Molecular Genetics and Metabolism Reports 26 (2021) 100702

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

Novel *GYS2* mutations in a Japanese patient with glycogen storage disease type 0a

Hiroyuki Iijima [a], Yasuhiko Ago [b], Ryoji Fujiki [c], Takaaki Takayanagi [d], Mitsuru Kubota [a,*]

[a] Department of General Pediatrics & Interdisciplinary Medicine, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
[b] Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan
[c] Department of Applied Genomics, Kazusa DNA Research Institute, 2-6-7 Kazusa-Kamatari, Kisarazu City, Chiba 292-0818, Japan
[d] Department of Pediatrics, Ebara Hospital, 4-5-10 Higashiyukigaya, Ohta-ku, Tokyo 145-0065, Japan

ARTICLE INFO

Keywords:
Diabetes mellitus
Fatty liver
Glycogen storage disease type 0a
Glycogenosis, glycogen synthase 2
Haploinsufficiency

ABSTRACT

Background: Glycogen storage disease type 0a (GSD 0a), caused by *GYS2* mutations, has a broad phenotypic spectrum, mostly associated with hypoglycemia. This disease has been characterized by the inability to store glycogen in the liver, leading to no hepatomegaly. Although the prevention of hypoglycemia has been considered the first therapeutic goal, the long-term complications remain unclear. In addition, few studies summarized clinical or biochemical features or examined genotype-phenotype correlation.

Case presentation: A 4-year-old Japanese boy was admitted to our hospital because of hypoglycemia. We suspected GSD 0a based on recurrent irritability episodes before feeding, fasting ketotic hypoglycemia, postprandial hyperglycemia/hyperlactatemia, and no hepatomegaly. Mutation analyses revealed novel mutations (p.His610fs and deletion of exons 8–10) in the *GYS2* gene. At 5 years old, his growth and development are normal. Fasting symptoms and hypoglycemia remain controlled by dietary management.

Review of literature: We summarized the clinical and biochemical features of 33 patients with GSD 0a and 27 different mutations in the *GYS2* gene. Nonspecific fasting symptoms (lethargy, drowsiness, nausea, and irritability) were found in 39% of patients, whereas 41% were asymptomatic. All patients had a combination of fasting ketotic hypoglycemia and postprandial hyperglycemia/hyperlactatemia. Hepatomegaly and hepatic steatosis were observed in 12% and 73% of patients. There was no genotype-phenotype correlation in patients with GSD 0a.

Conclusion: This is a clinical report of a Japanese GSD 0a patient with novel *GYS2* mutations and a review of cases. As secondary hepatic disorders may occur due to postprandial hyperglycemia, the treatment's ultimate goal is to prevent both hypoglycemia and hyperglycemia.

1. Introduction

Glycogen storage disease type 0a (GSD 0a; OMIM #240600) is a rare autosomal recessive metabolic disorder caused by mutations in the *GYS2* gene (*138571), which is located on chromosome 12p12 and consists of 16 exons [1]. The *GYS2* gene encodes hepatic glycogen synthase (GS), a key enzyme in glycogenesis, and catalyzes the addition of α-1,4-linked glucose to the growing glycogen chain. Impaired activity of GS results in the reduction of glycogen storage in the liver [2]. So far, only 37 cases with GSD 0a have been reported. Previous studies have described the broad phenotypic spectrum of GSD 0a — morning drowsiness and lethargy, seizure, development delay, growth failure, no hepatomegaly, fasting ketotic hypoglycemia, and postprandial hyperglycemia with hyperlactatemia. Although GSD 0a has been considered to have a good prognosis, few studies summarized clinical, genetic, and biochemical features. The long-term complications and genotype-phenotype correlation remain unclear. Here we report a Japanese case of GSD 0a caused by novel *GYS2* mutations and present a review of the literature to clarify the clinical picture of GSD 0a.

