International consensus guidelines for phosphoglucomutase 1 deficiency (PGM1-CDG): diagnosis, follow-up and management

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Abstract

Phosphoglucomutase 1 (PGM1) deficiency is a rare genetic disorder that affects glycogen metabolism, glycolysis, as well as one of the most important biological processes namely glycosylation. Previously known as GSD XIV, it was recently re-classified as a congenital disorder of glycosylation, PGM1-CDG. Owing to the important biochemical positioning of the affected enzyme, PGM1-CDG usually manifests as a multisystem disease. Due to the variety of clinical features of the many different CDG, the diagnosis can be difficult. Unlike most other CDG, PGM1-CDG has an effective treatment option, D-galactose, which has shown to improve many of the patients' symptoms. Therefore, early diagnosis and treatment in these patients are crucial. In this paper, our group of international experts suggests diagnostic, follow-up and management guidelines for PGM1-CDG. The guidelines are based on the best available evidence-based data and experts' opinions aiming to provide a practical resource for health care providers to facilitate early diagnosis and appropriate management of PGM1-CDG patients.

Key words

Congenital disorder of glycosylation, Phosphoglucomutase 1 deficiency, PGM1-CDG, Management guidelines.

Abbreviations

PGM1-CDG Phosphoglucomutase 1 deficiency**, CDG** congenital disorders of glycosylation**, GSD** glycogen storage disorder, **SIGN** Scottish Intercollegiate Guidelines Network, **EEG** electroencephalogram**, MRI** magnetic resonance imaging**, CNS** central nervous system**, FSH** follicle-stimulating hormone**, TBG** thyroxin-binding globulin, **IGFBP-3** insulin-like growth factor-binding protein 3, **AST** aspartate aminotransferase, **ALT** alanine aminotransferase**, CK** creatine kinase**, TPCRS** Tulane PGM1-CDG Rating Scale, **EMG** electromyography, **NCS** nerve conduction studies, **Tf** serum transferrin, **TIEF transferrin** isoelectric focusing, **CE** capillary electrophoresis**, HPLC** High-Performance Liquid Chromatography, **LC/MS** Liquid Chromatography /Mass Spectrometry, **ER** endoplasmic reticulum, **GA** Golgi apparatus

General background

PGM1-CDG (OMIM 614921), previously known as congenital disorder of glycosylation 1T (CDG1T) and glycogen storage disorder GSD XIV, is a rare autosomal recessive disorder caused by PGM1 (phosphoglucomutase 1) enzyme deficiency. This disorder was initially reported in two patients with diverse presentations. Thomson 1963[1] reported a child that presented with a cardiac conduction defect and skeletal myopathy. In contrast, Sugie 1988[2] identified it in an infant with recurrent vomiting, failure to thrive and hepatopathy.

PGM1 deficiency is caused by mutations in *PGM1*, which encodes PGM1, the predominant isoform of the phosphoglucomutase family. In most cell types, it represents around 90 % of total PGM activity and is expressed ubiquitously in different tissues. In contrast, PGM1 is absent in red blood cells, where PGM2 is the predominant isoform (Tegtmeyer 2014).

PGM1 catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate, and is involved in several crucial metabolic pathways such as glycolysis, glycogenolysis, glycogenesis and, indirectly, N-linked glycosylation (Quick 1974, Tegtmeyer 2014) [3]. Therefore, it is not surprising that this disorder was first categorized as a GSD (Stojkovic 2009) [4] and only later as a CDG (Timal 2012) [5].

1 Thomson WHS, Maclaurin JC, Prineas JW. Skeletal muscle glycogenosis: an investigation of two similar cases. J Neurol Neurosurg Psychiatry. 1963 Feb; 26:60-8

2 Sugie H, Kobayashi J, Sugie Y, Ichimura M, Miyamoto R, Ito T, Shimizu K, Igarashi Y. Infantile muscle glycogen storage disease: phosphoglucomutase deficiency with decreased muscle and serum carnitine levels. Neurology. 1988 Apr;38(4):602-5.

3 Quick CB, Fisher RA, Harris H. A kinetic study of the isozymes determined by the three human phosphoglucomutase loci PGM1, PGM2, and PGM3. Eur J Biochem. 1974 Mar 1;42(2):511-7. PMID: 4829444

4 Stojkovic T, Vissing J, Petit F, Piraud M, Orngreen MC, Andersen G, Claeys KG, Wary C, Hogrel JY, Laforêt P. Muscle glycogenosis due to phosphoglucomutase 1 deficiency. N Engl J Med. 2009 Jul 23;361(4):425-7

5 Timal S, Hoischen A, Lehle L, Adamowicz M, Huijben K, Sykut-Cegielska J, Paprocka J, Jamroz E, van Spronsen FJ, Körner C, Gilissen C, Rodenburg RJ, Eidhof I, Van den Heuvel L, Thiel C, Wevers RA, Morava E, Veltman J, Lefeber DJ. Gene identification in the congenital disorders of glycosylation type I by whole-exome sequencing.Hum Mol Genet. 2012 Oct 1;21(19):4151-61.

This disorder presents as two major phenotypes: a primary myopathic one and a multisystem one. The latter involves congenital malformations (cleft palate, bifid uvula, cardiac valves malformations, anal atresia, and vertebral anomalies), variable endocrine and hematological abnormalities, cardiac, muscle and hepatic involvement (Morava 2015) [6]. The prevalence of PGM1-CDG is unknown. Fifty seven molecularly confirmed PGM1-CDG patients have been reported from different ethnicities.

PGM1-CDG is one of the few inborn errors of metabolism with an effective treatment in the form of D-galactose (Tegtmeyer 2014). This monosaccharide can restart the glycosylation in PGM1-CDG patients by replenishing the depleted pools of UDP-glucose and UDP-galactose, necessary for the N-glycosylation (Radenkovic 2019), improving several symptoms. Early diagnosis and administration of D-galactose is thus essential for these patients.

To facilitate the diagnosis and management of PGM1-CDG, we offer detailed guidelines specifically focusing on the affected organ systems, diagnostic tools and treatment with D-galactose.

Methodology

An international group of experts in disorders of glycosylation reviewed the evidence base and developed management guidelines. A PubMed database search was performed from the date of the first clinical description till December 2019 using the following terms: carbohydrate-deficient glycoprotein syndrome *OR* congenital disorder of glycosylation type It *OR* PGM1-CDG *OR* phosphoglucomutase 1 deficiency. Neurological, ophthalmologic, cardiac, endocrine, hematological, immunological, gastrointestinal, hepatic, and musculoskeletal systems were reviewed. Next, a consensus was developed in each area of diagnosis, treatment, and management. For the most part, the evidence and resulting recommendations are considered experts' opinions because additional levels of evidence were not available in the literature. Evidence levels

6 Morava E, Wong S, Lefeber D. Disease severity and clinical outcome in phosphosglucomutase deficiency. J Inherit Metab Dis. 2015 Mar;38(2):207-9. doi: 10.1007/s10545-014-9769-5.PMID: 25288126

were classified in accordance with the Scottish Intercollegiate Guidelines Network (SIGN) methodology (http://www.sign.ac.uk) (Supplementary Figure 1).

Results

Thirty five articles were found through PubMed database search:13 case reports, 9 case series, 7 diagnostic papers and 6 reviews. A review of the 57 molecularly confirmed PGM1-CDG patients and affected systems are summarized in supplementary table 1, and the clinical presentations and suggested surveillance are summarized in supplementary table 2.

SYSTEMS SUMMARIES AND STATEMENTS

CONGENITAL MALFORMATIONS

Of 57 patients with PGM1-CDG reported to date, cleft palate was the most commonly reported anomaly (n=28) followed by bifid uvula (n=25), and Pierre-Robin sequence (n=15). The term “first branchial arch syndrome” (Pérez 2013[7]) was also used to describe the findings of a patient later reported as having Pierre-Robin sequence (Wong 2016[8]). In the largest cohort published to date, the presence of either bifid uvula, cleft palate or Pierre-Robin sequence was identified in 23/27 patients (Wong 2016). Other findings occasionally reported include prominent forehead, small face and depressed nasal bridge (Küçükçongar 2015[9]), hypertelorism, short neck, retrognathia, smooth philtrum and low set ears (Zeevaert 2016[10]), dysmorphic ears, preauricular tissue (Schrapers 2016[11]), undescended testes (Nolting 2017[12]), foreshortened esophagus (Preisler 2017[13]), anal atresia, and a missing lumbar vertebra (Wong 2016).

7 Pérez B, Medrano C, Ecay MJ, Ruiz-Sala P, Martínez-Pardo M, Ugarte M, Pérez-Cerdá C. A novel congenital disorder of glycosylation type without central nervous system involvement caused by mutations in the phosphoglucomutase 1 gene.J Inherit Metab Dis. 2013 May;36(3):535-42. doi: 10.1007/s10545-012-9525-7. Epub 2012 Sep 14.

8 Wong SY, Beamer LJ, Gadomski T, Honzik T, Mohamed M, Wortmann SB, Brocke Holmefjord KS, Mork M, Bowling F, Sykut-Cegielska J, Koch D, Ackermann A, Stanley CA, Rymen D, Zeharia A, Al-Sayed M, Marquardt T, Jaeken J, Lefeber D, Conrad DF, Kozicz T, Morava E. Defining the Phenotype and Assessing Severity in Phosphoglucomutase-1 Deficiency.J Pediatr. 2016 Aug; 175:130-136.e8. doi: 10.1016/j.jpeds.2016.04.021. Epub 2016 May 17

9 Küçükçongar A, Tümer L, Ezgü FS, Kasapkara ÇS, Jaeken J, Matthijs G, Rymen D, Dalgiç B, Bıdecı A, Hasanoğlu A. A case with rare type of congenital disorder of glycosylation: PGM1-CDG. Genet Couns. 2015;26(1):87-90

10 Zeevaert R, Scalais E, Muino Mosquera L, De Meirleir L, De Beaufort C, Witsch M, Jaeken J, De Schepper J PGM1 deficiency diagnosed during an endocrine work-up of low IGF-1 mediated growth failure. Acta Clin Belg. 2016 Dec;71(6):435-437. Epub 2016 May 24.

