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Phosphoglucomutase-1 deficiency: Early presentation, metabolic management and detection in neonatal blood spots

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ABSTRACT

Phosphoglucomutase 1 deficiency is a congenital disorder of glycosylation (CDG) with multiorgan involvement affecting carbohydrate metabolism, N-glycosylation and energy production. The metabolic management consists of dietary D-galactose supplementation that ameliorates hypoglycemia, hepatic dysfunction, endocrine

Abbreviations: ALT, ALanine amino Transferase; APTT, Activated Partial Thromboplastin Time; AST, ASpartate amino Transferase; ATIII, Antithrombin III; CDG, Congenital Disorder of Glycosylation; CDT, Carbohydrate Deficient Transferrin Test; CK, Creatine Kinase; CNS, Central Nervous System; DBS, Dried Blood Spot screening; ECC, Endogenous Creatinine Clearance; ECG, ElectroCardioGram; IGF, Insulin-like Growth Factors (1 and 3); LC-MS, Liquid-Chromatography Mass Spectrometry; LDH, Lactate Dehydrogenase; LOCGI, Lack of Complete Glycan Index; LOGI, Lack of Galactose Index; NGI, Normal Glycan Index; OGTT, Oral Glucose Tolerance Test; PGM1, Phosphoglucomutase 1; PT, Prothrombin Time; Q-ToF MS, Quadrupole Time-of-Flight Mass Spectrometry; TIEF, Transferrin Isoelectric Focusing; VSD, ventricular septal defect

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Galactose
Hypoglycemia
Exercise intolerance
Dilated cardiomyopathy

anomalies and growth delay. Previous studies suggest that D-galactose administration in juvenile patients leads to more significant and long-lasting effects, stressing the urge of neonatal diagnosis (0–6 months of age).

Here, we detail the early clinical presentation of PGM1-CDG in eleven infantile patients, and applied the modified Beutler test for screening of PGM1-CDG in neonatal dried blood spots (DBSs).

All eleven infants presented episodic hypoglycemia and elevated transaminases, along with cleft palate and growth delay (10/11), muscle involvement (8/11), neurologic involvement (5/11), cardiac defects (2/11). Standard dietary measures for suspected lactose intolerance in four patients prior to diagnosis led to worsening of hypoglycemia, hepatic failure and recurrent diarrhea, which resolved upon D-galactose supplementation. To investigate possible differences in early vs. late clinical presentation, we performed the first systematic literature review for PGM1-CDG, which highlighted respiratory and gastrointestinal symptoms as significantly more diagnosed in neonatal age.

The modified Butler-test successfully identified PGM1-CDG in DBSs from seven patients, including for the first time Guthrie cards from newborn screening, confirming the possibility of future inclusion of PGM1-CDG in neonatal screening programs.

In conclusion, severe infantile morbidity of PGM1-CDG due to delayed diagnosis could be prevented by raising awareness on its early presentation and by inclusion in newborn screening programs, enabling early treatments and galactose-based metabolic management.

1. Introduction

Phosphoglucomutase 1 (PGM1) deficiency was initially described in 2009 as a Glycogen Storage Disease (GSD, or muscle glycogenosis, type XIV) in an adult patient with exercise intolerance, elevated creatine kinase levels, and episodes of rhabdomyolysis [1–3]. In 2014, several recessive *PGM1* mutations were discovered in a larger cohort of patients showing a heterogeneous spectrum of symptoms including hypoglycemia, cleft uvula or palate, liver disease, growth delay, endocrine abnormalities, dilated cardiomyopathy, myopathy with or without rhabdomyolysis, and coagulation disturbance [4–10]. Although the CNS was initially considered unaffected in PGM1-deficient patients[8], recent studies suggest an association between PGM1 deficiency and neurological symptoms, including seizure and intellectual disability, which do not appear to be secondary to hypoglycemia [11,12]. In 2012 and 2014, the identification of defective serum transferrin glycosylation (showing both missing glycans and truncated glycans) in all PGM1-deficient patients led to the redefinition of this disorder as a Congenital Disorder of Glycosylation (CDG) [5,6]. PGM1 belongs to the phosphohexose mutase family and catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate, modulating carbohydrate metabolism, energy production and protein N-glycosylation [13,14]. Although the mechanisms leading to missing glycans has yet to be unveiled, the lack of galactose in truncated glycans (hypogalactosylation) [5,6] has been explained by galactose metabolism via UDP-glucose 4-epimerase and galactose-1-phosphate uridyltransferase, creating the basis for oral D-galactose supplementation [5,6,15]. A number of articles describing the effects of oral D-galactose supplementation report evidence of liver dysfunction recovery, restoration of endocrine function with resolution of hypogonadotropic hypogonadism, amelioration of coagulation, and reduced occurrence of rhabdomyolytic and hypoglycemic episodes [4,6,15,16]. Biochemically, the treatment showed a clear enhancement of the glycosylation of glycoproteins by reducing both missing glycans and truncated glycans, without any evidence of glycogen accumulation [6]. From a clinical perspective, it has been suggested that the beneficial effects of D-galactose, especially on the hepatic and endocrine functions, are more successful when the treatment is administrated to juvenile patients rather than post-pubertal patients [4,6]. Furthermore, frequent feeding and a standard glucose infusion during the acute phase of hypoglycemia have been reported to be insufficient to treat hypoglycemia in several pediatric PGM1-CDG patients [12].

As early recognition is crucial to prevent complications by initiating early intervention, and in consideration of the enormous heterogeneity in clinical presentations, we here focus on the earliest presenting symptoms in PGM1-CDG in eleven pediatric patients. In addition, we studied the presence of age-related clinical manifestations via a systematic literature review on 43 cases. Lastly, we tested the efficiency of the modified Beutler test [17] in detecting PGM1-CDG in dried blood spots (DBSs) from seven PGM1-CDG patients, including two neonatal DBSs, to assess the potential inclusion of this test in routine neonatal screening.

2. Materials and methods

2.1. Retrospective case series

2.1.1. Ethical approval and informed consent

Patient data was collected as part of standard clinical care. Informed consent on sharing anonym patient data was provided by the participants according to the regulations of the participating institutions. Early onset was defined as manifestation before the age of one year.

2.1.2. Biochemical and genetic diagnosis

Patients were diagnosed by CDG screening initially performed by Transferrin IsoElectric Focusing (TIEF) or Carbohydrate Deficient Transferrin (CDT) test based on clinical indication.

For the patients for which Q-ToF MS-based transferrin glycoprofiling was performed, three glycan-indexes were calculated as illustrated in the study by Abu Bakar et al. [18]: Normal Glycan Index (NGI), Lack of Complete Glycan Index (LOCGI) and Lack of Galactose Index (LOGI). The reference ranges were based on the Q-ToF MS analysis of plasma samples from a control group of 20 healthy volunteers (ages: > 18 years, $n = 5$; 2–18 years, n = 5; 2 years-1 month, n = 5; < 1 month, n = 5) to define normal transferrin glycosylation [18].

The genetic investigation of the *PGM1* gene was performed via Sanger sequencing or Whole Exome Sequencing, according to the diagnostic guidelines of each specific Country. The parents of the reported patients were all heterozygous for *PGM1* mutations.

The exonic location in the genetic sequence and the position in the amino acidic sequence of each mutation were determined using NCBI/Gene (*https://www.ncbi.nlm.nih.gov/gene/*) and UniProt (*http://www.uniprot.org/*), and confirmed by structural data from the literature [13,19,20].

