Vietnam Journal of Biotechnology **20**(1): 1-7, 2022

A HOMOZYGOUS VARIANT IN *G6PC* IN A VIETNAMESE PATIENT WITH GLYCOGEN STORAGE DISEASE TYPE IA

Nguyen Huu Hong Thu[1], Nguyen Thi Khanh Ly[1], Nguyen Thuy Duong[1,2,✉]

[1]*Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam*
[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam*

✉To whom correspondence should be addressed. E-mail: tdnguyen@igr.ac.vn

Received: 22.10.2021
Accepted: 25.2.2022

SUMMARY

Glycogen storage diseases (GSDs) are rare inherited metabolic disorders characterized by the absence of required enzymes for the glycogen degradation metabolism. GSD can be divided into more than 12 types based on enzyme deficiency and affected tissues, in which glycogen storage disease type Ia (GSD1a or von Gierke disease) is a liver-affecting form. GSD1a is an autosomal recessive inherited disease caused by mutations in the *G6PC* gene on chromosome 17q21. The present study reports a Vietnamese family with a 6-month-old male patient diagnosed with type Ia glycogen storage disease. A homozygous variant in the *G6PC* gene (NM_000151.3: c.518T>C; p.L173P) was detected in the proband using a comprehensive glycogen storage disease panel. This variant has been previously reported in ClinVar (Accession ClinVar: VCV000640818.3). The segregation of the variant was confirmed in ten people of a 3-generation family using Sanger sequencing. The results showed both parents were heterozygous for the variant. In addition, the variant c.518T>C in the *G6PC* gene was predicted to be deleterious using in silico prediction tools (SIFT, PolyPhen-2, Proven, REVEL, and MutPred2). Our results could help doctors decide on appropriate treatment and diet for the disease. Moreover, the study is also a contribution to molecular studies on GSD1a.

Keywords: GSD1a, *G6PC*, gene panel, Sanger sequencing, Vietnam.

INTRODUCTION

Glycogen storage diseases (GSD) or glycogenosis are rare inherited metabolic disorders connected with the body's glycogen metabolism. GSD results in the absence of required enzymes for the glycogen degradation process (Kanungo *et al.*, 2018a). Missense mutations in the genes responsible for encoding enzymes involved in the glycogen metabolism pathway can potentially cause several modified disorders in defect glycogenolysis. There are over 12 types of GSD depending on enzyme deficiency and affected tissues (Özen, 2007). GSD type 0, I, III, IV, and VI are liver-affecting forms, while type II, IIIA, V, and VII are muscle-specific forms. Still, complex types can affect both liver, muscles, and other areas of the body (Rake *et al.*, 2006).

GSD-I or von Gierke disease is an autosomal recessive inherited disease in 1 out of 100,000 individuals (Chou *et al.*, 2005a; Bennett, Burchell, 2013). GSD-I comprises four subtypes: glycogen storage disease Ia (MIM# 232200), which causes defects in the glucose-6-phosphatase-α (G6Pase-α) catalytic unit; glycogen storage disease Ib (MIM# 232220), which has been linked to a reduced level of glucose-6-phosphate transporter (G6PT),

glycogen storage disease Ic (MIM # 232240) caused by mutations in glucose-6-phosphate translocase and glycogen storage disease Id in putative glucose transporter (Yang Chou, Mansfield, 1999; Beyzaei, Geramizadeh, 2019). Glycogen storage disease type Ia (GSD1a) is caused by mutations in the *G6PC* gene on chromosome 17q21 (Kishnani *et al.*, 2014a), which encodes glucose-6-phosphatase (G6Pase). GSD1A, the most common type of GSD-I, accounts for about 80% of GSD-I cases (Chou *et al.*, 2005b; Ferns, Halpern, 2018).