11 Schrapers E, Tegtmeyer LC, Simic-Schleicher G, Debus V, Reunert J, Balbach S, Klingel K, Du Chesne I, Seelhöfer A, Fobker M, Marquardt T, Rust S.News on Clinical Details and Treatment in PGM1-CDG. JIMD Rep. 2016; 26:77-84. doi: 10.1007/8904_2015_471. Epub 2015 Aug 25.

12 Nolting K, Park JH, Tegtmeyer LC, Zühlsdorf A, Grüneberg M, Rust S, Reunert J, Du Chesne I, Debus V, Schulze-Bahr E, Baxter RC, Wada Y, Thiel C, van Schaftingen E, Fingerhut R, Marquardt T. Limitations of galactose therapy in phosphoglucomutase 1 deficiency. Mol Genet Metab Rep. 2017 Jul 31; 13:33-40

13 Preisler N, Cohen J, Vissing CR, Madsen KL, Heinicke K, Sharp LJ, Phillips L, Romain N, Park SY, Newby M, Wyrick P, Mancias P, Galbo H, Vissing J, Haller RG. Impaired glycogen breakdown and synthesis in phosphoglucomutase 1 deficiency. Mol Genet Metab. 2017 Nov;122(3):117-121. doi: 10.1016/j.ymgme.2017.08.007. Epub 2017 Aug 25.

Presentation (statement #1: grade of recommendation C)

PGM1-CDG can present with facial dysmorphic features, including micrognathia or retrognathia, bifid uvula, cleft hard palate, or full Pierre-Robin sequence. The combined frequency of these findings is 85 %.

Diagnosis and follow-up: (Statement #3: grade of recommendation D)

Assessment by a clinical geneticist (evaluating for dysmorphic features) should be done at the time of the diagnosis. If bifid uvula or cleft hard palate is found, evaluation by a craniofacial specialist is warranted. If Pierre-Robin sequence is present, evaluation by an otolaryngologist is warranted, as a tracheostomy may be required to ensure airway patency. Ideally, evaluation should take place at a multidisciplinary craniofacial clinic, with access to specialists in otolaryngology, oral and maxillofacial surgery, plastic surgery, speech-language pathology, and dentistry.

Treatment: (Statement# 4: grade of recommendation D)

There is no disorder-specific management for congenital malformations in PGM1-CDG patients. Standard care for cleft palate and Pierre-Robin sequence should be pursued, such as surgical repair, feeding intervention, and speech therapy.

NEUROLOGICAL INVOLVEMENT

Neurological phenotype is not a frequent presentation of PGM1-CDG. Normal neurodevelopment was stated in 17 PGM1-CDG patients. Motor delay was described in four patients (Ondruskova 2014[14], Nolting 2017, Ding 2018[15], Radenkovic 2019[16]). Cognitive impairments were noted in 14 patients, intellectual disability in 8, learning disabilities in 11 and speech delay in 2 (Ondruskova 2014, Tegtemeyer 2014[17], Wong 2016, Nolting 2017, Ding 2018). IQ was measured only in one patient and showed a global IQ of 78 at 6 years and 66 at 10 years indicating cognitive regression (Ondruskova 2014). In three patients, learning difficulties were stated as probably secondary to recurrent hypoglycemic episodes (Loewenthal 2015[18]). Epileptic seizures have been reported in three patients with developmental delay, and abnormal EEG was detected in one patient (Wong 2016, Ding 2018, Radenkovic 2019). No perinatal injuries or other plausible causes were reported. Brain MRI was normal in five patients and a thin pituitary was reported in one patient (Ding 2018). Other scarce neurological phenotypes included left hemiplegia due to carotid thrombosis in one patient (Zeevaert 2016), and sensorineural deafness in another patient

14 Ondruskova N, Honzik T, Vondrackova A, Tesarova M, Zeman J, Hansikova H. Glycogen storage disease-like phenotype with central nervous system involvement in a PGM1-CDG patient. Neuro Endocrinol Lett. 2014;35(2):137-41.

15 Ding Y, Li N, Chang G, Li J, Yao R, Shen Y, Wang J, Huang X, Wang X.Clinical and molecular genetic characterization of two patients with mutations in the phosphoglucomutase 1 (PGM1) gene.J Pediatr Endocrinol Metab. 2018 Jul 26;31(7):781-788. doi: 10.1515/jpem-2017-0551

16 Radenkovic S, Bird MJ, Emmerzaal TL, Wong SY, Felgueira C, Stiers KM, Sabbagh L, Himmelreich N, Poschet G, Windmolders P, Verheijen J, Witters P, Altassan R, Honzik T, Eminoglu TF, James PM, Edmondson AC, Hertecant J, Kozicz T, Thiel C, Vermeersch P, Cassiman D, Beamer L, Morava E, Ghesquière B. The Metabolic Map into the Pathomechanism and Treatment of PGM1-CDG. Am J Hum Genet. 2019 May 2;104(5):835-846.

17 Tegtmeyer LC, Rust S, van Scherpenzeel M, Ng BG, Losfeld ME, Timal S, Raymond K, He P, Ichikawa M, Veltman J, Huijben K, Shin YS, Sharma V, Adamowicz M, Lammens M, Reunert J, Witten A, Schrapers E, Matthijs G, Jaeken J, Rymen D, Stojkovic T, Laforêt P, Petit F, Aumaître O, Czarnowska E, Piraud M, Podskarbi T, Stanley CA, Matalon R, Burda P, Seyyedi S, Debus V, Socha P, Sykut-Cegielska J, van Spronsen F, de Meirleir L, Vajro P, DeClue T, Ficicioglu C, Wada Y, Wevers RA, Vanderschaeghe D, Callewaert N, Fingerhut R, van Schaftingen E, Freeze HH, Morava E, Lefeber DJ, Marquardt T. Multiple phenotypes in phosphoglucomutase 1 deficiency.N Engl J Med. 2014 Feb 6;370(6):533-42. doi:10.1056/NEJMoa1206605.

18 Loewenthal N, Haim A, Parvari R, Hershkovitz E. Phosphoglucomutase-1 deficiency: Intrafamilial clinical variability and common secondary adrenal insufficiency. Am J Med Genet A. 2015 Dec;167A (12):3139-43. doi: 10.1002/ajmg.a.37294. Epub 2015 Aug 19

(Nolting 2017). It is difficult to assess the impact of D-galactose supplementation in preventing CNS involvement in patients with PGM1-CDG.

Presentation (statement #1: grade of recommendation C)

Cognition, motor development, speech, and hearing can be affected in multisystem PGM1-CDG. Seizures can be primary or secondary to hypoglycemia .

Diagnosis and follow-up (Statement# 2: grade of recommendation D)

Neurological assessment should be done at the time of the diagnosis. EEG and brain MRI should be considered if clinically indicated.

Follow-up investigations should include early stimulation programs for the infants and toddlers, and psychometric tests and adaptations of educational programs when needed.

Treatment (Statement# 3: grade of recommendation D)

No specific treatment is available for the psychomotor delay and/or learning disabilities in PGM1-CDG besides early physical and speech therapy. Although the relationship between hypoglycemia and CNS involvement is not clear, diet or treatments to prevent CNS damage secondary to hypoglycemia are suggested.

OPHTHALMOLOGIC INVOLVEMENT

Ophthalmologic involvement is only reported with the multisystem PGM1-CDG. It has been reported in 6 patients. The most commonly reported ophthalmologic abnormalities were unspecified abnormal eye movements (n=2) (Wong 2016, Radenkovic 2018[19]) and strabismus (n=2) (Wong 2016). Less common findings were reading problems (n=1) (Ondruskova 2014), nasolacrimal duct obstruction and epiphoria (n=1) (Küçükçongar 2015). These results may be underestimated due to scarce data available with respect to ophthalmologic examination, management, and outcome.

Presentation (statement #1; grade of recommendation C)

PGM1-CDG can present with the following eye abnormalities; strabismus, abnormal eye movements, reading problems, nasolacrimal duct obstruction and/ or epiphoria.

Diagnosis and follow-up (statement #2; grade of recommendation C)

Ophthalmologic assessment is recommended standard of care of PGM1-CDG.

Management (statement #3; grade of recommendation C)

Ophthalmologic abnormalities appear to be rare and should be treated individually, including surgery, if necessary. Standard supportive treatment (e.g. glasses or orthoptic treatment) is recommended for the strabismus.

19 Radenkovic S, Witters P, Morava E. Central nervous involvement is common in PGM1-CDG.Mol Genet Metab. 2018 Nov;125(3):200-204. doi: 10.1016/j.ymgme.2018.08.008. Epub 2018 Aug 2

ENDOCRINE INVOLVEMENT

Endocrine and growth problems are common in PGM1-CDG, reported in 39 patients. Hypoglycemia was the main presentation, reported in 38 patients and mostly due to hyperinsulinism in young patients and responding to diazoxide (Wong 2017, Ding 2018, Radenkovic 2018, Zeevaert, 2016). Ketotic hypoglycemic episodes secondary to starvation and/or febrile events have also been reported (Loewenthal 2015, Radenkovic 2018) which suggests the complexity of the hypoglycemia origins at different ages (Morava 2014[20]). Hypoglycemic episodes can spontaneously resolve later in life (Loewenthal 2015). Oral administration of uncooked corn starch before bedtime was successful to prevent the hypoglycemia in one patient (Yokoi 2019[21]), while the administration of hydrocortisone (12 mg/m2/day) reduced hypoglycemic events in another patient (Loewenthal 2015). Hypogonadotropic hypogonadism was reported in 3 patients (Tegtemeyer 2014, Zeevaert 2016); two of them presented with delayed puberty and decreased serum follicle-stimulating hormone (FSH). In both patients, D-galactose supplementation (0.5 to 1.0 g/kg/day) resolved both features (Tegtemeyer 2014). Adrenal function deterioration was reported in 3 patients (Loewenthal 2015). High levels of serum thyroid-stimulating hormone (TSH) were reported in 3 patients and decreased levels of thyroxin-binding globulin (TBG) in 6 (Ondruskova 2014, Küçükçongar 2015, Wong 2017). Moreover, 7 patients presented with hypocortisolism and 5 of them had increased adrenocorticotropic hormone levels (Küçükçongar 2015, Lowenthal 2015, Zeevaert 2016, Ding 2018). Twelve patients presented with decreased levels of growth hormone or IGF1 (Pérez 2012, Küçükçongar 2015, Loewenthal 2015, Schrapers 2016, Zeevaert 2016, Nolting 2017, Ding 2018, Ferlund et al 2019) and nine patients showed decreased serum insulin-like growth factor-binding protein 3 (IGFBP-3) (Wong 2017, Ferlund et al 2019). Growth hormone therapy has been described in only 3 patients (Zeevaert 2017,

20 Morava E.Galactose supplementation in phosphoglucomutase-1 deficiency; review and outlook for a novel treatable CDG.Mol Genet Metab. 2014 Aug;112(4):275-9. doi: 10.1016/j.ymgme.2014.06.002. Epub 2014 Jun 21. Review

21 Yokoi K, Nakajima Y, Ohye T, Inagaki H, Wada Y, Fukuda T, Sugie H, Yuasa I, Ito T, Kurahashi H. Disruption of the Responsible Gene in a Phosphoglucomutase 1 Deficiency Patient by Homozygous Chromosomal Inversion.JIMD Rep. 2019;43:85-90. doi: 10.1007/8904_2018_108. Epub 2018 May 12.