2.1.3. Measurement of phosphoglucomutase activity in dried blood spots (DBS)

To measure PGM1 enzymatic activity the so called *modified Beutler test* was applied [17] on Guthrie heel-prick test cards (dried blood spots, DBS). The DBS samples were collected from seven biochemically and genetically confirmed PGM1-deficient patients, of which four belong to the cohort herein described (patients 3, 4, 6 and 8). For patient 6 and 8 neonatal DBS samples were available.

Table 1
Summary of the clinical presentation of PGM1 deficiency of the 11 reported patients.

Patient	1[a]	2[a]	3[a]	4[a]	5[a]	6	7[a]	8	9	10	11
Gender	F	M	M	M	M	F	M	M	F	M	F
Age (at first hospitalization)	At birth	3 m.o.	2 y.o.	At birth	At birth	3 m.o.	At birth	19 m.o.	At birth	At birth	At birth
Nationality	Somalian	Australian	Arabian	Arabian	Arabian	Irish	Guatemalan	Dutch	American	Irish	Pacific Islander
Consanguineous parents	Yes	No	Yes	Yes	Yes	No	No	No	No	No	No
Mutation(s)	c.689G > A p.G230E	c.157_158delinsG p.Q53Gfs[a]15	c.1544G > A	c.1544G > A	c.1544G > A	c.988G > C p.G330R	c.1014 T > A	c.988G > C p.G330R	c.313A > T p.K105X	c. 1378-2379delTC p.A461Kfs[a]2	c.1561C > T p.Arg521Ter
		c.1507C > T p.R503[a]	p.R515Q	p.R515Q	p.R515Q	c.661delC p.R221Vfs[a]13	p.S338R	c.1007C > G p.P336R	c.206 T > G p.M67R	c.87-88delCC p.F29Lfs[a]75	
Laboratory findings (before galactose treatment)[b]											
AST (U/L) [r.v.: < 38 U/L]	140	256	119–520	115–365	168–1245	620	1813	256	236	2219	294–725
ALT (U/L) [r.v.: < 50 U/L]	Normal	94	41–1429	Normal to 92	80–473	257	1003	Normal	61	1170	683–1613
Hypoglycemia (mmol/L Glc)	1.8	2.5	1.4–3	1.55	0.07–1.5	1.5–2.6	1.61	1.8–2.1	2.6	0.78	< 0.1
CK level (U/L) [r.v.: < 280 U/L]	311	500	3382	622	Normal	1500	1036	1357	1800	Normal	Normal
IGF1 (ng/mL)	N/A	41 [47–231]	5.87 [4.29–21.06]	4.77 [7.54–34.71]	3.26 [3.25–13.13]	< 3.3 [7–43]	> 21 [34–340]	Normal	N/A	63 [34–340]	N/A
IGFBP3 (ng/mL)	N/A	Normal	290 [290–1484]	319 [290–1484]	235 [290–1484]	N/A	810 [1100 − 3200]	Normal	N/A	816 [1100–3200]	Normal
Coagulation	Delayed PT, aPTT	Slightly delayed PT, aPTT	Normal	Normal	Normal	Normal	N/A	Delayed PT, aPTT	Normal	Delayed PT, aPTT	Delayed aPTT
Symptoms (before galactose treatment)[b]											
Oral cleft	CP	CP, PRS	No	CP	CP	CP, PRS	CP, PRS	PRS, BU	CP	CP, PRS	CP, PRS
Liver anomalies	No	Hepatomegaly	No	No	No	No	Steatohepatitis	Hepatomegaly	Steatohepatitis	Micro/Macro-vesicular steatosis	Micro/Macro-vesicular steatosis
Muscle anomalies	Hypotonia	Hypotonia	Lower leg pain, fatigue, weakness	Fatigue, weakness	Fatigue, weakness	No	Mild proximal weakness	No	Exercise intolerance	No	Hypotonia
Rhabdomyolysis	No	Yes	No	No	No	No	No	No	Yes	No	No
Heart anomalies	Enlarged heart, VSD	No	No	No	No	No	No	No	No	No	VSD, aorta coarctation, cardiomegaly
Growth delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes
Neurological symptoms	Delayed motor development	Delayed motor development	No	No	No	No	Abnormal EEG	No	No	Developmental Delay	Delayed motor development
Others	Asphyxia at birth, pulmonary hypertension	Persistent diarrhea	Facial dysmorphism, chocking attack with seizures, short limbs	Facial dysmorphism, short limbs, fused kidney, resuscitation at birth	Facial dysmorphism, mild left hydronephrosis, tachypnea at birth	Recurrent vomiting	Blue sclera, adrenal dysfunction, anticonvulsivant therapy	Fasting intolerance	Recurrent ear infections, delayed speech development	Hearing loss, undescended testes, feeding intolerance	Micrognathia, proteinuria, pulmonary hypertension, persistent diarrhea, blue sclera

[a] Previously published, see patient description.
[b] Abbreviations: r.v., reference values; Glc, glucose; PRS, Pierro-Robin sequence; BU, bifid uvula; CP, cleft palate; VSD, entricular septal defect; EEG, electroencephalography. IGF1 and IGFBP3 reference ranges from Mayo Clinic Laboratories guidelines (https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/36365).

2.2. Systematic review of the literature and statistical analysis

The systematic literature search was performed following the PRISMA protocol on PubMed/NIH database (http://www.ncbi.nlm.nih.gov/pubmed) in October 2019. The search queries and selection process are described in Supplementary Tables S1 and S2. The clinical details of the gathered PGM1-CDG patients are described in Table S3, divided in two groups based on younger age for which a detailed clinical description was available (age ≤ 1 year; > 1 year). In Table S3, the clinical symptoms are classified in 26 categories. For each of these categories, the number of positively diagnosed patients was counted (Table S4), and the χ2-test (corrected for multiple comparisons with Benjamini-Hochberg procedure) was applied to evaluate whether significant differences in early vs. late presentation were present (Table S5). The symptom 'puberty delay' was excluded by this analysis as not present neonatally. Likewise, the same χ2-test was applied to test significant gender-based differences in the recognition of the symptoms (Table S6).

3. Results

3.1. Retrospective case series

3.1.1. Patient 1 (Somalia)

Patient 1 [4,5] was born at 40 weeks from consanguineous parents with unknown birth parameters. A few minutes after birth she developed apnea requiring postnatal resuscitation. Further tests identified cardiomegaly and low O_2-saturation, and the ECG recorded at the 3rd day of life showed a ventricular septal defect (VSD) apical at the septum, and moderate pulmonary hypertension (Table 1). Other clinical findings evident at birth included cleft palate (affecting both soft and hard palate), mild muscular hypotonia, especially of the upper body, neck and head, and feeding difficulties with tendency to exhaustion (Tables 1, S3). Growth parameters remained under the 3rd centile by the age of 7 and 12 months of age. Her VSD spontaneously resolved at 6 months, but at the age of 11 months she was diagnosed with dilated cardiomyopathy, and she was noted to have a mild motor development delay and muscular weakness (Table 1). At the age of 18 months she had normal cognitive development, with some delayed language development mostly due to the cleft palate, for which no surgery procedures were initially performed due to the cardiac condition. Her serum glucose measurements revealed recurrent borderline hypoglycemia without clinical symptoms. Laboratory results initially showed normal CK levels, which later appeared slightly elevated occasionally. While ALT was normal, AST resulted mildly elevated (Table 1). ATIII activity was decreased suggesting defective coagulation.