In GSD1a, poor glucose-6-phosphatase results in the stocking of glycogen in the liver as glucose-6-phosphate cannot be cleaved by phosphate molecules. Its conversion of fructose and galactose is required to transfer them to the liver, kidneys, and intestines (Kanungo *et al.*, 2018b). The biochemical pathway of glycolysis is shunted due to the accumulation of G6P in the liver (Chou *et al.*, 2017). Excess G6P leads to the production of pyruvate and acetyl-CoA, resulting in lactic acid stagnation and triglycerides (Kishnani *et al.*, 2014b). Alternatively, G6P could participate in the pentose phosphate pathway by producing ribose-5-phosphate and excess uric acid. Infants affected with GSD1A may suffer from developmental delays, lactic acidosis, hyperuricemia, hyperlipidemia, or hepatomegaly due to glycogen stored in the liver (Cohen *et al.*, 1985; Matern *et al.*, 2002).

Herein, we present a 6-month-old male patient with glycogen storage disease type Ia caused by a homozygous variant in *G6PC* (NM_000151.3: c.518T>C; p.L173P) using a targeted gene panel. Molecular studies on GSD1A could help doctors develop the right treatment for the patient and provide genetic counseling.

SUBJECT AND METHODS

Study subject

Blood samples from all family members were taken after obtaining consent forms from the proband's parents. The study was approved by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology (No: 2-2019/NCHG-HĐĐĐ).

Targeted gene panel sequencing

Genomic DNA was extracted and purified from the proband's peripheral blood and nine family members using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (ThermoFisher Scientific, USA), following the manufacturer's protocol. A total of 43 genes were selected to design the targeted gene panel (Table 1). Genomic DNA obtained from the proband was enriched for targeted regions and run on NovaSeq 6000 (Illumina, USA). Short reads were mapped onto the human reference genome (UCSC hg19) using Burrows-Wheeler Aligner tool version 0.7.12 (Li, Durbin, 2009). Variant callings were performed following Genome Analysis Toolkit Best Practices (https://www.broadinstitute.org/gatk/index.php).

Table 1. Comprehensive glycogen storage disease panel.

Gene	Disease	Inheritance
	Primary genes in the glycogen storage disorder panel	
AGL	Glycogen storage disease III	AR
ALDOA	Glycogen storage disease XII	AR
ENO3	Glycogen storage disease XIII	AR
FBP1	Fructose-1,6-bisphosphatase deficiency	AR
G6PC	Glycogen storage disease Ia	AR
GAA	Glycogen storage disease II	AR
GBE1	Glycogen storage disease IV	AR

GYG1	Glycogen storage disease XV, polyglucosan body myopathy 2	AR
GYS1	Glycogen storage disease 0	AR
GYS2	Glycogen storage disease 0A	AR
LAMP2	Danon disease, glycogen storage disease IIb	XL
LDHA	Glycogen storage disease XI	AR
PFKM	Glycogen storage disease VII	AR
PGAM2	Glycogen storage disease X	AR
PHKA1	Glycogen storage disease IXd	XL
PHKA2	Glycogen storage disease IXa	XL
PHKB	Glycogen storage disease IXb	AR
PHKG2	Glycogen storage disease IX	AR
PYGL	Glycogen storage disease VI	AR
PYGM	Glycogen storage disease V	AR
SLC2A2	Glycogen storage disease XI, Fanconi-Bickel syndrome, neonatal diabetes mellitus	AR
SLC37A4	Glycogen storage disease Ib	AR
Additional fatty acid oxidation genes related to metabolic diseases		
ACADM	Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	AR
ACADS	Short-chain acyl-CoA dehydrogenase (SCAD) deficiency	AR
ACADSB	2-methylbutyryl-CoA dehydrogenase deficiency	AR
ACADVL	Long chain acyl-CoA dehydrogenase (VLCAD) deficiency	AR
CPT1A	Carnitine palmitoyltransferase I (CPT1) deficiency	AR
CPT2	Carnitine palmitoyltransferase II (CPTII or CPT2) deficiency	AR
ETFA	Glutaric acidemia type II; GA II	AR
ETFB	Glutaric acidemia type II; GA II	AR
ETFDH	Multiple acyl-CoA dehydrogenase deficiency (MADD)	AR
HADH	Medium/short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (M/SCHAD)	AR
HADHA	Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency Mitochondrial trifunctional protein (MTP) deficiency	AR
HADHB	Mitochondrial trifunctional protein deficiency	AR
HMGCL	3-hydroxy-3-methylglutaryl (3HMG)-CoA lyase deficiency	AR
HMGCS2	3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase deficiency	AR
MLYCD	Malonyl-CoA decarboxylase deficiency	AR
NADK2	2,4-dienoyl-CoA reductase deficiency (correlated)	AR
SLC22A5	Primary carnitine deficiency	AR
SLC25A20	Carnitine-acylcarnitine translocase (CACT) deficiency	AR
SLC52A1	Riboflavin transporter deficiency (correlated)	AD
SLC52A2	Brown-Vialetto-Van Laere syndrome 2 (BVVLS2)	AR
SLC52A3	Brown-Vialetto-Van Laere syndrome 1 (BVVLS1)	AR