Nolting 2017, Medrano 2019[22]). Furthermore, Nolting (2017) reported a patient whose growth rate did not improve upon treatment with D-galactose alone or in combination with uridine.

Presentations (statement #1: grade of recommendation C)

PGM1-CDG frequently presents with endocrine and growth problems, particularly, hyperinsulinemic hypoglycemia, hypogonadotropic hypogonadism, hypocortisolism, and delayed puberty.

Diagnosis and follow-up: (Statement# 2: grade of recommendation D)

The growth of PMG1-CDG patients as well as the serum levels of IGF-1, IGFBP3, TGB, TSH, free T4, ACTH, cortisol, and glucose should be assessed at the initial screening and regularly monitored.

In addition, serum cortisol and ACTH should be assessed at the time of diagnosis and monitored as clinically indicated.

Treatment: (Statement# 3: grade of recommendation D)

There is no disorder-specific management for endocrine dysfunction in PGM1-CDG patients, although D-galactose supplementation can be used for its management.

Hypoglycemia can be managed with the oral administration of uncooked corn starch before bedtime, in addition to frequent feedings and the use of complex carbohydrates.

Oral diazoxide therapy should be considered for hyperinsulinemic hypoglycemia.

Therapy with L-thyroxin is indicated for clinical hypothyroidism treatment.

Cortisol supplements are recommended to treat hypocortisolism.

22Medrano C, Vega A, Navarrete R, Ecay MJ, Calvo R, Pascual SI, Ruiz-Pons M, Toledo L, García-Jiménez I, Arroyo I, Campo A, Couce ML, Domingo-Jiménez MR, García-Silva MT, González-Gutiérrez-Solana L, Hierro L, Martín-Hernández E, Martínez-Pardo M, Roldán S, Tomás M, Cabrera JC, Mártinez-Bugallo F, Martín-Viota L, Vitoria-Miñana I, Lefeber DJ, Girós ML, Serrano Gimare M, Ugarte M, Pérez B, Pérez-Cerdá C. Clinical and molecular diagnosis of non-phosphomannomutase 2 N-linked congenital disorders of glycosylation in Spain. Clin Genet. 2019 May;95(5):615-626. doi: 10.1111/cge.13508. Epub 2019 Apr 3.

Growth impairment can be managed with growth hormone therapy, although not all patients respond to this treatment.

CARDIAC INVOLVEMENT

Twenty-four PGM1-CDG patients had cardiac manifestations as part of the multisystem involvement. .

The main cardiac abnormality was cardiomyopathy (CM) with dilated cardiomyopathy as the most common type (n=12) (Tegtmeyer 2014, Timal 2014, Loewenthal 2015, Zeevaert 2016, Wong et al 2016, Voermans 2017[23], Nolting 2017, Fernlund 2019[24]). CM resulted in cardiac arrest in 8 patients, and heart failure in 4 patients. There is unpublished data about one patient who initially had restrictive cardiomyopathy, which evolved into combined dilated restrictive cardiomyopathy with predominant restrictive findings (personal communication). Electrocardiogram (ECG) abnormalities were stated in five patients including long QT interval, ST wave elevation, T wave inversion, sinus tachycardia, and minor incomplete intraventricular conduction disturbance. Other echocardiogram abnormalities include mild left ventricular enlargement (n=3), septal defects (n=2) and valvular heart defects (n=2). Cardiac manifestation in PGM1-CDG might be underreported due to the absence of screening for cardiac involvement before the first presentation of clinical features. The documented age of onset for cardiac findings was variable: within the first 5 years (n=5), between 5 to 10 years (n=2) and after 10 years (n=6). The oldest age of onset was 49 years. There was limited information on the evolution of the cardiac manifestations with time but one patient was reported to have a normal cardiac function at 19 months of age but developed cardiomyopathy on follow-up. Six PGM1-CDG patients died from cardiac causes (Tegtmeyer 2014, Timal 2014, Loewenthal 2015, Zeevaert 2016). Sudden cardiac death in 12 and 13-year-old siblings diagnosed with PGM1-CDG was recently reported (Fernlund 2019).

23 Voermans NC, Preisler N, Madsen KL, Janssen MC, Kusters B, Abu Bakar N, Conte F, Lamberti VM, Nusman F, van Engelen BG, van Scherpenzeel M, Vissing J, Lefeber DJ. PGM1 deficiency: Substrate use during exercise and effect of treatment with galactose. Neuromuscul Disord. 2017 Apr;27(4):370-376. doi: 10.1016/j.nmd.2017.01.014. Epub 2017 Jan 19.

24 Fernlund E, Andersson O, Ellegård R, Årstrand HK, Green H, Olsson H, Gunnarsson C. The congenital disorder of glycosylation in PGM1 (PGM1-CDG) can cause severe cardiomyopathy andunexpected sudden cardiac death in childhood.Forensic Sci Int Genet. 2019 Nov; 43:102111. doi: 10.1016/j.fsigen.2019.06.012. Epub 2019 Jun 17.

Presentation (Statement #1: grade of recommendation C)

Cardiac involvement is mostly part of multisystem PGM1-CDG with variable age of presentation and onset. The most common cardiac feature is cardiomyopathy, specifically dilated cardiomyopathy. Conduction and structural heart abnormalities are less common.

Diagnosis andfFollow-up (statement #2: grade of recommendation D)

Cardiac assessment and baseline investigations should be done at the time of diagnosis and include creatine kinase MM (CK-MM), chest X-ray, echocardiogram and electrocardiogram (ECG). Further cardiac evaluation i.e. Holter monitor and exercise testing, should be done if clinically indicated.

Annual cardiac screening is recommended, especially in childhood and adolescence. Continued cardiology screening in affected adults should be considered.

Treatment (statement #3: grade of recommendation D)

There is no specific treatment for cardiac involvement in PGM1-CDG. Treatment of cardiac manifestations is based on current clinical practice and guidelines.

RESPIRATORY SYMPTOMS

Overt respiratory symptoms are rare manifestations of PGM1-CDG, reported in 8 patients due to congenital facial malformations, underlying heart disease, or exercise intolerance (in the muscular form) (Timal 2012, Tegtmeyer 2014, Lowenthal 2015, Schrapers 2016, Voermans 2017, Tian 2019[25]).

Upper airway obstruction (requiring a tracheostomy and placement of a tracheal tube) occurred in the neonatal period in one patient with Pierre Robin sequence (Lowenthal 2015). Two adult PGM1-CDG patients experienced breathing difficulties during physical exercise. In one patient, physical training led to considerable improvement, whereas galactose administration was reported to ameliorate the clinical status of the other patient (Schrapers 2016, Voermans 2017). Both patients showed mild to moderate cardiac involvement.

Surgical correction of congenital facial malformations could lead to severe bleeding episodes and needed repeated surgery in several patients (Wong, 2017). I would like to add how many patients had tracheostomy and how long. I know of two patients, one is already de-trached (Dr.Morava's comment)

Presentations (statement #1: grade of recommendation C)

PGM1-CDG seldom presents with overt respiratory symptoms. Breathing difficulties tend to occur when there are underlying congenital malformations (e.g. Pierre Robin sequence), severe cardiac dysfunction, especially and during intense physical exercise. Hence, respiratory involvement seems to be secondary to other clinical features.

Diagnosis and follow-up: (Statement# 2: grade of recommendation D)

The following investigations should be done at the time of the diagnosis: pulse oximetry, and more extensive exams upon clinical suspicion. In the

25 Tian WT, Luan XH, Zhou HY, Zhang C, Huang XJ, Liu XL, Chen SD, Tang HD, Cao L. Congenital disorder of glycosylation type 1T with a novel truncated homozygous mutation in PGM1 gene and literature review. Neuromuscul Disord. 2019 Apr;29(4):282-289. doi: 10.1016/j.nmd.2019.01.001. Epub 2019 Jan 6.

presence of congenital facial malformation, one should be wary of breathing difficulties. Surgical procedures such as tracheostomy and permanent correction of the malformations may be necessary.

Treatment: (Statement# 3: grade of recommendation D)
There is no specific treatment for respiratory symptoms in PGM1-CDG. Treatment of these manifestations is based on current clinical practice and guidelines. Early surgical correction of midline malformations is recommended.

GASTROINTESTINAL INVOLVEMENT

Hard and/or soft cleft palate (with or without bifid uvula) and/or without Robin-Pierre sequence, were the most frequently presented congenital anomalies of the gastrointestinal (GI) tract, (refer to congenital malformation section). Feeding difficulties were reported in only 5 patients; one patient with dysphagia did not have cleft palate (Voermans 2016). One patient had an imperforate anus (Zeevaert 2016). One obese patient had cholecystolithiasis (Ondruskova 2014).

Presentation (statement #1: grade of recommendation D)

Gastrointestinal symptoms are not characteristic of PGM1-CDG patients while bifid uvula/cleft palate are frequent in the multisystem form.

Diagnosis and follow-up (statement #2: grade of recommendation D)

The diagnosis of cleft palate and Robin-Pierre sequence is clinical. An ultrasound scan of the abdomen is advised to exclude other possible congenital anomalies of the GI tract.

Referrals to an oral and maxillofacial surgeon, nutritionist and speech therapist are recommended for the patients with cleft palate.

Treatment (statement #3: grade of recommendation D)

Treatment of the cleft palate should be guided by the oral and maxillofacial surgeons based on current practice and expertise. Patients with cleft palate should be followed up for feeding difficulties and possible need for feeding assistance.