At the age of 2 years CGH array revealed 5 large homozygosity areas, one of them also involving the *PGM1* locus. TIEF showed a mixed type I/II pattern and a homozygous missense variant was detected in PGM1 (c.689G > A (p.Gly230Glu) affecting the sugar-phosphate binding domain (IV) of the enzyme [13,19,20] (UniProt, NCBI/Gene) (Fig. 2).

After diagnosis, she was started on oral D-Galactose supplementation at the age of 3 years according to the protocol by Wong [4] (Fig. 1). Liver transaminase values, antithrombin values, LOCGI and LOGI on plasma transferrin improved, but did not fully normalize (Table 2). On her last review at the age of 7 years, she still showed mild muscular weakness. Her cleft palate has been surgically repaired after starting galactose treatment. She had no cognitive deficit but both body length and weight remained under the 3rd centile. The dilated cardiomyopathy (ejection fraction between 20 and 30%) is being treated with ACE inhibitors and diuretics, and has persisted till the most recent examination.

3.1.2. Patient 2 (Australia)

Patient 2 [4,5,12] was born at term of a normal pregnancy with a birth weight of 2.9 kg and length at 47.0 cm, both below the 5th centile. He was diagnosed at birth with Pierre Robin Sequence, bilateral cleft palate (affecting both soft and hard palate) and strabismus (Tables 1, S3). At 3 months of age, he was hospitalized for recurrent episodes of hypoglycemia, failure to thrive and persistent diarrhea (Table 1). The hypoglycemia events began after he was commenced on a lactose-free diet, as other attempts to manage the diarrhea were unsuccessful. He had episodes of both hyperinsulinemic and ketotic hypoglycemia, which could not be prevented by Diazoxide alone. Cessation of the lactose-free diet resulted in a reduction in hypoglycemia episodes, after which he was put on a frequent complex carbohydrate feeding protocol. A gastrostomy was performed at the age of 6 months to facilitate continuous overnight feedings. He had poor muscle tone with limited head control and delayed motor development, but cardiac examination was normal. He developed a growth delay with height under the 3rd percentile. The cleft palate required serial surgical interventions.

Laboratory analysis showed persistently mildly increased CK and hepatopathy with elevated ALT and AST (Tables 1, S3). His PT and APTT were slightly prolonged with 17.6 s, and 88.9 s, respectively, suggesting defective coagulation.

The CDT test was abnormal and *PGM1* gene sequencing showed a nonsense mutation inherited from the father, c.157_158delinsG (p.Q53Gfs*15), and a missense mutation inherited from the mother, c.1507C > T (p.R503*), affecting domains I and III, respectively [13,19,20] (UniProt, NCBI/Gene) (Fig. 2). D-Galactose supplementation was started at 10 months of age (Fig. 1), while continuing on Diazoxide. As result, the frequency of hypoglycemic episodes strongly reduced to only 1 to 2 times per year. His clotting, liver function tests and CK levels normalized, and his fasting tolerance extended (Fig. 1). LOCGI and LOGI on plasma transferrin slightly improved but did not fully normalize (Table 2).

3.1.3. Patients 3, 4 and 5 (United Arab Emirates)

Patients 3 and 4 were sons of consanguineous Arab parents and first cousins of patient 5, who was also son of consanguineous parents.

Patient 3 [21] was born after a normal pregnancy and delivery with a birth weight of 2.9 kg (below 5th centile) and length of 44.5 cm (below 1st centile). Short limbs were suspected prenatally and confirmed after birth. Cyanosis and severe bradycardia were developed at 17 h after birth, preceded by vomiting. Resuscitation was started immediately and he was intubated and admitted to NICU where he developed seizures. During that episode, his blood glucose was measured low (Table 1). He was treated with intravenous glucose and phenobarbital and put on a frequent feeding schedule for 3 months. The seizures did not recur.

He was again admitted for evaluation at the age of 2 years due to short but proportionate stature. Facial dysmorphic features were recognized, including large eyes with flat midface and short flat nose, frontal bossing, shallow orbits, long smooth philtrum, a thin downturning upper lip, prominent ears with simple helix (Tables 1, S3). He had normal developmental milestones. Skeletal survey, ECG, abdominal US and echo-Doppler did not show any anomalies. At 5 years of age, he showed normal cognitive and speech development. At the age of 6, blood sugar levels were measured repeatedly and he was diagnosed with recurrent hypoglycemia (Table 1) after fasting for longer than 10 h. Laboratory results showed elevated CK, AST and ALT, and low IGF-1, but normal coagulation (Tables 1, S3).

Hypoglycemic episodes disappeared and transaminases improved once he was started on galactose supplementation (Fig. 1), while CK levels remained variable. Galactose supplementation also fully rescued a peculiar symptom the patient was complaining about since the age of 6 years: frequent episodes of lower limb pain, lasting about 15 min, which sometimes made walking impossible. At the age of 8 years he was cognitively normal and his weight improved to the 10th centile,

Fig. 1. Overview of the complications and metabolic management in our patient cohort.
The different metabolic management strategies are highlighted as follows: lactose-free diet in blue color, feeding regimen with complex carbohydrates diet in green color, and galactose oral supplementation in yellow color. In the boxes below each timeline, the results of the different metabolic management strategies are summarized. For patient 9, although clinical details of her neonatal period (early presentation) are available, thus she was included in the cohort, the correct PGM1-CDG diagnosis was made during adulthood (26 years of age). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Glycosylation indexes from Q-ToF MS-based transferrin glycoprofiling of patients 1, 2, 6 and 8.

Patient	Age[a]	Therapeutic state	NGI[b]	LOCGI[c]	LOGI[d]
Controls	0-18[e] years	–	92.3–93.8	1.9–2.8	0.47–0.86
Patient 1	1 to 2 years	Before treatment	53,2	25,4	13,2
	3 to 4 years	Galactose supplementation (1 g/kg/day)	60,7	20,5	11,0
Patient 2	5 to 6 months	Before treatment	10,2	31,8	42,7
	10 to 12 months	Galactose supplementation (2 g/kg/day)	22,5	21,3	39,7
Patient 6	1 to 2 months	Before treatment	15,3	25,9	42,1
	3 to 4 months	D-Galactose supplementation (5.4 g/kg/day)	88,9	6,0	2,1
Patient 8[f]	2 to 3 months	Before treatment	21,1	23,3	34,2
	20 to 25 months	D-Galactose supplementation (3 g/kg/day)	87,8	7,1	1,6
	25 to 27 months	D-Galactose supplementation (1.1 g/kg/day)	58,3	21,8	12,0
	7 to 8 years	D-Galactose supplementation (1 g/kg/day)	78,4	8,2	4,9

[a] Patient age at sample collection.
[b] Normal glycan index.
[c] Lack of complete glycans index.
[d] Galactose lacked index.
[e] For establishment of reference ranges, plasma samples from a control group of 20 healthy volunteers (> 18 years, $n = 5$; 2–18 years, n-5; 1 month - 2 years, n = 5 and < 1 month, n = 5) were analyzed [18].
[f] For patient 8 extra measurements have been performed during D-Galactose supplementation. The complete list of results is reported in Table S8.

although the body length remained under 3rd centile.

