

# Glycogen storage disease-like phenotype with central nervous system involvement in a PGM1-CDG patient

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## Abstract

**OBJECTIVES:** A 10-year-old boy presented with cleft palate, hepatopathy, cholecystolithiasis, myopathy, coagulopathy, hyperlipidemia, hypoglycemia, hyperuricemia, short stature, obesity, hypothyroidism, microcephaly and mild intellectual disability. The multi-systemic manifestation involving certain distinct clinical features prompted us to search for a subtype of congenital disorders of glycosylation (CDG).

**METHODS:** The patient was screened for CDG by examining the distribution of transferrin (TRF) and apolipoprotein C-III (ApoC-III) sialylated isoforms using isoelectric focusing of serum. This was followed by spectrophotometric measurement of phosphoglucomutase 1 (PGM1) activity in fibroblasts and molecular analysis including sequencing and PCR-RFLP of *PGM1* gene. Selected bioinformatics tools were used to evaluate the data.

**RESULTS:** Increased relative levels of di-, mono- and asialotransferrin reflected a defect of *N*-glycosylation in the patient. Markedly decreased activity of PGM1 corresponding to less than 5% of control's was found. Sequencing of *PGM1* gene revealed the presence of two heterozygous missense mutations c.1010C>T (p.T337M) and c.1508G>A (p.R503Q), whose pathogenicity was confirmed by *in silico* analysis.

**CONCLUSION:** We report the first Czech patient with a glycosylation disorder due to PGM1 deficiency. Compared to the described cases, no dilated cardiomyopathy was noted in our patient. However, he suffered from a mild neurological impairment, which is an uncommon feature that extends the phenotype associated with PGM1-CDG. Lactose-rich diet, which was previously reported to have ameliorated the clinical symptoms in some PGM1-CDG patients, did not result in any improvement in our patient.

#### Abbreviations:

ALT	- alanine transaminase
ApoC-III	- apolipoprotein C-III
APTT	- activated partial thromboplastin time
AST	- aspartate transaminase
BMI	- body mass index
CDG	- congenital disorders of glycosylation
DNA	- deoxyribonucleic acid
ft4	- free thyroxine
GSD	- glycogen storage disease
IEF	- isoelectric focusing
IQ	- intelligence quotient
PCR	- polymerase chain reaction
PCR-RFLP	- polymerase chain reaction-restriction fragment length polymorphism
PGM1	- phosphoglucomutase 1
PMI	- phosphomannoisomerase
PMM2	- phosphomannomutase 2
SD	- standard deviation
SDS-PAGE	- sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBG	- thyroxine binding globulin
TRF	- transferrin
TSH	- thyroid-stimulating hormone

## INTRODUCTION

The family of disorders, labeled congenital disorders of glycosylation (CDG), is a rapidly expanding group of multisystemic and clinically heterogeneous diseases, characterized by defective glycosylation of proteins and lipids. In 1984, Dr. Jaeken was the first to report a case of twin patients with decreased transferrin sialylation (Jaeken *et al.* 1984), a novel syndrome later found to be caused by pathological mutations in the phosphomannomutase 2 gene. This disorder, now referred to as PMM2-CDG, is the most frequently diagnosed form of CDG. Since then, defects in more than 80 different genes leading to various degrees of glycoconjugate hypoglycosylation have been described (Freeze 2013). Considering the large number of genes potentially involved in the glycosylation process, more novel CDG types are expected to be discovered in the near future.

Diagnosing CDG is quite challenging, due to both the extreme variability of clinical symptoms and the non-specific manifestations of the disease. Common symptoms of CDG include psychomotor delay and intellectual disability, muscle hypotonia, seizures, strabismus, dysmorphism, microcephaly, growth retardation, hepatopathy and coagulopathy (Grunewald *et al.* 2002). Often, more typical signs such as inverted nipples and abnormal fat distribution are noted (Jaeken 2010; Honzik *et al.* 2012). Some CDG present cutis laxa, blindness, deafness or urogenital anomalies (Jaeken 2010; Ondruskova *et al.* 2012). The standard selective screening method consists of isoelectric focusing (IEF) of serum transferrin (TRF) and apolipoprotein C-III (ApoC-III). Based on the results of these tests and subsequent analyses, several CDG types can be recognized. However, many cases remain unsolved and are labeled CDG-Ix or CDG-IIx.