LIVER INVOLVEMENT

Liver involvement has not been described in patients with the muscular form (Stojkovic 2009, Voermans 2017). On the other hand, all patients with the multisystem form presented with intermittent or chronically elevated serum transaminases (Pérez 2012, Timal 2012, Preisler 2013[26], Ondruskova 2014, Tegtmeyer 2014, Küçükçongar 2015, Loewenthal 2015, Schrapers 2016, Wong 2016, Zeevaert 2016, Nolting 2017, Preisler 2017, Wong 2017, Ding 2018, Yokoi 2018, Medrano 2019, Tian 2019, Radenkovic 2019). A positive effect of D-galactose treatment was observed, with an improvement of serum aspartate aminotransferase (AST) values and a normalization of alanine aminotransferase (ALT) values (Wong 2017, Radenkovic 2019). Hepatomegaly was only described in 2 patients but could be underreported (Ondruskova 2014, Küçükçongar 2015). Liver biopsy, performed in 5 patients, showed steatosis, cholestasis and/or slight fibrosis (Tegtmeyer 2014). Cirrhosis has not (yet) been reported. Glycogen accumulation, defined by increased PAS-positive staining, was observed in two out of five liver biopsies (Tegtmeyer 2014). There is insufficient data about the evolution of liver involvement in PGM1-CDG. Episodes of acute hepatic failure were described in 5 patients, however, without details concerning the circumstances or the extent of presentation (Wong 2016). The occurrence of acute liver failure did not correlate with the overall severity of the phenotype (Wong 2016).

Presentation (statement #1: grade of recommendation C)

Elevated serum transaminases are the most common biochemical finding of liver involvement in PGM1-CDG. In addition, steatosis, cholestasis, fibrosis, and episodes of acute hepatic failure may occur.

Diagnosis and follow-up (statement #1: grade of recommendation D)

26 Preisler N, Laforêt P, Echaniz-Laguna A, Ørngreen MC, Lonsdorfer-Wolf E, Doutreleau S, Geny B, Stojkovic T, Piraud M, Petit FM, Vissing J. Fat and carbohydrate metabolism during exercise in phosphoglucomutase type 1 deficiency. J Clin Endocrinol Metab. 2013 Jul;98(7):E1235-40. doi: 10.1210/jc.2013-1651. Epub 2013 Jun 18.

Transaminases are the best markers to follow the disease progression and to use as treatment and compliance indicators. CDG screening tests should be considered in the instance of unexplained liver disease.

Liver tests and biochemical markers of the liver's synthetic capacity should be monitored yearly. Liver ultrasound should be performed at the time of diagnosis. Non-invasive elastography can be used to monitor the development of liver fibrosis.

Treatment (statement #1: grade of recommendation D)

D-Galactose treatment positively influences the transaminase levels in PGM1-CDG. Hepatotoxic medication should be avoided.

HEMATOLOGICAL/VASCULAR INVOLVEMENT

Twenty-nine reported PGM1-CDG patients presented hematological/vascular anomalies. The coagulation anomalies included both procoagulant and anticoagulant factors. The most common abnormality was antithrombin III deficiency, present in 14 patients, whilst prolonged aPTT and elevated PT were found in 5 and two patients, respectively. Low factor XI was reported in 6 patients and low factor VII, IX, X, and XIII in two patients. Additionally, reduced levels of protein S and C were described in three patients.

Isolated or recurrent thrombotic events were reported in 5 % of the patients. A cerebrovascular accident was the cause of death in an 8-year-old patient who had left carotid artery thrombosis after an episode of transient hemiplegia at the age of 4 (Timal 2012, Zeevart 2016). In another patient, recurrent thrombosis developed after resuscitation from a cardiac arrest (Tegtmeyer 2014).

Specific treatment for coagulation issues in PGM1-CDG patients was not reported. However, supplementation with D-galactose was found to improve antithrombin III levels in 5 patients (Wong 2017, Radenkovic 2019).

Presentations (statement #1: grade of recommendation C)

The main coagulation abnormality observed in PGM1-CDG is antithrombin III deficiency. PGM1-CDG can also be associated with factor VII, IX, X, XIand XIII deficiency, reduced protein C and S as well as increased PT and prolonged aPTT. Since procoagulant and anticoagulant factors can be affected, patients are susceptible to thrombotic and hemorrhagic events.

Diagnosis and follow-up: (Statement #3: grade of recommendation D)

At diagnosis, a complete blood count and hemostasis study should be performed, including PT and aPTT, antithrombin III, factors VII, IX, X and XIII, as well as protein C and S. Coagulation assessment should be done at least yearly.

Treatment: (Statement #4: grade of recommendation D)

There is no standard disease-specific treatment for coagulation anomalies in PGM1-CDG. Management procedures should be done according to

standard protocols considering the clinical status and history of the patient. D-galactose treatment improves antithrombin III levels in some patients.

MUSCULAR INVOLVEMENT

Muscular involvement is a predominant feature of PGM1-CDG and can be similar to that of muscle glycogenosis with exercise intolerance, fatigability, muscle weakness and attacks of rhabdomyolysis (Stojkovic 2009). Muscular manifestation can be the only symptom of the disease. The primary muscular form can be related to specific *PGM1* mutations (Wong 2016, March 1993). The degree of muscular involvement can be an important predictor of disease severity with more significant myopathy reported in severe phenotypes (Beamer 2015[27], Wong 2016, Radenkovic 2019). We reviewed data on 52 out of the 57 patients where the muscular symptoms were described in details. Any patients with only muscle symptoms?

Exercise intolerance was reported in 20 patients (Sugie 1988, Gehrmann 2003[28], Stojkovic 2009, Timal 2012, Tegtmeyer 2014, Ondruskova 2014, Voermans 2015, Wong 2016, Zeevaert 2016, Wong 2017, Radenkovic 2019). A second wind phenomenon, patient's better tolerance for aerobic exercise such as walking and cycling after approximately 10 minutes of rest, was explicitly reported as absent in the patients reported by Stojkovic 2009, Preisler 2013 and Tegtmeyer, 2014, and present in the patient reported by Wong 2016. Cramps provoked by exercise were reported in one patient (Stojkovic 2009).

Muscle weakness was reported in 18 patients, with very limited information on the pattern of muscle weakness. Only three patients were reported to have more weakness in the pelvic girdle and/or legs than in the upper extremities (Stojkovic 2009, Notting 2017, Tian 2019,). In one report, muscle weakness was proximal (Voermans 2017).

27 Beamer LJ. Mutations in hereditary phosphoglucomutase 1 deficiency map to key regions of enzyme structure and function.J Inherit Metab Dis. 2015 Mar;38(2):243-56. doi: 10.1007/s10545-014-9757-9. Epub 2014 Aug 29. Review.

28 Gehrmann J, Sohlbach K, Linnebank M, Böhles HJ, Buderus S, Kehl HG, Vogt J, Harms E, Marquardt T. Cardiol Young.Cardiomyopathy in congenital disorders of glycosylation. 2003 Aug;13(4):345-51. Review.

Hypotonia was mentioned in only three patients (Sugie 1988, Ondruskova 2014, Zeevaert 2016). Episodes of generalized hypotonia and hyporeflexia during episodes of metabolic decompensation were described in one patient (Sugie 1988).

Myopathic gait and muscle wasting was described in only 2 patients who had predominant muscle symptomatology (Thompson 1963, Tian 2019). Abnormal toe gait from the age of 2.5 years was the major complaint of the first patient who also had muscular wasting but no exercise intolerance nor muscular pain (Thompson 1963). The second one presented with life-long muscle weakness, fatigability, and slow myopathic gait; muscle wasting was not mentioned (Tian 2019).

Attacks of rhabdomyolysis were studied in 43 patients and occurred in 25 % of patients (11/43). Patients suffered most often from moderate attacks with levels of creatine kinase (CK) from 10 000 to 50 000 IU/l(Tegtmeyer 2014, Wong 2017). Severe rhabdomyolysis with renal failure was not described in any patient. The age of rhabdomyolysis was seldom mentioned; it occurred from infancy to adulthood. In one patient the first and only attack happened at 52 years (Voermans 2017).

Chronic CK elevation was present in 65 % of patients (13/20) and in almost half of them (6/13) it was without any history of rhabdomyolysis. The maximum CK levels in those patients were 1.5 – 13 fold the upper normal limit (i.e. 300 - 2600 IU/l). Serum myoglobin was reported only in 4 patients (Marquardt 2014, Ondruskova 2014, Wong 2016, Wong 2017, Tian 2019) with normal levels. Other laboratory markers of muscle damage were not mentioned.

Muscular involvement is part of the Tulane PGM1-CDG Rating Scale (TPCRS) (Wong 2016). The exact muscular subscore was mentioned in only 12 out of 52 patients, but it can be partially derived from the information in the articles in 46/52 patients. Moderate degree of myopathy (i.e. 2 TPCRS points) with generalized weakness, rhabdomyolysis, and/or exercise-related pain was stated in 11 patients. Mild involvement with localized muscle weakness, and/or exercise intolerance (i.e. 1 TPCRS point) was present in 14 patients. Normal muscle tone was reported in 21 patients.

None of the patients was wheelchair-dependent or had respiratory compromise due to the myopathy.

Clinical neurophysiological studies (electromyography (EMG), nerve conduction studies (NCS) were reported only in five patients. The EMG in two patients showed a myopathic pattern (Tian 2019, Stojkovic 2009). In another patient, EMG showed the myopathic pattern only during voluntary contraction consisting of polyphasic low-voltage potentials of short duration (quadriceps, gastrocnemii) (Thompson 1968). Normal nerve conduction studies and EMG were stated in two patients (Wong 2016, Zeevaert 2016).

Muscle biopsy was performed in 12 patients; 10 biopsies were abnormal, showing myopathic changes (increase of internal nuclei or fiber size variation) and/or accumulation of fat or glycogen (Thompson 1963, Sugie 1988, Stojkovic 2009, Timal 2012, Tegtmeyer 2014, Morava 2014, Tian 2019) The fat accumulation, indicative for glycogen storage disease, was reported in 6 of these ten patients. In two patients, the biopsy was normal (Pérez 2013.Wong 2016)

Twenty patients were treated with oral D-galactose in variable doses (0.5 – 2.5 g/kg/day). The duration varied between 4.5 and 16.5 months. Six patients reported a positive effect on exercise intolerance and fatigability, and in one the effect was not clear. The number of rhabdomyolysis episodes did not decrease in any of the questioned 14 patients. CK elevation decreased after initiation of D-galactose (Wong 2016, Radenkovic 2019).