Patient 4 [21] was born at term after a normal pregnancy and delivery, with a birth weight of 3.0 kg and length of 47.0 cm, which both remained under the 3rd centile. The newborn required perinatal resuscitation, including intubation and ventilation. As for his brother, short limbs were suspected prenatally and confirmed after birth. Several facial dysmorphic features were diagnosed including posterior cleft palate with micrognathia, flat midface, long philtrum, thin down-turning upper lip, and prominent ears with simple helix (Tables 1, S3). Several apnea events due to the cleft palate ultimately lead to tracheostomy, removed at the age of 1 year, followed by surgically resolution of cleft. Ultrasound kidney showed an ectopic fused right kidney. DMSA scan at the age of 7 months revealed only a left sided conglomerate kidney, the renal parenchyma of the left and fused right kidneys were difficult to separate (renal function ECC 71% left and 29% right). ECG and ECHO-Doppler of the liver were normal. Around 2 years of age also this patient developed recurrent episodes of hypoglycemia very similar to the ones of his brother (Table 1). Laboratory studies performed at the age of 2.5 years, showed low IGF1 and elevated AST and ALT (Tables 1, S3). Since then, ALT became normal but AST has been often mildly increased, with a maximum of 115 IU/L. CK was monitored for the first time at age of 3.5 years, when it was elevated (Tables 1, S3).

Galactose treatment 1 g/kg/day was started at the age of 5 years (Fig. 1), after which the hypoglycemia episodes disappeared. Although galactose treatment improved the appetite, he did not show significant improvement on IGF1 and growth delay. During the most recent evaluation, at the age of 6 years, the patient weight fell between the 10th and 25th centiles, while the length remained below the 3rd centile.

For *patients 3* and *4* TIEF showed a mixed type I/II pattern. Genetic investigations showed a homozygous mutation in exon 9 of *PGM1* (c.1544G > A (p.R515Q)) (NCBI/Gene). This mutation is located in the IV domain of the protein and affects a critical site for substrate binding [13,19,20] (NCBI/Gene) (Fig. 2).

Patient 5 [21] was born from an uncomplicated pregnancy with Apgar scores 4/9. At birth, his weight was 2.3 kg and his length 44.0 cm (both below 3rd centile). Small mandible, small tongue and a cleft of both the soft and hard palate were identified at birth (Tables 1, S3). He required tube feeding, which was maintained till 2 months of age. Mild left hydronephrosis was detected, which resolved around 3.5 years of age. He presented with growth delay as his height consistently remained below the 3rd centile, with normal weight. Apart from the proportionate short stature, other clinical examinations like ECG, ECHO and abdominal ultrasound of the liver did not show any anomaly.

At the age of 19 months, he was kept fasting in preparation for the palate surgery, but 6 h later he exhibited irritability and sweating,

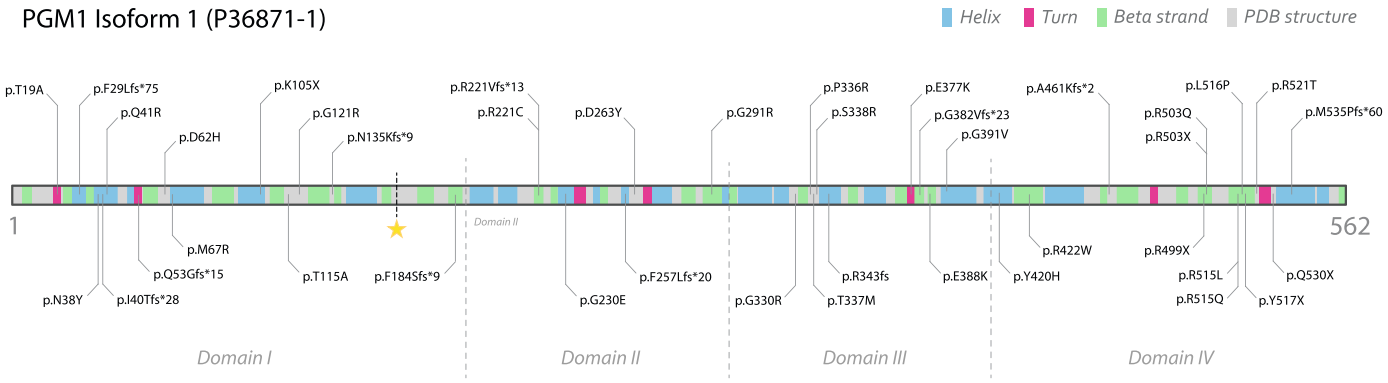

Fig. 2. Map of all PGM1 protein mutations reported in 54 PGM1-CDG patients.
Visualization of all amino acidic mutations affecting PGM1 protein ever reported in the literature till October 2019 (including our ten patients presented hereabove), displayed on the secondary structure of PGM1 (UniProt). The map does not display the mutations *inv(1)(p31.1p32.3)* [24] and *c.1145-1G > C* [1,4,5,31], affecting intron 1 and a splicing donor site on introns 7–8, respectively. The secondary structure was obtained from UniProt database (*UniProt Consortium, Nucleic Acids Res. 2019 Jan 8;47(D1):D506-D515*). Legend: alpha helixes indicated in blue; beta sheets in green; turns in pink; areas with structure predicted from *Protein Data Bank* (PDB) indicated in grey; catalytic residue (Serine 117) indicated by the star symbol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which evolved into tonic-clonic convulsions. At that time his blood glucose level was extremely low, confirming severe hypoglycemia. All these symptoms could be completely rescued by normal formula feeding. After the diagnosis of PGM1-CDG was made, frequent feeding was encouraged however he kept having hypoglycemia episodes, even with fasting periods of around 3 h. Laboratory analysis showed low IGF-1 and increased AST and ALT (Tables 1, S3). CK was also monitored but it appeared normal in all the evaluations performed.

Sequence analysis showed the homozygous mutation (c.1544G > A (p.R515Q)) as previously detected in patients 3 and 4 of the same family, and TIEF confirmed a mixed CDG type I/II pattern.

At the age of 3 years oral galactose supplementation (1 g/kg/day) was commenced, while frequent feeding continued, and the hypoglycemic episodes disappeared (Fig. 1).

At the age of 3 years, he still presented short stature but normal cognitive development. The surgical repair of the palate failed twice and cleft is present to this day, which led to poor language skills. Severe muscle weakness occurred as a post anesthesia complication for his first failed cleft palate repair, without hyperthermia: he needed ventilation for a week and could not adequately move for approximately 3 weeks after. His muscular condition gradually improved, but he easily got fatigued for several following months and for even a longer period he dragged his right leg when walking. The second failed repair was done after galactose treatment administration according to the protocol by Wong [4], after which he did not develop any muscle related complication.

3.1.4. Patient 6 (Ireland)

Patient 6 was born to non-consanguineous healthy Irish parents after an uncomplicated pregnancy with birth weight of 3.4 kg. At birth she was diagnosed with Pierre-Robin sequence with cleft palate (affecting both hard and soft palate), which made nasogastric tube feeding and airway assistance with a nasopharyngeal airway necessary. Due to a suspected lactose-intolerance based on recurrent vomiting in the first weeks of life, she was put on a lactose-free infant formula, after which her condition worsened. At 2 months of age she was hospitalized due to severe hypotonia, although she was alert. Cardiac assessment identified a dilated left ventricle with good ventricular function (Tables 1, S3). Recurrent hypoketotic hypoglycemia of unknown origin (possibly mild hyperinsulinism but the investigations were inconclusive) required continuous feeding (Tables 1, S3).