The current approach to disease gene identification increasingly relies on the application of next-generation sequencing techniques. Recently, a novel type of CDG due to phosphoglucomutase 1 (PGM1; EC 5.4.2.2) deficiency was identified in four patients from three families (Timal *et al.* 2012; Stojkovic *et al.* 2009; Pérez *et al.* 2013). Herein, we report the first Czech patient with novel mutations in the *PGM1* gene. The patient presented with multi-organ dysfunction including attacks of ketotic hypoglycemia. No dilated cardiomyopathy was documented. Central nervous system involvement observed in our patient is an uncommon feature that extends the phenotype associated with PGM1-CDG.

## MATERIAL AND METHODS

### Clinical report

The patient was born after 37 weeks of gestation, with a birth weight of 2.75 kg and length of 47 cm, following an uneventful pregnancy. He is the second child of non-consanguineous parents of Caucasian origin. His early postnatal adaptation was complicated by hypotonia and cleft palate involving both the soft palate and part of the hard palate. At 6 months, he presented with a developmental delay and persistent muscular hypotonia. Although his motor milestones were significantly delayed, he made gradual progress during the first two years of life. He was able to walk with support at 17 months of age. At that time, he was diagnosed with cholelithiasis and hypothyroidism (ft4 12.4 pmol/l, controls 11.5–22.7; TSH 6.4 mIU/l, controls 0.5–6.0) with low levels of thyroxine-binding globulin (TBG 13 mg/l, controls >19). Surgical closure of the cleft palate was postponed 15 times due to recurrent otitis media.

At 6 years old, upon evaluation by a psychologist, the patient was reported to have borderline intellectual functioning (IQ 78). Short stature (108 cm; –2.85 SD), obesity (23.5 kg; +2.79 SD) and microcephaly (49.5 cm; 3<sup>rd</sup> centile) were also documented. Laboratory investigations showed elevated serum transaminases (ALT 1.69 µkat/l, controls <0.69; AST 4.0 µkat/l, controls <0.63), creatine kinase (12 µkat/l, controls <2.3), uric acid (413 µmol/l, controls <340), triglycerides (6.7 mmol/l, controls <1.5), and lactate (5 mmol/l, controls <2.3), as well as mild coagulopathy (APTT 41.8 sec; antithrombin III 32%; protein C 44%; protein S 67%, controls 70–120) with low factor XI (19%; controls 70–120). In addition, a low fasting glucose level (2.4 mmol/l, controls >3.3) accompanied with ketonuria was noted. Abdominal ultrasound demonstrated mild hepatomegaly. Echocardiography was normal.

On his most recent routine clinical visit at the age of 10.5 years, the height of the patient remained far below the 3<sup>rd</sup> centile (126 cm; –2.73 SD), and he had severe obesity (51 kg; BMI 32; +5.7 SD) and a head circumference of 52 cm (20<sup>th</sup> centile). He displayed distractibility, hyperactivity and inattentiveness. At school, he struggles in reading and requires special assistance.

Psychological showed IQ decline in the patient, confirming mild intellectual disability (IQ 66). Abdominal ultrasound revealed one gallstone within the gallbladder and showed mild hepatomegaly. Treatments include dietary intervention to prevent hypoglycemia, allopurinol to keep uric acid within the reference range and levothyroxine substitution to normalize thyroid gland function. However, hepatopathy (transaminases 4- to 6-fold above upper control values), hyperlipidemia, mild coagulopathy and mildly elevated creatine kinase persist.

#### Ethics

This study was approved by the Ethics Committee of the General University Hospital in Prague. All blood and tissue samples were analyzed with informed consent from the parents of the patient.

#### CDG screening in serum

The patient was screened for defective *N*-glycosylation using IEF and SDS-PAGE of TRF. To detect alterations in protein *O*-glycosylation, IEF of ApoC-III was performed. The methods were carried out as previously described (Wopereis *et al.* 2007).

#### Enzyme assays

Cultivated fibroblasts were grown in DMEM (E15-843, PAA Laboratories GmbH) supplemented with 10% fetal calf serum and antibiotics (P11-002, PAA Laboratories GmbH). The cells were harvested by trypsinization and stored at  $-80^{\circ}\text{C}$ .