Note: XL: X-linked, AD: autosomal dominant, AR: autosomal recessive.

PCR and Sanger sequencing

The causative variant was validated on the proband (III-1) and his family members (I-1, I-2, I-3, I-4, II-1, II-2, II-3, II-4, II-5). The target site and flanking regions were amplified using designed primers (primer sequences are available upon request). Purified PCR products were sequenced using ABI Big Dye Terminator v3.1 Sequencing Standard Kit (Applied Biosystems, CA) on ABI 3500 Genetic Analyzer sequencer (Applied Biosystems).

In silico prediction

Conservation at amino acid position 173 was evaluated using a tool designed by the UCSC Genome Browser Group (https://genome.ucsc.edu/) (Blanchette, Kent, *et al.*, 2004). In addition, the pathogenicity of the variant was predicted using several tools, including SIFT (Sorting Intolerant From Tolerant) (Ng, Henikoff, 2003), PolyPhen 2 (Polymorphism Phenotyping v2) (Adzhubei *et al.*, 2013), Proven (Choi, Chan, 2015), REVEL (Ioannidis *et al.*, 2016), and MutPred2 (Pejaver *et al.*, 2020).

RESULTS

Patient presentation

A 6-month-old male Vietnamese patient was diagnosed with metabolic disorder/glycogen storage diseases in Vietnam National Children's Hospital. He had jaundice and swelling all over his body. He was in an entire edematous state, without convulsions or infections. Several examinations were performed on the patient, including biochemical blood analyses, physical examination, and abdominal sonography. The right liver's size measurement at ~140 mm and 3 cm under the ribs indicated that his liver was evenly enlarged through sonography. Biochemical analysis showed a high level of lactatemia 4.94 nmol/l (reference range: 0.6 - 2.20 nmol/l), alanine aminotransferase (ALT) 393 UI/l (reference range: 7 – 40 UI/l), aspartate aminotransferase (AST) 368 UI/l (reference range: 20 – 40 UI/l), and ammonia 93 µmol/l (reference range: 11 – 32 µmol/l). All clinical tests suggested liver dysfunction. Also, the patient suffered from severe hypoglycemia with a dangerously low fasting blood glucose level of 0.94 mmol/l (reference range: 3.9 - 5.5 mmol/l). The rest of the family members appeared to be healthy.

Genetic analysis

A homozygous missense variant (NM_000151.3: c.518T>C; p.L173P) was detected in the *G6PC* gene in the proband using gene panel sequencing. This variant has been previously reported in ClinVar (Accession ClinVar: VCV000640818.3). The segregation analysis was confirmed by Sanger sequencing. In the family, the individuals I-2, I-3, II-2, and II-3 carried a heterozygous variant while the others (I-1, I-4, II-1, II-4, and II-5) were wild-type (Figure 1A, 1B).

In silico analysis

The alignment of multiple sequences showed that the amino acid L173 was highly conserved across species (Figure 1C). *In silico* prediction tools, including SIFT, PolyPhen-2, Proven, REVEL, and MutPred2, were used to computationally predict the variant's effect on the protein function at the molecular level (Figure 1D). Overall, the missense variant *G6PC* p.L173P was predicted to be pathogenic.