Further laboratory investigations revealed elevated AST, ALT and CK (Tables 1, S3). Low levels of ceruloplasmin (57 U/L, normal range 90–270) and IGF1 (< 3.3 U/L, normal range 7–43) were detected, while coagulation was normal (Table S3).

Following a TIEF pattern suggestive of PGM1-CDG, genetic investigation identified two mutations: c.661delC (p.R221Vfs*13) affecting domain II, and c.988G > C (p.G330R) affecting domain III, the latter predicted to be pathogenic (13,19,20) (UniProt, NCBI/Gene) (Fig. 2).

After diagnosis at 2 months of age, she was commenced on lactose-reach diet from infant cow-milk derived formula (estimated to provide about 5.4 g/kg/day of galactose from lactose) (Fig. 1). Both LOCGI and LOGI on plasma transferrin measured via LC-MS progressively improved upon galactose-containing diet (Table 2). In addition, she underwent Percutaneous Enteral Gastrostomy (PEG) tube placement to facilitate feeding. Laboratory tests on the normal infant feeding regime showed improvement of all abnormal results that had been observed when she was on galactose-free diet. Current D-Galactose intake derived from lactose-containing products at the age of 3.5 years of age is estimated to be 1.7 g/kg/day, which helped normalizing transaminases, thyroid hormones, IGF1 and IGFBP3 levels.

During her latest assessment at 3 years of age, she presented with short stature, weight on 75th centile for age and height on 2nd centile. Muscle tone and development are normal apart from speech development delay. Repeated echocardiography and cardiac assessment were normal. She no longer requires airway support but continues to need some PEG feeds to supplement her oral intake, while awaiting for palatoplasty.

3.1.5. Patient 7 (United States of America)

Patient 7 [21] was born at 36 weeks gestation from non-consanguineous parents with Guatemalan origin, with birth weight of 2.4 kg and length of 43.0 cm, both below the 10th centile. He was hospitalized at birth due to transient hypoglycemia and cleft palate, without anomalies detected on brain MRI and ultrasound (Tables 1, S3). His parents had a history of a term male stillbirth and a term male who died of unknown cause on day 5 after birth who presented cleft palate, unilateral renal agenesis and heart malformation (no investigations in the direction of PGM1-CDG diagnosis were performed). After palatoplasty, the patient was lost for follow-up. He presented at age of 28 months with faltering weight (2nd percentile), short stature (3rd percentile), blue sclera and mild proximal weakness (Tables 1, S3).

Liver ultrasound showed increased diffuse echogenicity while liver biopsy showed severe steatohepatitis, greatly increased lipids with both microvesicular and macrovesicular steatosis, but mitochondria and glycogen content were normal (Tables 1, S3). At the same age, an anticonvulsant-based therapy was commenced to treat his seizures. The patient continued to show recurrent severe hypoglycemia with an inappropriate corticosteroid response, supporting the hypothesis of adrenal dysfunction. Laboratory analysis revealed low levels of IGF-1 and IGFBP3, and elevated CK, AST and ALT (Tables 1, S3).

Upon clinical suspicion of PGM1-CDG, TIEF was performed, confirming the mixed type I/II pattern. The diagnosis was confirmed by genetic investigation that revealed a missense mutation, c.1014 T > A (p.S338R), in exon 6 encoding domain IV of the enzyme [3,19,20] (UniProt, NCBI/Gene) (Fig. 2).

At 28 months of age the patient was started on oral galactose and remained on 1 g/kg/day long term (Fig. 1). The therapy improved all laboratory parameters, including cholestasis and hypoglycemia within 4 months.

3.1.6. Patient 8 (the Netherlands)

Patient 8 is a son of non-consanguineous parents born at term after a normal pregnancy with a weight of 3.2 kg and length of 49.0 cm (both values under 15th centile). He was diagnosed with Pierre Robin anomaly with cleft uvula, which caused minimal feeding difficulties. At the age of 19-months, he was referred to the hospital due to hypoketotic hypoglycemic episodes, suspected hyperinsulinism and moderate hepatomegaly (Tables 1, S3). He had no cardiomyopathy, no psychomotor development delay or other clinically relevant features.

Due to lactose intolerance, the patient was maintained on a dairy-free diet (estimate galactose intake < 0,35 g/kg/day). At this age, his height fell between the 5th and 10th centile, and recurrent hypoglycemic episodes without ketosis (Table 1). He showed a variable fasting tolerance decreasing from 2 to 6 h. The patient was started on tube feeding with feeding frequency of every 2 h with extra cornstarch (four times 0.5 g/kg/day) but the hypoglycemia persisted. Laboratory investigations at this age detected increased AST, ALT and LDH, minimally elevated CK, and abnormal coagulation (Tables 1, S3, S7). He had normal levels of uric acid, triglycerides and cholesterol, insulin, GH, TSH and free T4 and IGF1/IGFBP3 (Tables S3, S7). Mild fasting lactic acid elevation in blood was reported (3.0 mmol/L). Endocrine studies, including blood glucagon, adrenalin and noradrenalin levels were normal during hypoglycemic episodes. Insulin levels were measurable during two hypoglycemic episodes (relative hyperinsulinism of 5 μU/mL and 8 μU/mL). During the oral glucose tolerance test (OGTT), persistent hyperglycemia lasted for two hours (10.3–11.0 mmol/L), with significant increased insulin in response to 1 g/kg glucose (from < 1.5 to 62 μU/mL) but no lactic acid elevation.

Abnormal TIEF was suggestive for PGM1-CDG, which was confirmed by genetic investigations that identified two mutations on exon

5, c.988G > C (p.Gly330Arg) and c.1007C > G (p.Pro336Arg), affecting PGM1 sugar-binding domain (III) [13,19,20] (UniProt, NCBI/Gene) (Fig. 2).

At 20 months of age, the patient was started on D-galactose supplementation with a dose of 3 g/kg/day, later adjusted to 1 g/kg/day (Fig. 1). Galactose treatment led to normalization of coagulation and transaminase levels, reduction of hypoglycemia, hepatomegaly rescue and growth rate improvement (height between 25-50th centile) (Fig. 1, Table S7). During therapy both LOCGI and LOGI on plasma transferrin improved (Tables 2 and S8). The fasting intolerance also ameliorated and at 3 years of age tube feeding was removed, but cornstarch supplementation continued.

3.1.7. Patient 9 (United States of America)

Patient 9 was born after a normal pregnancy at term, with a 3.6 kg birth weight and normal birth parameters. She had a traumatic delivery by forceps, with significant caput succedaneum. She stayed at the hospital for 2 days. She had feeding problems and was diagnosed with a posterior, small midline cleft (Tables 1, S3). She had low blood sugar levels in infancy and needed frequent feeding including night feeding till the age of 2 years. She had normal motor development, walking at 12 months. Her speech was delayed, and at 2 years she started speech therapy, and had clear words only at 4 years of age (Tables 1, S3). She also suffered from recurrent ear infections. Laboratory tests showed elevated CK, AST and ALT levels (Tables 1, S3).

She was diagnosed with exercise intolerance at the age of 4 years, based on not being able to run. She had collapse episodes due to exercise. She developed a mild hypothyroidism (Tables 1, S3). She had her first rhabdomyolysis at the age 18 years, with hospital admission, and several recurrent episodes till her diagnosis at 26 years. All episodes happened after strenuous exercise. Cardiac evaluation showed no heart involvement so far.