Phosphoglucomutase (PGM1) activity was measured in cultivated fibroblasts as previously described (Timal *et al.* 2012) with minor modifications. Activity assay was performed in the final volume of 500  $\mu\text{l}$  reaction mixture and 20  $\mu\text{l}$  of the cell extract, corresponding to 60–80  $\mu\text{g}$  of protein, was used for one measurement. Phosphoglucomutase activities were expressed as nmol/min/mg protein. The protein level was determined according to Lowry (Lowry *et al.* 1951). Phosphomannoisomerase (PMI) activity was measured as a control enzyme, according to (Van Schaftingen & Jaeken 1995).

#### Molecular analysis

Using the Primer3Plus software, primers were designed to amplify the 11 exons and their flanking regions of the *PGM1* gene. Primer sequences and specific PCR conditions are available on request. Genomic DNA was extracted from peripheral blood using the standard procedures of protease digestion, phenol-chloroform extraction and ethanol precipitation. PCR amplicons were then sequenced using the ABI PRISM 3100/3100-Avant Genetic Analyzer (Applied Biosystems). The frequency of the identified causal missense sequence variants was ascertained by PCR-RFLP (BssSI, Hpy188I) in 100 healthy Czech control samples. The web servers SIFT, PolyPhen-2, MutPred, SNPs&GO, nsSNPAna-

lyzer and PANTHER were used to evaluate the possible pathogenicity of the identified missense substitutions as described elsewhere (Sim *et al.* 2012; Adzhubei *et al.* 2010; Li *et al.* 2009; Calabrese *et al.* 2009; Bao *et al.* 2005; Mi *et al.* 2013).

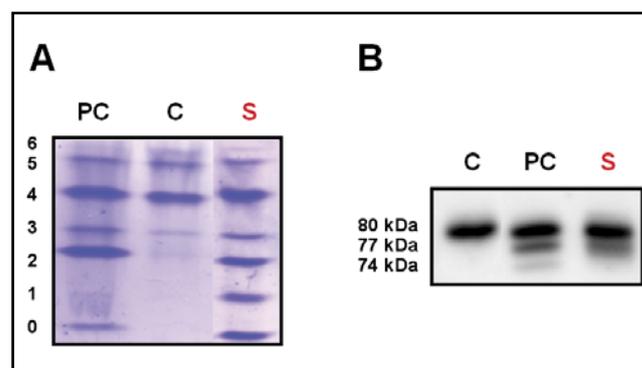
## RESULTS

#### Biochemical findings

Elevated levels of di-, mono- and asialotransferrin relative to fully glycosylated TRF forms were found in patient's serum (type 2 pattern; Figure 1A); a TRF polymorphism was excluded by IEF of TRF after neuraminidase treatment. Analysis of TRF by SDS-PAGE showed a shift towards lower molecular weights, representative of TRF isoforms with incomplete glycan chains (Figure 1B). A physiological distribution of ApoC-III glycoforms was detected. Fibroblasts from the patient showed a clearly deficient phosphoglucomutase activity (2.2 nmol/min per mg protein; controls 53–87;  $n=5$ ). IEF of TRF in serum samples taken after 5 months of lactose treatment showed no changes in the profile of TRF hyposialylated isoforms (data not shown).

#### Molecular analysis

Two heterozygous missense variations c.1010C>T (p.T337M) and c.1508G>A (p.R503Q) were identified in the *PGM1* gene of the patient. In 100 healthy Czech controls, neither of the sequence variants was detected by PCR-RFLP. Protein alignment revealed that the affected codons are evolutionary conserved. All applied online bioinformatics tools unanimously assessed the p.T337M and p.R503Q substitutions in the *PGM1* gene as disease-causing. Unfortunately, PANTHER was not able to classify the effect of p.R503Q.



**Fig. 1.** Biochemical screening for CDG in serum from the patient. **A:** Samples from a patient with PMM2-CDG syndrome (PC), a healthy control (C) and our subject (S) were analyzed by isoelectric focusing of transferrin (pH 5-7). Numbers on the left indicate sialic acid content on protein (asialo- to hexasialotransferrin). **B:** Transferrin analyzed by SDS-PAGE (12% gel) in sera of a control (C), PMM2-CDG patient (PC) and the subject (S).