Presentation (Statement #1: grade of recommendation C)

Muscular involvement is one of the predominant features of PGM1-CDG. The main skeletal muscle manifestations are exercise intolerance, fatigability, muscle weakness and attack(s) of rhabdomyolysis. The disease can progress to significant generalized myopathy in about half of the patients with skeletal muscle involvement.

Diagnosis and follow-up (Statement# 3: grade of recommendation D)

Initial and annual neurological evaluation is recommended. According to the disease progression, neurophysiological studies should be performed.

Muscle biopsy investigation is not required to diagnose PGM1-CDG and the invasiveness of this procedure limits its indication to research purposes. Because of the risk of marked myoglobinuria, serum creatine kinase and myoglobin level determination are warranted during any acute intercurrent infections and each time when the patient complains about sudden episodes of myoglobinuria, muscle pain, swelling and /or weakness. In case of rhabdomyolysis, treatment should be focused at prevention of kidney failure and metabolic abnormalities.

Treatment: (Statement# 4: grade of recommendation D)

Dietary D-galactose supplementation should be considered in all patients since a positive effect on exercise intolerance, fatigability and reduction of creatine kinase elevation has been observed.

MALIGNANT HYPERTHERMIA SUSCEPTIBILITY

There is limited information in the literature about surgeries and anesthetic agents used in PGM1-CDG patients. The most common reported surgery is cleft palate repair. Malignant hyperthermia was reported in only 2 patients of 22 operated PGM1-CDG patients (one of whom had a history of exercise intolerance, and rhabdomyolysis was reported in both patients (Tegtmeyer 2014, Marquardt 2014). Detailed anesthetic information, including anesthetic agents used for surgery, was only reported in one patient (Marquardt 2014). Halothane was the anesthetic agent used for cleft palate repair and it was associated with malignant hyperthermia. The diagnosis was based on the clinical grading scale previously reported by Larach 1994[29]. Propofol and remifentanyl were reported to cause minor rise in CK in the same patient (Marquardt 2014). Pseudocholinesterase activity, when measured, was reported to be low and this was associated with risk of prolonged recovery from anesthetic agents in children (Tegtmeyer 2014, Soliday 2010[30]).

Presentation (Statement #1: grade of recommendation D)

Malignant hyperthermia was reported in PGM1-CDG in association with the use of Halothane

Diagnosis and follow-up (statement #2: grade of recommendation D)

Malignant hyperthermia may be a risk, particularly in PGM1 patients with skeletal myopathy and rhabdomyolysis. Pre-anesthetic evaluation is strongly recommended for PGM1 patients undergoing surgery. Pseudocholinesterase activity can be measured in patients with PGM1-CDG.

Treatment (Statement #2: grade of recommendations D)

29 Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert GA, Kaplan RF, Muldoon SM, Nelson TE, Ording H. A clinical grading scale to predict malignant hyperthermia susceptibility. Anesthesiology. 1994 Apr;80(4):771-9

30 Soliday FK, Conley YP, Henker R. Pseudocholinesterase deficiency: a comprehensive review of genetic, acquired, and drug influences. AANA J. 2010 Aug;78(4):313-20

Caution should be exercised with the use of anesthetic agents, particularly depolarizing muscle relaxants and volatile anesthetic agents in patients with PGM1-CDG.

ADULT PRESENTATION

There are 15 adult patients with PGM1-CDG described in the medical literature, including one patient who was described as a 13 year-old and subsequently as a 32 year adult (Pérez 2012, Medrano 2019). PGM1-CDG patients can live into adulthood but frequently present to medical care in infancy with classic presentations of PGM1-CDG, including congenital anomalies, hypoglycemia, hepatopathy, and cardiomyopathy. Some adult patients had mild presentations that delayed diagnosis, including recurrent exercise-induced myopathy with and without rhabdomyolysis, muscular fatigue, and short stature. Severe symptoms can persist into adulthood, including hypoglycemia, cardiomyopathy, and hepatopathy.

Presentation (Statement #1: grade of recommendation D)

Adult patients presenting with recurrent myopathy or rhabdomyolysis should be evaluated for PGM1-CDG.

Diagnosis and Follow up (Statement# 3: grade of recommendation D)

Adult PGM1-CDG patients should continue to have ongoing health screening at least annually.

Treatment: (Statement# 4: grade of recommendation D)

Treatment of adult presentation is based on the standard of care of adults.

DIAGNOSTIC TOOLS

1) BIOCHEMICAL DIAGNOSIS

1.1 Transferrin and N-glycan analysis

The serum transferrin (Tf) analysis by isoelectric focusing (TIEF), capillary electrophoresis (CE), High-Performance Liquid Chromatography (HPLC), or by Liquid Chromatography coupled with Mass Spectrometry (LC/MS) has been used in diagnostic testing of different GDG. (supplementary table 3)

All the above-mentioned methods are based on monitoring glycosylation changes in serum Tf. This protein is abundant in serum and has two N-linked glycan attached to the Tf protein. The most abundant Tf species in healthy controls is Tf which bears two complete oligosaccharide chains (tetrasialotransferrin), which is represented by a major band on IEF gel. CDG-I presents with increased transferrin missing both or one oligosaccharide chain represented by two additional IEF bands (asialotransferrin, disialotransferrin) consistent with missing glycan chains (type 1 pattern), while CDG-II has transferrin with truncated oligosaccharide chains represented by more IEF bands (asialo, monosialo, disialo, trisialo, tetrasialotransferrin)(type 2 pattern).

The majority of reported PGM1-CDG patients presented with a mixed TIEF-1/2 pattern, a phenomenon so far seen only in PGM1-CDG. This profile suggests that this disorder affects both ER (CDG-I) and Golgi apparatus (CDG-II)-associated glycosylation. This has partially been explained by the depletion of UDP-glucose and UDP-galactose in PGM1-CDG patients. These sugar nucleotides are essential glycan building blocks involved in ER and GA glycosylation processes respectively (Radenkovic 2019).

The most commonly used method in the diagnosis of CDG is TIEF; it has been performed in more than 81 % of the reported PGM1-CDG patients (Perez 2012, Timal, 2012, Ondruskova 2014, Socha 2014, Tegtmeyer 2014, Kucukcongar 2015, Lowenthal 2015, Schrapers 2015, Zeevaert 2016, Nolting 2017, Preisler 2017, Wong 2017, Wong 2017, Abu Bakar 2018, Yokoi 2018)

TIEF is a fast and easily applicable laboratory technique in clinical settings. However, it is not the ideal method, as the results are not always conclusive and often fluctuate, which is particularly common in the first months of life. Moreover, abnormal TIEF patterns sometimes resolve on growing older, regardless of the patient's disease progression. In addition, the changes in TIEF are not limited to CDG and can be seen in other conditions, such as liver pathologies, endocrine anomalies and infections (Bean 1995[31], Bean 1998[32], Charlwood 1998[33], Helander 2001[34], Kaphalia 2014[35]). Therefore, additional methods assessing patient's glycosylation status are highly recommended.

More robust and useful methods including different Mass-Spectrometry MS techniques like LC/MS are generally recognized as being capable of identifying PGM1-CDG (Tegtmeyer 2014). They also offer the possibility to monitor the patients' response to treatment (Voermans 2015, Wong 2017)

The LC/MS pattern of PGM1-CDG is unique and characteristic for all confirmed patients. It is a pathognomonic pattern of transferrin missing one entire glycan while the remaining glycans are truncated at the galactose level (Tegtmeyer 2014).

Analysis of intact transferrin (Van Scherpenzeel 2015[36]) and total plasma N-glycoproteins by HPLC-CHIP-QTOF LC-MS (Abu Bakar 2018[37]) has also

31 Bean P, Sutphin MS, Liu Y, Anton R, Reynolds TB, Shoenfeld Y, Peter JB. Carbohydrate-deficient transferrin and false-positive results for alcohol abuse in primary biliary cirrhosis: differential diagnosis by detection of mitochondrial autoantibodies. Clin Chem. 1995 Jun;41(6 Pt 1):858-61.

32 Bean P, Husa A, Liegmann K, Sundrehagen E. Semi-automated carbohydrate-deficient transferrin in primary biliary cirrhosis: a pilot study. Alcohol Alcohol. 1998 Nov-Dec;33(6):657-60

33 Charlwood J, Clayton P, Keir G, Mian N, Winchester B.Defective galactosylation of serum transferrin in galactosemia.Glycobiology. 1998 Apr;8(4):351-7

34 Helander A, Eriksson G, Stibler H, Jeppsson JO. Interference of transferrin isoform types with carbohydrate-deficient transferrin quantification in the identification of alcohol abuse. Clin Chem. 2001;47(7):1225-33

35 Kaphalia BS. Biomarkers of acute and chronic pancreatitis. In: Biomarkers in Toxicology, edited by Gupta RC, editor. San Diego, CA: Elsevier/Academic, 2014, p. 279–289. doi:10.1016/B978-0-12-404630-6.00016-6

36 van Scherpenzeel M, Steenbergen G, Morava E, Wevers RA, Lefeber DJ. High-resolution mass spectrometry glycoprofiling of intact transferrin for diagnosis and subtype identification in the congenital disorders of glycosylation. Transl Res. 2015 Dec;166(6):639-649.e1.

37 Abu Bakar N, Voermans NC, Marquardt T, Thiel C, Janssen MCH, Hansikova H, Crushell E, Sykut-Cegielska J, Bowling F, MØrkrid L, Vissing J, Morava E, van Scherpenzeel M, Lefeber DJ. Intact transferrin

been reported and could additionally be used to screen for different CDG including PGM1-CDG. However, total plasma N-glycan profiling does not give insight into the absence of glycan forms, given the fact that it depends on relative protein abundance. To circumvent this problem, the authors have specifically defined 3 glycan indexes which can be extracted from transferrin QTOF spectra and can be used to recognize PGM1-CDG.