Transferrin glycoprofiling showed abnormal glycans, pointing towards PGM1-CDG, later confirmed by *PGM1* gene sequencing that showed compound heterozygosity for two mutations both affecting domain I of the enzyme [13,19,20] (UniProt, NCBI/Gene) (Fig. 2): c.313A > T (p.L105X) and c.206 T > G (p.M67R).

She recently started D-Galactose therapy with a dose of 1 g/kg/day (Fig. 1) that led to normalization of liver function tests, and no further episode of rhabdomyolysis occurred.

3.1.8. Patient 10 (Ireland)

Patient 10 was a male born at 38 weeks to a primigravid 34 years old mother with prenatal history of intrauterine growth retardation and maternal PKU. The weight at birth was 2.5 kg. The patient was hypotonic at birth requiring prolonged resuscitation with chest compression and epinephrine with poor Apgar scores. A severe Pierre-Robin Sequence complicated the intubation during the delivery (Tables 1, S3). After resuscitation, he was transferred to a neonatal intensive care unit, he had a 72-h cool cap protocol due to the hypoxic-ischemic event. He had low set ears and was later diagnosed with a cleft of the soft palate when he was taken to the operation room to have his end tracheal tube changed for a larger size, and to have a mandibular expander placed. Feeding initially was nasogastric, and was changed to a gastric tube at four months of age. He remained 47 days in the NICU, and was transferred to a transitional care facility before going home at about three months of age. He failed his newborn screening hearing test. Initial labs were significantly abnormal due to his newborn stress, but resolved prior to discharge. At six months of age, he was readmitted for a liver biopsy due to increasing AST and ALT, the liver biopsy revealed micro- and macrovesicular steatosis, but no mitochondrial abnormality or storage material were noted (Tables 1, S3).

During admission, a metabolic consultation was requested resulting in, the only identified laboratory abnormality of an transferrin isoform pattern suggestive of PGM1 deficiency. A CDG molecular panel confirmed compound heterozygous mutations in the *PGM1* gene: c.87-88delCC (p.F29Lfs*75) affecting domain I and c. 1378-2379delTC (p.A461Kfs*2) located close to domain IV [13,19,20] (UniProt, NCBI/Gene) (Fig. 2).

After diagnosis, he was started on D-Galactose treatment (Fig. 1), which resulted in a gradual improvement of his lab parameters. At this point he has been on galactose therapy for seven months.

3.1.9. Patient 11 (Pacific islander)

Patient 11 is the daughter of non-consanguineous parents born at 37 weeks gestation after a normal pregnancy with a weight of 2.7 kg and length of 48.3 cm (both below 3rd centile). Clinical investigations showed coarctation of the aorta and perimembranous VSD, and Pierre-Robin sequence with U-shaped cleft palate and retro-micrognathia (Tables 1, S3). At 3 weeks of age she developed acute respiratory distress and cardiomegaly (Tables 1, S3) and was transferred for surgical correction of the coarctation, performed at 6 weeks of age. Post-operation, she decompensated needing cardiorespiratory resuscitation and underwent tracheostomy 3 days later. She developed severe liver dysfunction which later improved, but transaminitis did not normalize (Tables 1, S3). Recurrent hypoketotic hypoglycemia within 3 h after feeding was documented and treated with frequent high glucose (11 mg/kg/min) feeds. The hypoglycemia was nonresponsive to glucagon (single test). At 4 months of age she underwent gastrointestinal tube insertion and a liver biopsy, which showed non-specific and minimal macrovesicular steatosis, lymphocytic infiltration and periportal fibrosis. However, electron microscopic examination of the liver revealed scattered myelinoid bodies which have lamellar appearance with an empty center and often noted along the bile free bile canaliculi. Mitochondria degeneration was observed, along with mild to moderate amounts of micro and macrovesicular fat drops. No developmental delay was noticed.

Whole genome sequencing reported homozygosity of the known pathogenic nonsense mutation c.1561C > T (p.R521T) in the *PGM1* gene affecting domain IV [13,19,20] (UniProt, NCBI/Gene) (Fig. 2), confirmed biochemically by identification of characteristic glycosylation abnormalities by mass spectrometry of transferrin.

Laboratory investigations identified increased AST, ALT and LDH levels, along with abnormal coagulation parameters (Tables 1, S3). She had normal levels of CK, uric acid, triglycerides and cholesterol, insulin, GH, TSH, free T4 and IGFBP3 (Tables 1, S3).

At 4 months of age, the patient's weight and length were around the 3rd centile, while her length was around the 3rd centile. Galactose supplementation was commenced at this age, with an initial dose of 0.5 g/kg/day, later increased to match her needs (Fig. 1). The therapy led to an overall clinical improvement, including normalization of coagulation and transaminase levels, resolution of hypoglycemia on continuous feeds, and growth rate improvement (at 11 months of age, weight 34th centile, length 24th centile). Her diarrhea predated galactose therapy and did not worsen. TBG levels were 6.3 pre galactose therapy and 13.5 μg/ml after 4 months of therapy. Glycosylation of plasma transferrin improved. By 12 months of age, the patient was stable except for her respiratory state, was sitting with support, socially interactive and trying to roll. Cleft repair and attempt at removal of tracheal tube is pending.

3.2. Measurement of enzymatic activity in DBS

In view of the severe clinical symptoms presenting neonatally, the often long diagnostic delay and the positive response on metabolic management with galactose at juvenile age, PGM activity was assessed in neonatal DBS samples, available from patients 6 and 8, and in two DBS samples from infantile patients 3 and 4. PGM activity was assessed via the modified Beutler test [17], which was previously used to successfully detect deficient PGM activity in DBSs obtained from PGM1-CDG patients during life. In all four cases, deficiency of PGM activity (< 20 U/L) was detected (Table 3). In addition, deficient activity was

Table 3
Results of DBS test for PGM1 deficiency.

Modified Butler test on DBS			
	Age at sample collection	PGM activity [U]	GALT activity [U]
			Control enzyme
Patient 6	Neonatal	7.3	4.5
Patient 8	Neonatal	7.7	4.8
Patient 3	Infantile	4.3	2.6
Patient 4	Infantile	5.2	2.8
Patient A[a]	Pubertal	3.6	4.3
Patient B[a]	Adult	13.0	5.3
Patient C[a]	Adult	4.1	3.0
Reference values	–	> 20	> 2

[a] Measurement of the DBS from this patient have been reported in Tegtmeyer et al. [6].

detected in other three available samples of PGM1-CDG adult patients (A, B and C) (Table 3). GALT activity as control was normal for all samples.

These results indicate the potential to add PGM1 deficiency to future neonatal screening regimes to prevent early onset clinical symptoms by improved metabolic management.

3.3. Systematic review of the literature and comparison of clinical presentations

To compare the description of early presentation with literature and to identify possible age-related clinical presentations of PGM1 deficiency, we performed a systematic literature review on clinical symptoms in PGM1-CDG.

The systematic review of the literature led to 198 papers (Table S2), of which 17 case reports were included [1,4–6,8,11,16,21–31]. The article by Radenkovic et al. [12] was also included despite being a review as it provided unreported clinical details on previously published patients, with particular attention for CNS symptoms. One patient identified in a conference poster from 2012 was also included after contacting directly the author (Dr. D. Rymen) to gather further information on the case [32]. Collectively, 43 PGM1 deficient patients were reported till October 2019, ranging from 0 to 53 years of age (Table S3), which were combined with the 11 patients here presented, ending up with a total number of 54 patients. From this cohort, 43 mutations affecting *PGM1* gene have been identified (Table S3, Fig. 2) but, as reported in previous studies [4–6,13,33], no genotype-phenotype correlation emerged.

