in our patients, even at higher doses. Blood glycosylation improved or normalized in three of five patients (P1, P3, P5), which we were able to monitor by PGM1 therapy monitoring index (PGM1-TMI) (Figure 1, Supplementary Table 1). In addition, liver transaminases and coagulation factors improved or normalized in three of five patients (P1, P3, P5), CK levels significantly improved in two patients (P3, P5), while hypoglycemia resolved in two patients (P3, P5) (Table 2). It is important to note, though, that two of the pediatric patients received cornstarch or frequent feeding in combination with the D-gal treatment, which might have additionally improved hypoglycemia. P1 experienced recurring, severe episodes of rhabdomyolysis and hypoglycemia while on D-gal. In addition, elevated CK was measured for the first-time during D-gal treatment in P4. P2 experienced adverse effects on therapy, and no clinical improvement was observed, which led to the temporary discontinuation of D-gal. Moreover, three of the patients (P1–P3) had persistent cardiac abnormalities which did not resolve or improve on treatment.

One of the explanations as to why D-gal is ineffective in treating cardiac presentation in PGM1-CDG is that D-gal is unable to treat structural cardiac changes caused by PGM1 deficiency. In the heart, PGM1 protein interacts with ZASP protein, which in case of PGM1 deficiency can result in dilated cardiomyopathy.[42] Yet, this theory does not explain the presence of other cardiac abnormalities seen in PGM1-CDG patients such as long QT interval, ST wave elevation, T wave

inversion, and tachycardia.[12] In addition, previous investigations have shown that abnormal glycosylation can negatively affect action potential and channel signaling, resulting in prolonged cardiac action potential and shorter refractory times, providing a link between electrocardial signaling and abnormal glycosylation.[43] In addition, many of the structural proteins, such as collagen, are glycosylated, and its abnormal glycosylation could lead to incorrect collagen fibril assembly.[44] Collagen is one of the main structural heart proteins, which form extracellular matrix (ECM) and provide scaffold for the myofibril formation and play a crucial role in the correct heart function.[45] Therefore, the incorrect glycosylation of these proteins could lead to structural heart abnormalities. In addition, the remodeling capacity of ECM after birth quickly dissipates, limiting the time for the successful therapeutic interventions that could treat these structural abnormalities.[46]

Considering D-gal is able to improve glycosylation,[11] these observations suggest that altered glycosylation alone cannot explain the pathobiology of cardiac abnormalities in PGM1-CDG. Other pathomechanisms could, therefore, be at play. For instance, mitochondrial dysfunction and disturbed energy metabolism, which have been frequently associated with cardiac abnormalities,[47–52] could contribute to cardiac presentation but have never been studied in the context of PGM1-CDG. Apart from understanding the pathomechanism behind cardiac dysfunction in PGM1-CDG, specific biomarkers related to cardiac abnormalities, which would facilitate development and evaluation of potential therapies, are also lacking.

Therefore, deeper understanding of the cardiac changes elicited by PGM1 deficiency as well as cardiac-specific biomarkers are necessary to propose and trial therapies that would specifically target cardiac presentation in this disorder.

Conclusion

In conclusion, we describe here five PGM1-CDG patients and expand the phenotype of PGM1-CDG and report on the long-term effects of D-gal treatment in these patients. Finally, we pinpoint the limitations of D-gal in treating cardiac symptoms in PGM1-CDG, underlying the importance of developing novel, more efficient, therapies

that would treat the cardiac presentation in PGM1-CDG.

Declarations

Ethics approval and consent to participate

Ethical approval to develop this case series was granted by the Mayo Clinic IRB: 19-870051; ClinTrial.gov NCT04199000 (Clinical and Basic Investigations into Congenital Disorders of Glycosylation). The ethical approval for the D-galactose supplementation was granted by Mayo Clinic IRB: 18-760072, ClinTrial.gov NCT04198987 (monosaccharide therapy in CDG).

Consent for publication

Written informed consent for publication of their clinical details was obtained from the patient or the patients' guardian as a part of standard protocol related to Frontiers in CDG Consortium (FCDGC) Natural History study (Mayo Clinic IRB: 19-870051; ClinTrial.gov NCT04199000). A copy of the consent form is available for review with the editor.

Author contributions

Silvia Radenkovic: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

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Availability of data and materials
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Supplemental material
Supplemental material for this article is available online.

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