Other used methods for N-glycan analysis in CDG include MALDI-TOF (Xia 2013[38], Nolting 2017), ESI-MS (Lacey 2001[39]) and ESI-QTOF (Chen 2019[40]). Though MALDI-TOF is a more preferred method in CDG diagnostics due to its ease of use, robustness and the low amount of material necessary for the analysis, it isnot superior to other methods for this particular CDG.

The analysis of other highly glycosylated proteins such as apoC-III is commonly used during CDG diagnosis as a complementary analysis to transferrin analysis (Wopereis 2003[41], Lefeber 2014[42], Ondruskova 2015[43]). Most PGM1-CDG patients have a normal apoC-III profile (Tegtmeyer 2014, Wong 2017, Abu Bakar 2018), with only a few exceptions (Ondruskova 2015).

and total plasma glycoprofiling for diagnosis and therapy monitoring in phosphoglucomutase-I deficiency. Transl Res. 2018 Sep; 199:62-76

38Xia B, Zhang W, Li X, Jiang R, Harper T, Liu R, Cummings RD, He M. Serum N-glycan and O-glycan analysis by mass spectrometry for diagnosis of congenital disorders of glycosylation. Anal Biochem. 2013 Nov 15;442(2):178-85

39 Lacey JM, Bergen HR, Magera MJ, Naylor S, O'Brien JF. Rapid determination of transferrin isoforms by immunoaffinity liquid chromatography and electrospray mass spectrometry. Clin Chem. 2001 Mar;47(3):513-8.

40 Chen J, Li X, Edmondson A, Meyers GD, Izumi K, Ackermann AM, Morava E, Ficicioglu C, Bennett MJ, He M. Increased Clinical Sensitivity and Specificity of Plasma Protein N-Glycan Profiling for Diagnosing Congenital Disorders of Glycosylation by Use of Flow Injection-Electrospray Ionization-Quadrupole Time-of-Flight Mass Spectrometry. Clin Chem. 2019 May;65(5):653-663

41 Wopereis S, Grünewald S, Morava E, Penzien JM, Briones P, García-Silva MT, Demacker PN, Huijben KM, Wevers RA.Apolipoprotein C-III isofocusing in the diagnosis of genetic defects in O-glycan biosynthesis. Clin Chem. 2003 Nov;49(11):1839-45

Lefeber DJ, Morava E, Jaeken J. How to find and diagnose a CDG due to defective N-glycosylation. J Inherit Metab Dis. 2011 Aug;34(4):849-52

Ondrušková N, Honzík T, Kytnarová J, Matoulek M, Zeman J, Hansíková H. Isoelectric Focusing of Serum Apolipoprotein C-III as a Sensitive Screening Method for the Detection of O-glycosylation Disturbances. Prague Med Rep. 2015;116(2):73-86

42 Lefeber DJ, Morava E, Jaeken J. How to find and diagnose a CDG due to defective N-glycosylation. J Inherit Metab Dis. 2011 Aug;34(4):849-52

43 Ondrušková N, Honzík T, Kytnarová J, Matoulek M, Zeman J, Hansíková H. Isoelectric Focusing of Serum Apolipoprotein C-III as a Sensitive Screening Method for the Detection of O-glycosylation Disturbances. Prague Med Rep. 2015;116(2):73-86

Statement #1 (grade of recommendation: B)

PGM1-CDG patients present with a mixed type 1 and type 2 TIEF pattern (a combination of asialo, monosialo, disialo, trisialo and tetrasialotransferrin).

1.1.2 Therapy monitoring using Tf and N-glycan analysis

LC/MS and MALDI-TOF based methods can be used for monitoring D-galactose therapy that improves the restauration of incomplete N-glycans faster than that of unoccupied glycosylation sites. The latter is easily monitored by MALDI-TOF approach. For the practical and routine clinical laboratory use the HPLC-CHIP-QTOF LC/MS of total plasma N-glycans method can also be used to closely monitor the effect of D-galactose therapy outcome in PGM1-CDG patients using 3-glycan index (Abu Bakar 2018).

Statement #1 (grade of recommendation: B)

LC-MS platforms are the recommended methodology for monitoring therapy effectiveness in PGM1-CDG patients.

1.2 Enzymatic studies

Most PGM1-CDG patients have severely diminished PGM1 enzymatic activity.. Fibroblasts and leukocytes (red blood cells should not be used as PGM1 is absent) are commonly used for enzymatic studies. PGM1 activity can also be measured in muscle tissue but due to the most reliable assay, fibroblasts are usually preferred (Tegtmeyer et al, 2014).

The enzymatic activity of PGM1 in reported patients ranges from undetectable to 20 % of controls (Stojkovic 2009, Perez 2012, Timal 2012, Preisler 2013, Beamer 2014, Lee 2014, Ondruskova 2014, Socha2014, Tegtmeyer 2014, Wong 2016Zeevaert 2016, Nolting 2017, Preisler 2017 Voermans 2017, Wong 2017, Yokoi 2018, Radenkovic2019). Unusually, PGM1 activity doesn’t correlate with the severity of the patient’s phenotype and the differences in activities of other PGM isoforms have been proposed as one of the possible explanations (Tegtmeyer 2014, Wong 2016). It is important to note that, although enzymatic studies are

informative, they are not substantial for assessing the severity of the patient's phenotype and predicting the outcome.

Statement#1 (Grade of recommendation: D)

PGM1 enzymatic assays are frequently used in PGM1-CDG as complementary analysis and most of the patients present with <20 % enzymatic activity. Patient fibroblasts or leukocytes can be used for enzymatic studies. However, the final diagnosis is based on molecular testing.

1.3 Other biochemical analyses

Galactose-related metabolites (blood galactose-1-P levels, urine galactitol levels)can be measured in order to screen for PGM1 deficiency (Tegtmeyer 2014) and to monitor the safety of D-galactose therapy (Wong 2017). Interestingly, red blood cell galactose-1-P is paradoxically elevated in untreated PGM1-CDG patients in a mild to moderate degree (Tegtmeyer 2014). Therefore, galactosemia screening based on galactose-1-P levels in newborn screening (NBS) can show false positive results in PGM1- deficient patients. This marker could be used in early disease screening and potentially NBS. Patients who are started on galactose therapy should be monitored, as high levels of galactose-1-P could be toxic. The maximum daily D-galactose limit which is considered to be safe is 50 g (De Smet 2009[44]) and increased galactitol excretion has only been reported in patients receiving more than 50 g/day (Wong 2017).

In order to understand the pathomechanisms of the disease, an extended analysis of galactose metabolism has been reported (Radenkovic 2019). This approach used a variety of (tracer) MS techniques such as GC-MS and LC-MS, to elucidate the role of galactose in the treatment of PGM1-CDG. These labor-intensive and costly techniques are highly informative but not used in clinical practice.

Statement#1 (Grade of recommendation: C)

44 De Smet E, Rioux JP, Ammann H, Déziel C, Quérin S. FSGS permeability factor-associated nephrotic syndrome: remission after oral galactose therapy. Nephrol Dial Transplant. 2009 Sep;24(9):2938-40

Galactose-1-phosphate is mildly increased in untreated patients with PGM1-CDG. In order to monitor the safety of D-galactose therapy, galactose related metabolites (galactitol in urine) can be tested.

1.4 Sensitivity of biochemical testing

Though the majority of PGM1-CDG patients present with a mixed TIEF pattern, some patients can present with a type 1 or a type 2 pattern (Socha 2014, Perez 2012, Kucukcongar 2015, Zeevaert 2016, Nolting 2017). Therefore, PGM1-CDG should not be excluded solely on the basis of the TIEF results. Complementary glycosylation analysis, enzymatic assays and genetic testing should be included.

Statement #1 (Grade of recommendation: D)

Though the majority of PGM1-CDG patients present with a mixed TIEF pattern, some patients present with a type 1 or a type 2 pattern in which case it is recommended to use other methods in order to make a correct diagnosis.

1.5 Pre-analytical requirements for biochemical testing

Detailed statements on transferrin analysis sensitivity and the pre-analytical requirements for the biochemical testing are given in the PMM2-CDG guidelines (Altassan 2019[45]).

1.6 False positives

Aberrant glycosylation patterns can also be detected in individuals affected by other genetic disorders with secondary glycosylation defects such as galactosemia (Charlwood 1998) or hereditary fructose intolerance (Adamowitz 2007[46]). Note that galactosemia shows also a combined TIEF type1/type 2 pattern! Moreover, various conditions affecting the liver (e.g. alcoholism, infections, immune disorders) can similarly affect

45 Altassan R, Péanne R, Jaeken J, Barone R, Bidet M, Borgel D, Brasil S, Cassiman D, Cechova A, Coman D, *et al*. International clinical guidelines for the management of phosphomannomutase 2-congenital disorders of glycosylation: Diagnosis, treatment and follow up.J Inherit Metab Dis. 2019 Jan;42(1):5-28
46 Adamowicz M, Płoski R, Rokicki D, Morava E, Gizewska M, Mierzewska H, Pollak A, Lefeber DJ, Wevers RA, Pronicka E. Transferrin hypoglycosylation in hereditary fructose intolerance: using the clues and avoiding the pitfalls. J Inherit Metab Dis. 2007 Jun;30(3):407. Epub 2007 Apr 2

glycosylation, leading to mainly Golgi-related glycan abnormalities (glycan truncation).

Statement #1 (Grade of recommendation: C)

The presence of other clinical conditions should also be excluded in case of abnormal TIEF results.

2) MOLECULAR DIAGNOSIS

2.1 Genetic testing

PGM1-CDG can further be confirmed by standard genetic tests such as Sanger sequencing, whole-exome sequencing (WES) or homozygosity mapping.

PGM1-CDG is located on chromosome 1 and contains 11 exons. So far, 41 different variants in PGM1 have been reported spanning across the whole PGM1 gene. The most common variant is c.112A>T (n=9) located in the first exon, followed by c.988G>C (n=5) and c.787G>T (n=4). The majority of variants are missense (24) or frameshift/nonsense variants (11), with only a few splicing (4) and gross rearrangement type of variants reported (1). The frequency of compound heterozygotes and homozygotes is approximately similar in PGM1-CDG while deletions (n=2) and inversions (n=1) are very rare. (Supplementary Table 4)

Statement #1 (grade of recommendation: A)

Genetic testing is the standard method to confirm PGM1-CDG diagnosis.