Taken all together (regardless age-based or gender-based distinction), elevated transaminases (96%), growth delay (89%), hypoglycemia (89%) and cleft palate or bifid uvula (87%) resulted to be the most frequently reported symptoms, in line with what previously reported (5,6) (Table S4). The frequencies of the most severe symptoms,

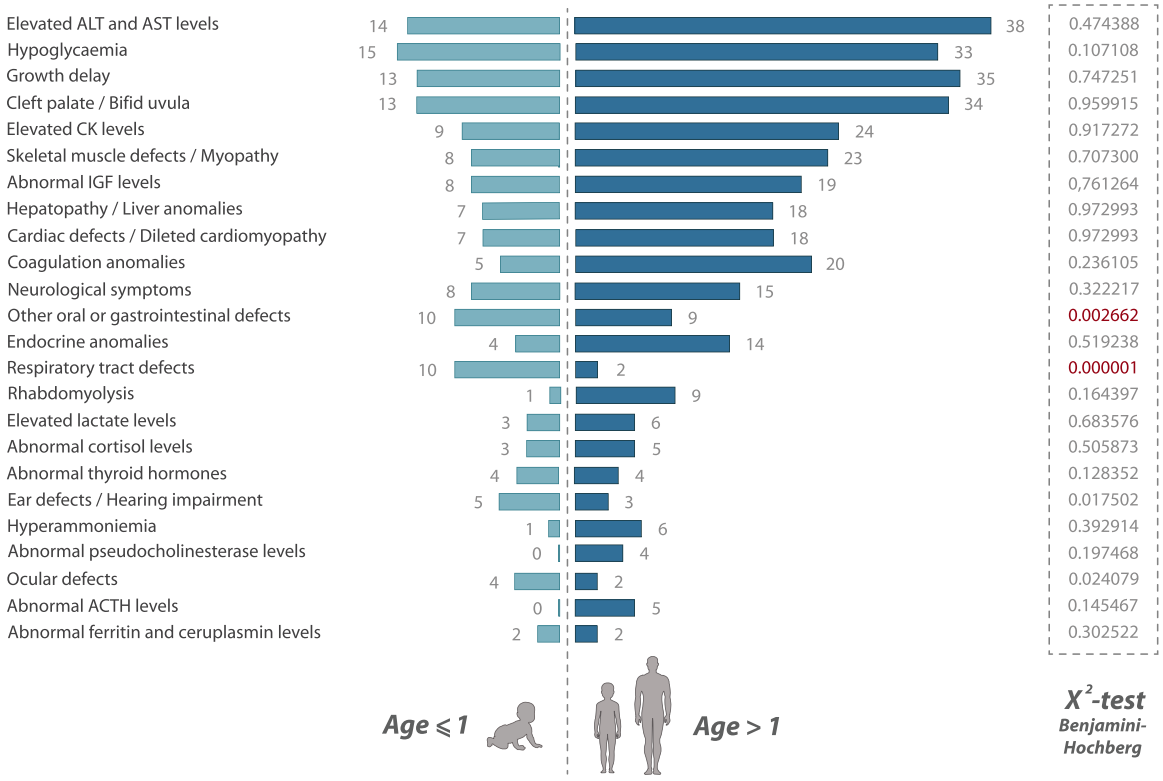

Fig. 3. Summary of the clinical features reported for 54 PGM1 patients.
The plot represents the clinical details reported for the 54 PGM1 patients, which include the ten patients of our cohort and the patients gathered through the systematic review of the literature (October 2019). In figure, the 54 patients have been divided into two groups based on their age: (A) patients who had their first referral to a medical center for PGM1-related symptoms at an age younger than 1 year (14 cases), and (B) patients who had their first referral after 1 year of age (39 cases). On the left, the list of categories in which the symptoms have been sorted as reported in Table S2. Note that the category 'Puberty delay' has not been included in the figure has this symptom is not diagnosable in neonatal and pediatric patients. For each category, the number of patients belonging to group A manifesting that certain symptom is reported in light blue (left bars), while the number of patients belonging to group B is reported in dark blue (right bars). For each category, the results of the Benjamini-Hochberg χ2-test are displayed in the dashed box on the right. The numbers highlighted in red color represent significant or borderline values, indicating symptoms that are diagnosed with a significant or borderline different between group A and group B. Non-significant values are shown in grey color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

namely defective cardiac functionality and DCM (46%), hepatic defects (46%) and myopathy or other muscle symptoms (57%), found in our analysis match or even exceed the frequencies described in previous studies [4–6] (Table S4). Neurological symptoms such as seizures, paralysis, hypotonia, intellectual disability and psychomotor impairment were described in 43% of these patients (Table S4), supporting the recently proposed hypothesis that PGM1-CDG also affects the CNS [11,12].

Out of 54 patients, 15 cases (28%) presented detailed description of the early clinical presentation (before 1 year of age) of PGM1 CDGs (Tables S3, S5). The list of the early symptoms from these 15 cases was compared to symptoms reported in patients older than 1 year of age (39 cases, 72%) to highlight age-dependent differences in the clinical presentation. Respiratory tract symptoms and oral/gastrointestinal defects (other than cleft) were the only symptoms significantly more frequently reported at a juvenile age (Fig. 3, Table S5). No significant age-based differences were reported in the frequency of recognition of the main PGM1-CDG symptoms, such as hypoglycemia and endocrine disfunction. When dividing the cohort by gender (19 females; 35 males), only thyroid hormonal imbalance resulted significantly more frequently reported in female patients (Table S6).

4. Discussion

PGM1 deficiency is a recently described multi-organ CDG characterized by highly heterogeneous clinical presentations, whose pathogenesis is related with the involvement of PGM1 enzyme in glycogen metabolism, glucose homeostasis and protein glycosylation. Dietary supplementation with D-Galactose has been proved to be beneficial, and it has been suggested that the treatment is more successful and long-lasting when administered at a juvenile age [4,6]. In view of the potential inclusion of PGM1 in neonatal screening programs, we here (i) focused on the earliest presenting symptoms (0–6 months of age) and their metabolic management in 11 cases, (ii) investigated differences between early vs. late presentation in a first systematic review, and (iii) assessed the efficacy of the modified Beutler test in detecting PGM1 deficiency in DBSs.

As reported in previous studies on large PGM1-CDG patients, the early presentation in our infantile cohort confirmed the elevated heterogeneity of the symptoms which characterized this CDG. Hypoglycemic episodes and elevated transaminases were confirmed as the most frequent PGM1-CDG symptoms also in our cohort, as present in all eleven patients, followed by cleft palate and growth delay that were instead reported in ten cases. Regarding the most severe symptoms, skeletal muscle symptoms (mainly muscular weakness) were reported in eight patients, although in some cases such symptoms were recognized later in life (after 6 months of age). Only two patients manifested cardiac defects neonatally, although we do not exclude with aging and consequential increase of physical activity some functional anomalies might arise. Lastly, five patients presented delayed motor development, corroborating the previously suggested hypothesis of a CNS involvement in PGM1-CDG [11,12], which appear evident already at a neonatal age.