2. Case report

A 4-year-old boy was referred to our hospital because of hypoglycemia. He was the first child of non-consanguineous Japanese parents

* Corresponding author.
E-mail address: kubota-mt@ncchd.go.jp (M. Kubota).

https://doi.org/10.1016/j.ymgmr.2020.100702
Received 4 November 2020; Received in revised form 23 December 2020; Accepted 23 December 2020

and had no significant family history, including diabetes mellitus. He was born after an uneventful pregnancy of 40 weeks, weighing 2768 g, and had an uneventful postnatal course. However, he had recurrent episodes of irritability that improved with feeding. His baseline diet was high in carbohydrates, and he frequently consumed juice. He fed at night until he was 3-year-old. At age 4, fasting ketotic hypoglycemia (glucose, 30–50 mg/dL; β-hydroxybutyrate 2.7–4.5 mmol/L) without hyperinsulinism was found during his hospitalization due to acute tonsillitis. His height and weight were 105.7 cm (+0.5 SD) and 17.6 kg (+0.5 SD), respectively. His developmental milestones and physical examination were normal, without hepatomegaly or neurological abnormalities. The laboratory data in the fasting state indicated normal levels of serum transaminases (AST 29 IU/L and ALT 15 IU/L), creatine phosphokinase (89 U/L), ammonia (55 μmol/L), hemoglobin A_{1c} (4.8%), insulin-like growth factor-1 (96 ng/mL), without hyperlipidemia, hyperuricemia, or hypothyroidism. The acylcarnitine profile was normal. The amino acid analysis revealed a low plasma level of alanine (0.14 mmol/L) in the fasting state. Oral glucose tolerance test revealed an exaggerated and prolonged increase of blood lactate (0 min, 13 mg/dL; 60 min, 40 mg/dL; 120 min, 49 mg/dL) and glucose (0 min, 53 mg/dL; 60 min, 163 mg/dL; 120 min, 149 mg/dL). Glucagon stimulation test (0.03 mg/kg) on fasting yielded a poor response regarding blood glucose, with 49 mg/dL before, and 55 mg/dL 30 min after stimulation. A urine organic acid analysis revealed a massive lactate and pyruvate level after feeding, but was normal in the fasting state. Abdominal ultrasonography, echocardiography, and brain magnetic resonance imaging showed no abnormal findings. Psychological testing with the Wechsler Pre-School and Primary Scale of Intelligence Third Edition (WPPSI III) yielded a full-scale IQ score of 100. Although no liver biopsy was performed and GS activity was not measured, his clinical features and biochemical findings suggested GSD 0a. He was given uncooked cornstarch in the evening to prevent fasting hypoglycemia. We adjusted the dose of uncooked cornstarch to 30 g (1.7 g/kg) based on the results of continuous blood glucose monitoring. He was recommended to avoid skipping dinner, follow a low glycemic index carbohydrate and high protein diet, and have additional uncooked cornstarch before exercise. He was able to sleep through the night after beginning this dietary management. At 5 years old, his growth (height 113.1 cm, +0.6SD; weight 21.0 kg, +0.6SD) and physical examination were normal. Morning hypoglycemia and postprandial hyperglycemia were controlled with this regimen.

3. Materials and methods

3.1. Mutation analysis

Written informed consent in accordance with the Declaration of Helsinki was obtained from the patient's parents, in addition to consent for publication. Genomic DNA was extracted from the peripheral blood leukocytes of the patient. Mutation analysis was performed using the NextSeq Sequencing System (Illumina, San Diego, CA, USA) at the Kazusa DNA Research Institute based on a DNA panel consisting of 193 genes used in a previous study [3]. Variants in protein-coding exonic regions and their 10-base flanking regions were detected by the method previously described [4]. To confirm whether a large deletion exists, a long PCR was performed to amplify exons 7, 8, 9, and 10 of the *GYS2* gene with the following primers: forward, 5′-TTGTTACTGTTGCTGTATTTCAT-3′; and reverse, 5′-AATGTTCCAAATTAGCACATTCC-3′. DNA electrophoresis in 1% agarose gel was performed to confirm the size of amplified fragments. A shorter DNA fragment than the control was analyzed by Sanger sequencing to detect the break point.

3.2. Review of literature

We collected medical history, clinical symptoms, biochemical data, pathological data, and *GYS2* gene mutation data in patients with GSD 0a reported by December 2020. Nonspecific symptoms such as lethargy, drowsiness, nausea, and irritability that occurred in the fasting state and improved with diet were defined as nonspecific fasting symptoms.

4. Results

4.1. Mutation analysis

Next-generation sequencing revealed a heterozygous novel mutation of *GYS2*, c.1827dupA (p.His610fs). In addition, a heterozygous deletion of exons 8–10 of *GYS2* was suspected because the depth of sequencing coverage was low compared with a control (Fig. 1A). Depth of coverage for each nucleotide position implied not only a large deletion of exons 8 and 9, but also that the 5′ part of exon 10 was deleted heterozygously (Fig. 1B).