2.2 Genotype-phenotype correlations

As mentioned earlier, PGM1-CDG phenotypes do not correlate with PGM1 enzymatic activities. Furthermore, several PGM1 variants do not correlate with phenotype severity, clinical outcome or response to D-galactose therapy.

One possible explanation is the existence of different PGM isoforms. For example, PGM2, which is the predominant PGM isoform in red blood cells, may be more active in some patients and therefore able to take over the role of PGM1 to some extent (Tegtmeyer 2014). However, PGM1 remains

the most important PGM isoform responsible for the interconversion of glucose-6-P into glucose-1-P and the discrepancy in the activities of different isoforms can provide only a partial explanation. Therefore, further studies are needed to understand the lack of the correlation between the genotype and phenotype in PGM1-CDG.

Statement #1 (grade of recommendation: C)

No genotype/phenotype correlation has been seen in PGM1-CDG. A partial explanation can come from the existence of different PGM isoforms, but more insight is needed in order to understand these discrepancies.

3) Common laboratory findings

Standard laboratory testing in PGM1-CDG usually reveals low or fluctuating glucose levels as well as elevated serum transaminases. Decreased coagulation factors including antithrombin-III, factor IX, factor XI, etc. and prolonged PT and aPTT are also common. In addition, the majority of PGM1-CDG patients, especially those with exercise intolerance, present with high creatine kinase (CK) levels. Other laboratory findings include altered plasma concentrations of cortisol, ACTH, GH, IGF1, IGFBP3, gonadotrophins (LH, FSH) and thyroid hormones (Stojkovic 2009, Perez 2012, Tegtmeyer 2014, Ondruskova 2014, Lowenthal 2015, Kucukcongar 2015, Voermans 2017, Preisler2017, Nolting 2017, Wong 2017, Ding 2018, Yokoi 2018, Radenkovic 2019, Tian 2019, Medrano 2019, Tian 2019). Global protein hypoglycosylation is also a common finding in PGM1-CDG (Abu Bakar 2018).

Spontaneous improvement of overall glycosylation, as has been seen in adult CDG patients,, especially in CDG-I (Witters 2019[47]) has never been observed in PGM1-CDG.

Monitoring mentioned laboratory findings is helpful during D-galactose therapy. Coagulation parameters and liver enzymes usually normalize

47 Witters P, Honzik T, Bauchart E, Altassan R, Pascreau T, Bruneel A, Vuillaumier S, Seta N, Borgel D, Matthijs G, Jaeken J, Meersseman W, Cassiman D, Pascale de L, Morava E. Long-term follow-up in PMM2-CDG: are we ready to start treatment trials? Genet Med. 2019 May;21(5):1181-1188

during the first year of the treatment. Similarly, CK levels improve on long term D-galactose therapy while glucose levels tend to fluctuate without additional nutritional intervention, frequent complex carbohydrate treatment or diazoxide therapy (Wong 2017, Radenkovic 2019). In addition, the overall glycosylation of secretory glycoproteins improves on D-galactose therapy and is easily monitored as well (Abu Bakar 2018). The improvement of the patients' laboratory findings correlates with their outcome and therefore it is highly informative for the therapy compliance as well (Wong 2017, Radenkovic 2019).

Statement #1 (Grade of recommendation: B)

Low blood sugar levels, elevated transaminases, and decreased antithrombin activity are the most consistently altered biochemical markers in patients with PGM1-CDG. Similarly, changes in other laboratory parameters such as aPTT, factor VIII, factor XI, CK, LH, FSH are frequent. Hence, their levels should be closely monitored, especially during therapy. Liver enzymes, thyroid hormones as well as coagulation factors normalize on long term D-galactose therapy and are easily monitored, while blood sugar levels might fluctuate regardless of D-galactose therapy.

4) Prenatal testing, screening and genetic counseling

Although the detection of PGM1 deficiency is possible from dried blood spots using a modified Beutler test (Tegtmeyer 2014), currently there is no newborn screening available for PGM1-CDG. Prenatal genetic testing can be done if the parents are known carriers of PGM1-CDG or if there is a family history of PGM1-CDG. TIEF of fetal plasma results are not conclusive and therefore not recommended (Matthijs 1998[48]).

Genetic counseling is an important part of PGM1-CDG diagnosis and should be offered to any parents with a child suspected or diagnosed with PGM1-CDG.

Statement #1 (grade of recommendation B)

48 Matthijs G, Schollen E, Cassiman J-J, Cormier-Daire V, Jaeken J, van Schaftingen E. Prenatal diagnosis in CDG1 families: beware of heterogeneity. Eur J Hum Genet. 1998;6(2):99-104.

Molecular analysis of *PGM1* is the gold standard of the diagnosis at any age in any individual with a suspected PGM1 deficiency. However, after birth, additional biochemical studies might be needed in case of the detection of novel genetic variants.

D-Galactose therapy

D-Galactose therapy in PGM1-CDG is able to restart the glycosylation by replenishing nucleotide sugar pools UDP-glucose and UDP-galactose necessary for ER linked glycosylation and Golgi linked glycosylation, respectively (Radenkovic 2019[49]).

Regarding the clinical aspect, D-galactose supplement was trialed in more than 20 PGM1-CDG patients and has shown marked improvement of most symptoms including exercise intolerance, fatigability, hypogonadism, delayed puberty, rhabdomyolysis and the frequency of hypoglycemias (Tegtmeyer 2014, Schrapers 2015, Wong 2017, Nolting 2017, Radenkovic 2019). On the laboratory side, long-term follow-up of PGM1-CDG patients trialed on galactose showed an improvement in transaminase levels, normalization of antithrombin III and FSH levels and reduction in CK levels (Tegtmeyer 2014, Morava 2014, Schrapers 2016, Voermans 2017, Wong 2017, Radenkovic 2019). In addition to D-galactose therapy, enrichment of the diet with complex carbohydrates is essential to maintain normal blood glucose levels in all PGM1-CDG patients (Witters 2017[50]).

N-glycosylation changes were monitored by different methods in multiple patients (Tegtemeyer 2014, Schrapers 2016, Nolting 2017, Voermans 2017, Wong 2017, Abu Bakar 2018, Chen 2019) during galactose therapy and showed sustained improvement in all except one (Nolting 2017).

The trialed galactose dose was ranging between 0.5 to 3 g/kg / day (maximum dose 50 g /day) and showed no adverse effects in most patients (Tegtmeyer 2014, Schrapers 2016, Voermans 2017, Nolting 2017, Wong 2017, Radenkovic 2019). One patient did not tolerate higher doses of D-galactose and was finally prescribed the maximum dose of 0.3 g/kg/day (Zeevaert 2016).

49 Radenkovic S, Bird MJ, Emmerzaal TL, Wong SY, Felgueira C, Stiers KM, Sabbagh L, Himmelreich N, Poschet G, Windmolders P, Verheijen J, Witters P, Altassan R, Honzik T, Eminoglu TF, James PM, Edmondson AC, Hertecant J, Kozicz T, Thiel C, Vermeersch P, Cassiman D, Beamer L, Morava E, Ghesquière B.The Metabolic Map into the Pathomechanism and Treatment of PGM1-CDG. Am J Hum Genet. 2019 May 2;104(5):835-846. doi: 10.1016/j.ajhg.2019.03.003. Epub 2019 Apr 11.

50 Witters P, Cassiman D, Morava E. Nutritional Therapies in Congenital Disorders of Glycosylation (CDG). Nutrients. 2017 Nov 7;9(11). pii: E1222. doi: 10.3390/nu9111222. Review.

It is important to note, however, that although D-galactose is able to alleviate multiple symptoms and improve patient outcome, it is not an ideal treatment. The patients have to consume high amounts of this sugar as well as other complex carbohydrates, and a consult with a dietician is recommended.

Characteristic (Statement #1: grade of recommendation D)
Oral D-galactose supplementation is a recommended treatment for PGM1-CDG patients presenting with hypoglycemia, rhabdomyolysis, coagulopathy, and or hepatopathy.

Method of administration (Statement #2: grade of recommendation D)

The recommended galactose dose starts at 0.5-3 g/kg/dose orally 4 to 5 times daily. The maximum recommended dose is 50 g/ day.

Monitoring of treatment (Statement #3: grade of recommendation D)

Monitoring of transaminases (ALT, AST), anticoagulation factors (ATIII) as well as CK levels is recommended especially at the start of treatment. In addition, N-glycosylation improvements should be monitored for treatment efficacy. Methods such as intact transferrin analysis, or more potent LC/MS methods can be used.

Undesirable effects (Statement #4: grade of recommendation D)

Higher doses of galactose treatment might not be tolerated in all patients and therefore should be carefully considered. Doses higher than 50 g/day should be avoided as galactose can result in the increase of galactose-1-P and galactitol which could be toxic.

Table 1: the most commonly reported phenotypes in PGM1-CDG		
System*	Phenotype	no. of patients
malformations	cleft palate	28
	bifid uvula	25
	Pierre-Robin sequence	15
Neurologic	developmental delay	11
	cognitive impairment	14
	intellectual disability	8
	learning disabilities	11
Endocrine	hypoglycemia	38
	hypocortisolism	7
	high adrenocorticotropic hormone	5
	low GH or IGF1	11
	low IGFBP-3	8
Cardiac	dilated cardiomyopathy	12
	structural abnormalities	7
	abnormal ECG (long QT interval, ST elevation, T inversion, others)	5
Hepatic	increased transaminases	42
	steatosis	7
	acute liver failure	5
Hematologic	coagulopathy	29
Muscular	rhabdomyolysis	42
	exercise intolerance	20
	muscle weakness	18
	chronic CK elevation	13

*Only the phenotypes reported in 5 or more patients are listed here.