DISCUSSION

We identified a homozygous missense variant c.518 T>C (p.L173P) in the *G6PC* gene. According to the public database of HGMD, more than 130 mutations in *G6PC* have been reported to be associated with GSD1a, including missense, nonsense, insertion/deletion, and splicing mutations. The c.518T>C in the *G6PC* gene alters the codon CTT to CCT, changing leucine to proline at position 173. Amino acid sequences from 170^{th} to 176^{th} containing two active sites (Arg-170, His-176) involved in a phosphorylated reaction. Therefore, Leu-173, located in the same conserved segment, possibly co-participates in the catalytic reaction.

Furthermore, the consensus results regarding pathogenicity obtained from all bioinformatics tools supported the strong effect of the mutation on G6Pase catalytic action. Therefore, changing leucine to proline may cause the dysfunction of G6Pase.

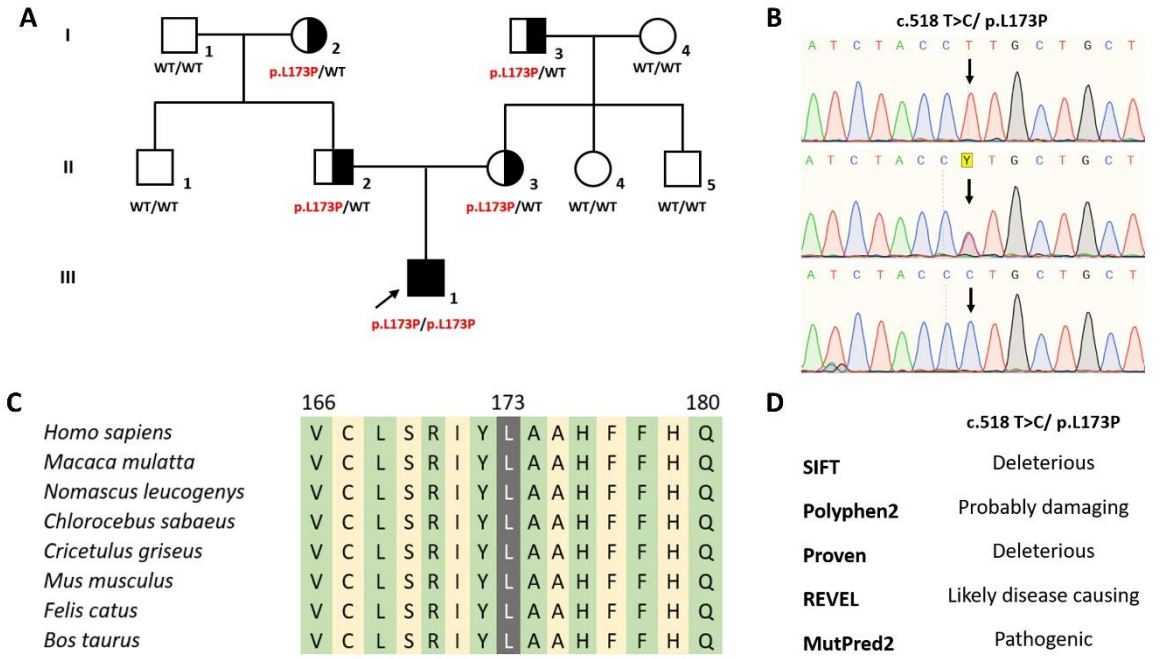

Figure 1. Pedigree analysis of the studied family. (A) the pedigree showing the segregation of the *G6PC* variant, (B) chromatograms for the variant p.L173P: wildtype, heterozygous missense variant, and homozygous missense variant; (C) Evolutionary conservation of G6PC p.L173 across different species. (D) Prediction of the effect of the missense variant.