## DISCUSSION

We report the first Czech patient with PGM1-CDG. He demonstrated multi-organ dysfunction, including impairment of the liver and gallbladder (hepatomegaly, hepatopathy and gallstone), muscle (exercise intolerance, elevated creatine kinase), the endocrine system (hypothyroidism, short stature, obesity and ketotic hypoglycemia), the brain (microcephaly, mild intellectual disability, hyperactivity and attention deficit), blood clotting and metabolism of uric acid (elevated uric acid), lipids (hyperlipidemia) and lactate (hyperlactacidemia). Although craniofacial dysmorphism, inverted nipples and atypical fat pads were not present, it is worthwhile to note that the patient had a cleft palate. Our finding of a mild impairment of the central nervous system is an uncommon feature that broadens the spectrum of PGM1-CDG previously reported as a non-neurological CDG (Pérez *et al.* 2013), however we cannot fully exclude other causes not related to PGM1 deficiency. In contrast to the four cases reported in the literature, dilated cardiomyopathy and attacks of myoglobinuria were not documented in our patient (Table 1).

Clinical and laboratory abnormalities such as short stature, hepatomegaly, hepatopathy, myopathy, hypertriglyceridemia, hyperlactacidemia, hyperuricemia and ketotic hypoglycemia prompted the diagnosis of a glycogen storage disease. The mild course of disease and absence of nephromegaly were not compatible with the diagnosis of GSD type I. GSD type III, VI and IX were excluded. Stojkovic *et al.* (Stojkovic *et al.* 2009) found

a remarkable similarity of PGM1 deficiency in their patient to McArdle's disease (glycogen storage disease type V) due to recurrent muscle cramps, exercise intolerance and attacks of myoglobinuria. The authors suggested that PGM1 deficiency be added to the list of rare glycogenolytic disorders and designated it as GSD type XIV (Stojkovic *et al.* 2009). In 2013, Preisler *et al.* observed increased exercise tolerance in the same PGM1-CDG patient after glucose infusion (Preisler *et al.* 2013).

Screening for CDG in our patient revealed a pathological transferrin profile, namely a type 2 pattern because of the increase of monosialotransferrin, pointing to a defect in the *N*-glycan remodeling in the Golgi. However it has to be noted that a type 2 pattern can hide a type 1 pattern (CDG I/II, as seen in galactosemia (Sturiale *et al.* 2005)). Thus, an associated *N*-glycan assembly defect is possible. Because the patient exhibited clinical symptoms suggestive of a glycogen storage disease, we investigated phosphoglucomutase activity. The PGM1 assay confirmed markedly decreased PGM1 activity (<5%) compared to healthy controls.

Based on molecular analysis, the patient was found to be a compound heterozygote for c.1010C>T (p.T337M) and c.1508G>A (p.R503Q) in the *PGM1* gene. The mutation c.1010C>T is unique and had not been characterized before. By utilizing selected *in silico* servers, the detected sequence variations were confirmed to be deleterious.

According to the study done by van Scherpenzeel and colleagues (van Scherpenzeel *et al.* 2012), galactose treatment in PGM1-CDG patients seems to be benefi-

**Tab. 1.** Clinical and laboratory data in our patient compared to previously described patients.

	Stojkovic <i>et al.</i> 2009	Timal <i>et al.</i> 2012	Timal <i>et al.</i> 2012	Pérez <i>et al.</i> 2012	Current report (2014)
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
<i>PGM1</i> gene mutations	c.343A>G/ c.1145-1G>C	c.1507C>T	c.415G>C	c.871G>A/ c.1144+3A>T	c.1010C>T/ c.1508G>A
Amino acid changes	n.d.	p.R503X	p.G121R	p.G291R/ p.R343fs	p.T337M/ p.R503Q
Gender	male	male	female	male	male
Age at time of publication	35 years	died at 8 years	16 years	13 years	10 years
Cleft palate/ Pierre-Robin sequence	n.d.	-	+/+	+/+*	+/-
Short stature	n.d.	n.d.	n.d.	+	+
Intellectual disability	n.d.	n.d.	n.d.	-	mild (IQ 66)
Myopathy	+	-	+	+	+
Dilated cardiomyopathy	n.d.	+	+	-	-
Hypoglycemia	n.d.	-	-	-	+
Hepatopathy	n.d.	+	+	+	+
Coagulopathy	n.d.	+	+	n.d.	+

abbreviations: n.d.: not determined; \* described as first branchial arch syndrome

cial. Because galactose as a prescription medication is not available in Czech Republic, we started the patient on a lactose-rich diet (40–50 g of lactose during therapy, 10 g before). Unfortunately, during the 5-month period of treatment, the diet modification did not result in either clinical improvement or amelioration of laboratory abnormalities, and his obesity severely worsened.

Our findings update the clinical and mutational spectrum of PGM1 deficiency and enable us to provide genetic counseling to the affected family.

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