The long PCR amplified another short DNA fragment that was not amplified from the control sample, which is consistent with the suspicion of a heterozygous large deletion (Fig. 2A). Subsequently, Sanger sequencing of the amplified shorter DNA fragment revealed the deletion, c.1063-591_1262del1947 (Fig. 2B).

4.2. Review of literature

We summarized 33 patients with GSD 0a from 27 different families (including our patient) (Table 1). Nonspecific fasting symptoms such as lethargy, drowsiness, nausea, and irritability were the most common (39%). Patients also presented with short stature (29%), seizures (22%), mental retardation (15%), and hepatomegaly (12%). Forty percent of the patients were asymptomatic and diagnosed from family history or laboratory tests during the differential diagnosis process. The age of onset for symptomatic patients ranged from 0 to 4 years old, with more than half around one year old, while the median age of diagnosis was five years old. Most of the symptomatic patients presented hypoglycemia symptoms in the morning after the night feeding cessation.

Fasting ketotic hypoglycemia and postprandial hyperglycemia/hyperlactatemia were found in 100% of patients. Glucosuria and hepatic steatosis were identified in 67% and 73% of patients. Normal glycemic response to glucagon was observed in 31% (fasting state) and 89% (fed state). Liver biopsy showed a decrease in glycogen content (89%) and GS activity (100%). A summary of *GYS2* gene mutations is presented in Fig. 3.

5. Discussion

This patient had recurrent episodes of irritability due to fasting hypoglycemia. GSD 0a was clinically diagnosed based on fasting ketotic hypoglycemia, postprandial hyperglycemia/hyperlactatemia, and no hepatomegaly. Genetic analyses revealed a heterozygous novel mutation, c.1827dupA (p.His610fs), and a heterozygous deletion, c. 1063-591_1262del1947 (exons 8–10) in the *GYS2* gene. We regarded these mutations as pathogenic because the former is a frameshift mutation whose downstream mutation (p.Asp668Asn) was reported to be pathogenic [5]; the latter is a large deletion, including ADP-binding pocket [2] and a missense mutation p.Gly382Glu in exon 8 was pathogenic [6].

5.1. Clinical and biochemical features

This review shows that symptoms of GSD 0a are variable and often nonspecific, and most of the symptomatic patients took several years from onset to diagnosis. These findings are consistent with previous reports of difficulty in diagnosing GSD 0a. However, we also found that symptoms of GSD 0a were likely to occur around the age of one year after the night feeding cessation. The late presentation and normal growth of our patient may have been contributed by frequent high-carbohydrate diets and nighttime feeding. Physicians should consider this diagnosis when a patient has hypoglycemic symptoms in the morning after stopping nighttime feeding.