Table 2: Clinical presentation in patients with PGM1 deficiency and suggested surveillance		
Phenotype		Suggested surveillance frequency
Congenital malformations	cleft palate, micrognathia, bifid uvula, Robin-Pierre sequence, vertebral malformations, anal atresia	Complete physical examination at the time of diagnosis and r
Neurological	cognitive delay, seizure	Complete physical examination at the time of diagnosis and y brain MRI if clinically indicated
Ophthalmological	strabismus, abnormal eye movements, nasolacrimal duct obstruction and/ or epiphoria	Eye exam at the time of diagnosis and monitoring if clinically
Endocrine	hypothyroidism, hypogonadotropic hypogonadism, delayed puberty, hyperinsulinism	Assessment of growth at the time of diagnosis and on follow- at the time of diagnosis and regularly monitored. Serum cortisol and ACTH levels at the time of diagnosis; furth
Cardiac	cardiomyopathy (dilated cardiomyopathy), structural heart abnormalities	Electrophysiology (ECG) and echocardiography at the time of
Muscle	exercise intolerance, myopathy, rhabdomyolysis	CK at the time of diagnosis, then if clinically indicated (during clinically indicated
Liver	Elevated transaminases, steatosis, cholestasis, fibrosis, acute hepatic failure	Transaminases and hepatic function at time of diagnosis and
Hematological	anthithrombin III, factors XI, VII, IX, X and XI deficiencies low proteins C and S, increased PT and prolonged aPPT	Coagulation profiles at the time of diagnosis and monitored r
Metabolic	hypoketotic and ketotic hypoglycemia	Glucose level at the time of diagnosis and during illnesses wit
Other	malignant hyperthermia	Caution is advised in regard of choice of anaesthetics

Table 3: N- glycosylation analysis methods in PGM1-CDG				
Method	**Sample type**	**Instrument**	**Pros**	**Cons**
TIEF (Transferrin Isoelectric focusing)	Plasma/Serum	PHAST machine	Easily applicable, fast, cheap, PGM1-CDG specific mixed CDG-I/II pattern, no enzymatic digestion	Results are difficult to b not high-throughput, false positive in several liver conditions, false negative at very y sometimes in older age
Intact transferrin* (transferrin glycoprofiling)	Plasma/Serum	MALDI-TOF/ MS	Rapid, easy to use, more accurate than TIEF, easy to implement, no enzymatic digestion, high-throughput, can be compared to the existing databases, protein specific glycosylation	Lower sensitivity compa lower accuracy and spe difficult to quantify
	Plasma	(nano) LC-chip-QTOF MS	High-throughput, no enzymatic digestion, detailed glycan assessment, PGM1-CDG specific, robust, protein specific glycosylation, semiquantitative (internal standards)	Detailed analysis and in data Time consuming, more No information about t of other proteins (Could require verificat TOF spectra)
Released N-glycans (total glycoprofiling)	Plasma/Serum	MALDI TOF/MS	Rapid, accurate, easy to implement, can be compared to existing databases	Enzymatic digestion wit Lower sensitivity, lower
	Plasma	HPLC-chip QTOF LC/MS	Small sample volume, more informative than MALDI, monitoring of therapy, PGM1-CDG specific	Enzymatic digestion wit Requires more time an detailed analysis and de disease specific marker
	Fibroblasts	MALDI TOF/TOF MS	Fibroblasts can be cultured potentially being an indispensable source of sample, compared to the plasma/serum sample which has to be obtained several times and is limited in volume	Enzymatic digestion wit Not validated for PGM1 High variability, Glycan profile can be in confluency and culturin

Apart from transferrin, other highly glycosylated proteins can be analysed (ApoCIII, IGFPB3 etc.).

Table 4: Molecular data of the reported PGM1-CDG patients

No.	Publication	Age (at the time of publication)/Gender	Genetic variant	Protein change
1	Stojkovic, 2008	35 yr/ M	c.343A>G c.1145-1G>C (intronic)	p.Thr115Ala
2	Timal, 2012	8 yr /M	c.361G>C HOM	p.Gly121Arg
3	Timal, 2012	16 yr /F	c.1507C >T HOM	p.Arg503Ter
4	Perez *et al*, 2014	13 yr/ M	c.871G>A c.1144 + 3A>T	p.Gly291Arg
5	Ondruskova *et al*, 2014	10 yr/ M	c.1010C>T c.1508G>A	p.Thr337Met p.Arg503Gln
6	Tegtmeyer *et al*, 2014	12 yr /M	c.1547T>C HOM	p.Leu516Pro
7	Tegtmeyer *et al*, 2014	17 yr/ M	c.1547T>C HOM	p.Leu516Pro
8	Tegtmeyer *et al*, 2014	19 yr/ F	c.988G>C c.1129G>A	p.Gly330Arg p.Glu377Lys
9	Tegtmeyer *et al*, 2014	33 yr/ M	c.787G>T c.788A>G	p.Asp263Tyr p.Asp263Gly
10	Tegtmeyer *et al*, 2014	19 yr/ M	c.122A>G c.1495C>T	p.Gln41Arg p.Arg499Ter
11	Tegtmeyer *et al*, 2014	26 yr/ M	c.1145-222G>T HOM	p.Gly382ValfsTer23
12	Tegtmeyer *et al*, 2014	20 yr/ F	c.1145-222G>T HOM	p.Gly382ValfsTer23
13	Tegtmeyer *et al*, 2014	23 yr/ F	c.122A>G c.871G>A	p.Gln41Arg p.Gly291Arg
14	Tegtmeyer *et al*, 2014	9 yr/ F	c.112A>T HOM	p.Asn38Tyr
15	Tegtmeyer *et al*, 2014	11 yr/ M	c.787G>T c.1551C>A	p.Asp263Tyr p.Tyr517Ter
16	Tegtmeyer *et al*, 2014	9 yr/ M	c.55A>G c.1600-523G>A	p.Thr19Ala p.Met535ProfsTer60
17	Tegtmeyer *et al*, 2014	5 yr/ M	c.184G>C HOM	p.Asp62His
18	Tegtmeyer *et al*, 2014	6 yr/ M	c.184G>C HOM	p.Asp62His
19	Tegtmeyer *et al*, 2014	10 yr/ M	c.1162G>A c.1547T>C	p.Glu388Lys p.Leu516Pro
20	Tegtmeyer *et al*, 2014	3 yr/ F	c.551delT HOM	p.F184Sfs*9
21	Tegtmeyer *et al*, 2014	30 yr/ F	c.787G>T c.661delC	p.Asp263Tyr p.Arg221ValfsTer13
22	Küçükçongar *et al*, 2015	19 mo/ F	c.551delT HOM	p.F184Sfs*9
23	Lowenthal *et al*, 2015	14 yr/ M	c.112A>T HOM	p.Asn38Tyr
24	Lowenthal *et al*, 2015	6 yr/ M	c.112A>T HOM	p.Asn38Tyr
25	Lowenthal *et al*, 2015	17 yr/ F	c.112A>T HOM	p.Asn38Tyr
26	Lowenthal *et al*, 2015	13.5 yr/ M	c.112A>T HOM	p.Asn38Tyr
27	Lowenthal *et al*, 2015	29 yr/ M	c.112A>T HOM	p.Asn38Tyr
28	Lowenthal *et al*, 2015	2 yr/ M	c.112A>T HOM	p.Asn38Tyr
29	Lowenthal *et al*, 2015	5 yr/ M	c.112A>T HOM	p.Asn38Tyr

30	Zeevaert *et al*, 2016	8 yr/ M	c.1162G>A c.1547T>C	p.Glu388Lys p.Leu516Pro
31	Wong *et al*, 2016	3 yr/ F	c.689G>A HOM	p.Gly230Glu
32	Wong *et al*, 2016	2 yr/ F	c.1598G>T HOM	p.Arg515Leu
33	Wong *et al*, 2016	2 yr/ M	c.157-158delinsG c.1507C>T c.661C>T	p.Gln53GlyfsTer15 p.Arg503Ter p.Arg221Cys
34	Wong *et al*, 2016	21 yr/ F	c.1264C>T c.1588C>T	p.Arg422Trp p.Gln530Ter
35	Wong *et al*, 2016	2 yr/ M	c.988G>C c.1007C>G	p.Gly330Arg p.Pro336Arg
36	Wong *et al*, 2016	2 yr/ M	c.112A>T HOM	p.Asn38Tyr
37	Voermans *et al,* 2017	53 yr/ M	c.988G>C c.1264C>T c.1258T>C	p.Gly330Arg p.Arg422Trp p.Tyr420His
38	Nolting *et al*, 2017	10 yr/ M	c.771delT HOM	p.Phe257LeufsTer20
39	Wong *et al*, 2017	19 mo/ F	c.661C>T c.1258T>C	p.Arg221Cys p.Tyr420His
40	Abu Bakar *et al*, 2018	21 yr/ F	c.316T>C HOM	p.Ile106Val
41	Abu Bakar *et al*, 2018	18 yr/ M	c.787G>T c.788A>G	p.Asp263Tyr p.Asp263Gly
42	Abu Bakar *et al*, 2018	18 yr/ F	c.871G>A HOM	p.Gly291Arg
43	Abu Bakar *et al*, 2018	7 yr/ M	c.988G>C c.1007C>G	p.Gly330Arg p.Pro336Arg
44	Abu Bakar *et al*, 2018	1 yr/ F	c.661delC c.988G>C	p.Arg221ValfsTer13 p.Gly330Arg
45	Abu Bakar *et al*, 2018	3 yr/ F	c.419G>A c.1597C>T	p.Gly140Asp p.Gln533Ter*
46	Ding *et al*, 2018	4 yr/ M	c.119delT HOM	p.Ile40ThrfsTer28
47	Ding *et al*, 2018	7 yr 4mo/ F	c.1172G>T c.1507C>T	p.Gly391Val p.Arg503Ter
48	Yokoi *et al*, 2018	12 yr/ M	inv(1)(p31.1p32.3)	-
49	Radenkovic *et al*, 2019	16 yr/ F	c.1508G>A HOM	p.Arg503Gln
50	Radenkovic *et al*, 2019	4 yr/ M	c.1544G>A HOM	p.Arg515Gln
51	Radenkovic *et al*, 2019	6 yr/ M	c.1544G>A HOM	p.Arg515Gln
52	Radenkovic *et al*, 2019	8 yr/ M	c.1544G>A HOM	p.Arg515Gln
53	Radenkovic *et al*, 2019	4 yr/ M	c.1014C>T HOM	p.Ser388Arg
54	Tian *et al*, 2019	49 yr/ M	c.405delT HOM	p.Asn135LysfsTer9
55	Fernlund *et al*, 2019	13 yr/ F (diseased before the publication)	c.689G>A HOM	p.Gly230Glu
56	Fernlund *et al*, 2019	12 yr/ M (disease before the publication)	c.689G>A HOM	p.Gly230Glu
57	Fernlund *et al*, 2019	15 yr/ M	c.689G>A HOM	p.Gly230Glu