In the majority of these neonatal patients, the dietary supplementation with D-Galactose ameliorated (or even fully rescued) some of the most frequent symptoms, especially hypoglycemic episodes, liver disease, endocrine dysfunction and growth delay (Fig. 1), confirming what hinted by several studies since 2014 [4,6].

Nevertheless, the effects of D-Galactose supplementation on cardiac and muscular symptoms has yet to be clearly documented. Interestingly, in our cohort three patients manifested different extents of muscle symptom improvement after commencing D-Galactose supplementation. In patient 2, which did not display any severe muscle symptom, galactose treatment helped normalizing the hematic CK levels, suggesting a possible beneficial effect on general muscle metabolism. In patient 3, D-Galactose supplementation rescued an unusual muscle symptom present since 6 years of age, consisting of transient but pain of the lower limbs. This represents the first evidence of direct galactose-induced improvement of muscle symptoms in a PGM1-deficient patient. Patient 5 (first cousin of patient 3) also reported a different muscle symptom that did not occur any longer after starting galactose treatment. While attempting surgical reparation of the cleft palate, this patient manifested a severe post-anesthesia muscular weakness which required a week of artificial ventilation. The patient required several months to recover his muscular functionality and long-term repercussions (e.g. reduced mobility of the right leg, ease to muscle fatigue) persisted. By the time the patient was commenced on galactose supplementation, his muscular condition was fully recover, so a clear effect could not be observed. Nonetheless, when the palatoplasty was reattempted, no extreme muscle weakness occurred and the surgery was successfully performed. Although more subtle, this clinical evidence might also pinpoint towards possible galactose-induced effects on muscular symptoms, which however still require more clinical and molecular investigations.

An early diagnosis however is not only critical to allow a prompt D-Galactose supplementation, but also to avoid the risk of metabolic mismanagement. In our cohort of eleven infantile patients, nine of which were diagnosed and received galactose treatment over six months after birth (Fig. 1), four patients (2, 6, 8 and 11) were taken off galactose containing diets due to suspicion of lactose-intolerance. In all cases the lactose-free regimen worsened some of the symptoms, in particular hypoglycemia and hepatic dysfunction (Fig. 1).

Neonatal diagnosis of PGM1-CDG can be facilitated on one side by raising awareness about the early clinical presentation among the clinical community, and on the other by creating new diagnostic tools applicable perinatally to integrate the traditional diagnostic pipeline.

Regarding the early clinical presentation, we extended our cohort by including other 43 patients gathered via systematic literature review to assess the presence of early presentation-specific differences for PGM1-CDG (Table S3). By comparing early vs. late clinical presentations, no significant differences were identified for the most frequent PGM1-CDG symptoms, such as hypoglycemia, liver dysfunction and cleft palate. Respiratory and oral/gastrointestinal symptoms (other than cleft) resulted to be the only two symptoms significantly more reported at a young age (Fig. 3). Although these two clinical features might result handy to orientate the neonatal diagnosis towards PGM1-CDG when presenting along with the most frequent symptoms, they have not been reported in all infantile cases (10/15) and thus cannot help to recognize all neonatal cases.

Similarly, when grouping the patients by gender, only one symptom show significant gender-based difference: the imbalance of thyroid hormones is more frequently diagnosed in female patients. No significant gender-based differences have been found for the other symptoms, although the rapidly growing number of PGM1-CDG patients diagnosed will help in future to better define their incidence. The prevalences here presented reflect the fact that some clinical features are not yet evident neonatally (e.g. delayed motor development), and thus they are more difficult to identify as early presenting symptoms. Another bias is introduced as a result of the improved knowledge, increased attention and new diagnostic strategies achieved in the recent years for PGM1-CDG, which allow a better and faster diagnosis in currently juvenile patients. Older patients whose clinical details were used to delineate the late presentation of this CDG in our study, have faced a far more elevated risk of misdiagnosis, due to the very recent PGM1-CDG characterization [1,6,7], to its rarity and its symptomatic spectrum overlapping with other disorders [15]. And often for these cases early clinical details are not available for retrospective studies.

In order to prevent serious complications at neonatal age and provide appropriate metabolic management, we further investigated the efficacy of the modified Beutler test [17] in detecting deficient PGM activity in neonatal DBS [34–36]. Similarly to the successful identification of deficient PGM activity in non-neonatal blood spots of PGM1-

CDG patients [6,17], we successfully detected PGM deficiency in DBS of PGM1-CDG patients derived from newborn screening cards (Table 3). This result opens the way to perform larger projects to establish the specificity and sensitivity of this test for implementation of PGM1-CDG in newborn screening programs. When positive, the screening result in combination with the characteristic clinical symptoms (e.g. cleft palate, hypoglycemic episodes, liver dysfunction) will lead to the application of appropriate biochemical and genetic diagnostic tests that will ultimately confirm PGM1-CDG diagnosis perinatally.

5. Conclusion

Although the molecular mechanisms explaining how galactose can improve some of the symptoms are yet to be fully clarified, this case series show the beneficial effects of galactose supplementation on the clinical symptoms in infantile PGM1-CDG patients. Taken together, these clinical evidences further enhance the urge of a rapid neonatal diagnosis for PGM1-CDG to avoid the exacerbation of the symptoms, and to enable a prompt galactose administration to improve the overall medical condition of the patients. The combination of elevated transaminases, growth delay, cleft palate (or bifid uvula) and episodic hypoglycemia should raise a high suspicion for this CDG. However, due to its clinical heterogeneity this is not always obvious, thus a DBS-based newborn screening for PGM1-CDG could play a fundamental role in enabling prompt neonatal diagnosis and immediate D-Galactose-based metabolic management.

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Contributors' statement

- F. Conte: clinical data interpretation; glycomics indexes calculation; systematic review of the literature and statistical analysis; manuscript writing (including figures and tables).
- E. Morava: clinical observation of patients 7, 8 and 9; designed of galactose therapy of patient 1, and 7-9; reviewed and revised the manuscript.
- N. Abu Bakar: glycomics measurement for patients 1, 2, 6 and 8.
- S. B. Wortmann: clinical observation and design of galactose therapy for patient 8, final manuscript revision.
- A. Jonge Poerink: clinical observation of patient 8, interpretation of data, manuscript revision.
- S. Grunewald: clinical observation and follow-up of patient 2, final manuscript revision.
- E. Crushell: clinical observation and follow-up of patient 6, final manuscript revision.
- L. Al-Gazali: clinical observation of patients 3, 4 and 5, final manuscript revision.
- M. de Vries: clinical observation of patient 8 and therapy monitoring; final manuscript revision.
- L. Mørkrid: clinical observation of patient 1, final manuscript revision.
- J. Hertecant: clinical observation of patients 3, 4 and 5; final manuscript revision.
- K.S. Brocke Holmefjord: clinical observation of patient 1; final manuscript revision.
- D. Kronn: clinical observation of patient 10; final manuscript revision.
- A. Feigenbaum: clinical observation of patient 11; final manuscript revision.
- R. Fingerhut: dried blood spot (DBS) analysis for eight (pediatric and adult) PGM1 patients.
- S.Y. Wong: unpublished clinical details of previously reported patients included in the systematic review; final manuscript revision.
- M. van Scherpenzeel: biochemical analysis; final manuscript revision.
- N.C. Voermans: clinical data interpretation; final manuscript revision.
- D.J. Lefeber: designed the study; biochemical data analysis; reviewed and revised the manuscript.

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Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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