The mutation c.518T>C (p.L173P) was reported to be pathogenic previously (Li *et al.*, 2007; Lu *et al.*, 2016). The first report was a 3-year-old female carrying compound heterozygotes p.L173P and p.R83H. The patient showed no clinical phenotypes until 2 years old and no sign of metabolic acidosis during treatment. However, the second report was from a 3-month-old Chinese female with GSD1a who also carried a homozygous missense mutation c.518T>C (p.L173P). She showed clinical phenotypes similar to our proband, including hypoglycemia, lactic acidosis, and hepatocellular dysfunction (Lu *et al.*, 2016). Patients were treated with a dietary therapy of raw cornstarch. Patients with milder conditions have shown improvement after treatments. Therefore, our patient could be treated with this treatment regimen since his symptoms are mild. If the patient was in the early stage of development and could have a low level of pancreatic amylase for hydrolyzing raw starch, dietary therapies should be considered. Due to the side effect of developing renal disease and HCA/HCC, leading to mortality in the long-term treatment (Weinstein, Wolfsdorf, 2002), liver-kidney transplantation or gene therapy will be recommended to the patient in the future. Several cases of GSD1a were reported to be metabolically normalized after liver transplantation, making this become a promising strategy for GSD1a treatment (Kasahara *et al.*, 2009). Somatic gene therapy is a promising approach using an adeno-associated virus (AAV) vector that carries murine (Ghosh *et al.*, 2006), canine (Chou *et al.*, 2002), and human G6Pase-α (Koeberl *et al.*, 2006). However, it is still an open research area to explore, develop and clinically test.

CONCLUSION

This article reports a 6-month-old male patient with a homozygous variant (NM_000151.3: c.518T>C; p.L173P) in the *G6PC* gene and typical symptoms of glycogen storage disease type Ia. The segregation of the variant was confirmed in the family using Sanger sequencing. Insight about genetic causes helps doctors diagnose precisely the type of metabolic disorder/ glycogen storage diseases, which are quite diverse to distinguish clinically, and unnecessary tests will not be executed. In addition, this variant can be used as a genetic marker for prenatal diagnosis.

Acknowledgments: *The study was supported by the Institute of Genome Research, Vietnam Academy of Science and Technology. We express our gratitude to all the family members who consented to participate in our study.*

REFERENCES

Adzhubei I, Jordan DM, Sunyaev SR (2013) Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet.*

Bennett K, Burchell A (2013) *Von Gierke Disease*. In: *Brenner's Encyclopedia of Genetics: Second Edition*. Academic Press, 304–307.

Beyzaei Z, Geramizadeh B (2019) Molecular diagnosis of glycogen storage disease type I: A review. *EXCLI J* 18: 30–46.

Blanchette M, Kent WJ, Riemer C, Elnitski L, Smith AFA, Roskin KM, Baertsch R, Rosenbloom K, Clawson H, Green ED, Haussler D, Miller W (2004) Aligning multiple genomic sequences with the threaded blockset aligner. *Genome Res* 14(4): 708–715.

Choi Y, Chan AP (2015) PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 31(16): 2745–2747.

Chou JY, Kim GY, Cho JH (2017) Recent development and gene therapy for glycogen storage disease type Ia. *Liver Res* 1(3): 174–180.

Chou J, Matern D, Mansfield B, Chen Y-T (2005a) Type I Glycogen Storage Diseases: Disorders of the Glucose-6- Phosphatase Complex. *Curr Mol Med* 2(2): 121–143.

Chou J, Matern D, Mansfield B, Chen Y-T (2005b) Type I Glycogen Storage Diseases: Disorders of the Glucose-6- Phosphatase Complex. *Curr Mol Med* 2(2): 121–143.

Chou JY, Zingone A, Pan CJ (2002) Adenovirus-mediated gene therapy in a mouse model of glycogen storage disease type 1a. In: *Eur J Pediatr, Supplement*.

Cohen JL, Vinik A, Faller J, Fox IH (1985) Hyperuricemia in glycogen storage disease type I. Contributions by hypoglycemia and hyperglucagonemia to increased urate production. *J Clin Invest* 75(1): 251–257.

Ferns JM, Halpern SH (2018) *Glycogen storage diseases*. In: *Consults in Obstetric Anesthesiology*. Academic Press, 235–240.

Ghosh A, Allamarvdasht M, Pan CJ, Sun MS, Mansfield BC, Byrne BJ, Chou JY (2006) Long-term correction of murine glycogen storage disease type la by recombinant adeno-associated virus-1-mediated gene transfer. *Gene Ther* 13(4): 321–329.

Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell IJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh CL, Wiklund F, Catalona WJ, Foulkes WD, Mandal D, Eeles RA, Kote-Jarai Z, Bustamante CD, Schaid DJ, Hastie T, Ostrander EA, Bailey-Wilson JE, Radivojac P, Thibodeau SN, Whittemore AS, Sieh W (2016) REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am J Hum Genet* 99(4): 877–885.

Kanungo S, Wells K, Tribett T, El-Gharbawy A (2018a) Glycogen metabolism and glycogen storage disorders. *Ann Transl Med* 6(24): 474–474.

Kanungo S, Wells K, Tribett T, El-Gharbawy A (2018b) Glycogen metabolism and glycogen storage disorders. *Ann Transl Med* 6(24): 474–474.

Kasahara M, Horikawa R, Sakamoto S, Shigeta T, Tanaka H, Fukuda A, Abe K, Yoshii K, Naiki Y, Kosaki R, Nakagawa A (2009) Living donor liver

transplantation for glycogen storage disease type Ib. *Liver Transplant* 15(12): 1867–1871.

Kishnani PS, Austin SL, Abdenur JE, Arn P, Bali DS, Boney A, Chung WK, Dagli AI, Dale D, Koeberl D, Somers MJ, Burns Wechsler S, Weinstein DA, Wolfsdorf JI, Watson MS (2014a) Diagnosis and management of glycogen storage disease type I: A practice guideline of the American College of Medical Genetics and Genomics. *Genet Med* 16(11): 1–29.

Kishnani PS, Austin SL, Abdenur JE, Arn P, Bali DS, Boney A, Chung WK, Dagli AI, Dale D, Koeberl D, Somers MJ, Burns Wechsler S, Weinstein DA, Wolfsdorf JI, Watson MS (2014b) Diagnosis and management of glycogen storage disease type I: A practice guideline of the American College of Medical Genetics and Genomics. *Genet Med* 16(11): 1–29.

Koeberl DD, Sun BD, Damodaran T V, Brown T, Millington DS, Benjamin DK, Bird A, Schneider A, Hillman S, Jackson M, Beaty RM, Chen YT (2006) Early, sustained efficacy of adeno-associated virus vector-mediated gene therapy in glycogen storage disease type Ia. *Gene Ther* 13(17): 1281–1289.

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14): 1754–1760.

Li DZ, Liao C, Tang XW (2007) Prenatal diagnosis of glycogen storage disease type Ia, presenting a new mutation in the glucose-6-phosphatase gene. *Prenat Diagn* 27(7): 685–686.

Lu Y, Wang L, Li J, Wu B, Wu H, Luo Y, Jin ZB, Shan X (2016) Molecular genetic analysis and phenotypic characteristics of a consanguineous family with glycogen storage disease type Ia. *Mol Med Rep* 14(4): 3251–3254.

Matern D, Seydewitz H, Bali D, Lang C, Chen Y-T (2002) Glycogen storage disease type I: diagnosis and phenotype/genotype correlation. *Eur J Pediatr* 161(1): S10–S19.

Ng PC, Henikoff S (2003) SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res* 31(13): 3812–3814.

Özen H (2007) Glycogen storage diseases: New perspectives. *World J Gastroenterol* 13(18): 2541–2553.

Pejaver V, Urresti J, Lugo-Martinez J, Pagel KA, Lin GN, Nam HJ, Mort M, Cooper DN, Sebat J, Iakoucheva LM, Mooney SD, Radivojac P (2020) Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. *Nat Commun* 11(1).

Rake JP, Visser G, Smit GPA (2006) *Disorders of carbohydrate and glycogen metabolism*. In: *Physician's Guide to the Treatment and Follow-Up of Metabolic Diseases*. Springer, Berlin, Heidelberg, 161–180.

Weinstein D, Wolfsdorf J (2002) Effect of continuous glucose therapy with uncooked cornstarch on the long-term clinical course of type 1a glycogen storage disease. *Eur J Pediatr* 161(1): S35–S39.

Yang Chou J, Mansfield BC (1999) Molecular genetics of type 1 glycogen storage diseases. *Trends Endocrinol Metab* 10(3): 104–113