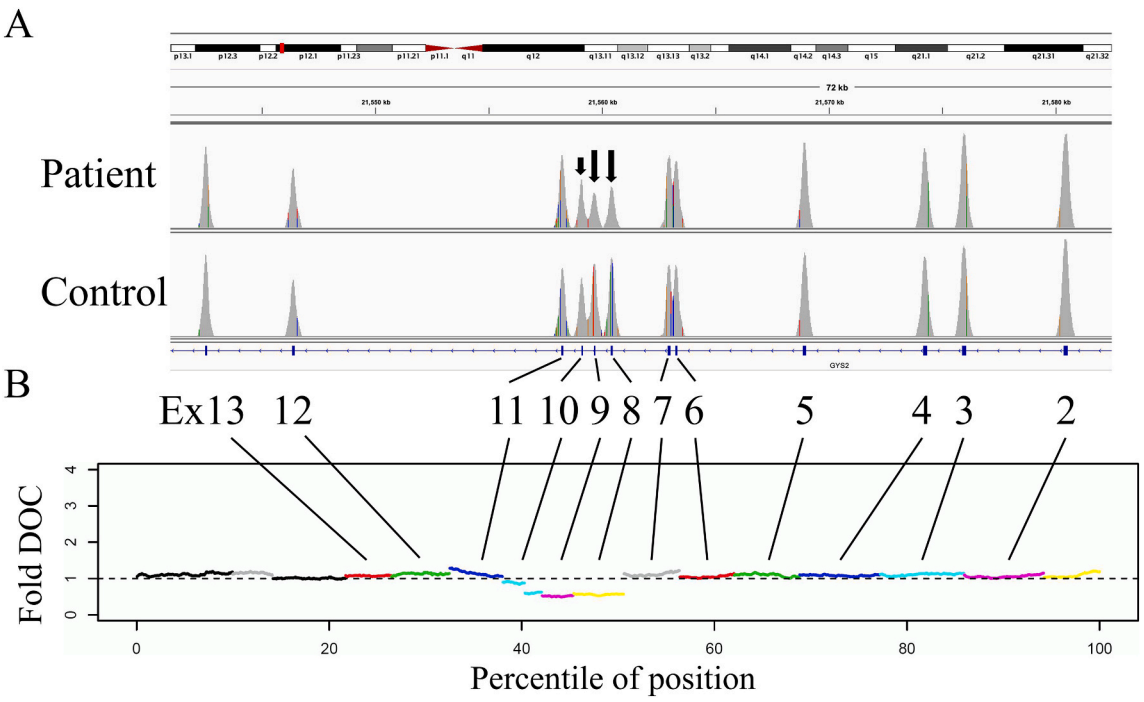

Fig. 1. A. Representation of the next-generation sequencing data coverage depth using the Integrative Genomics Viewer. A suspected heterozygous deletion of *GYS2* exons 8–10 was detected.
B. Representation of the depth of coverage for each nucleotide position in all exons of the *GYS2* gene. The horizontal axis indicates where each nucleotide is located in the protein-coding exonic regions, as a percentile. The vertical axis shows how many folds the DOC (depth of coverage) for each nucleotide is compared to that for the control. The exons next to each other are painted in different colors to distinguish them. A heterozygous deletion of exons 8, 9, and the 5′ region of exon 10 was suspected.

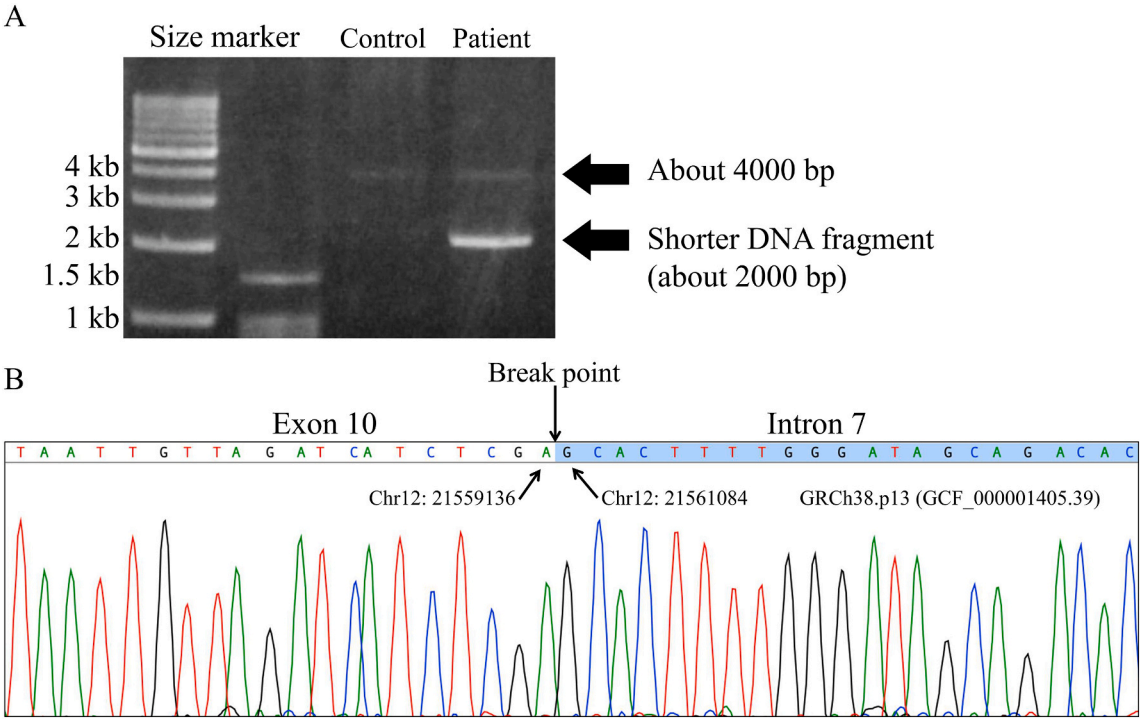

Fig. 2. A. The DNA electrophoresis in 1% agarose gel.
In the control case, only a single DNA fragment (approximately 4000 bp) was observed. On the contrary, a shorter DNA fragment (>2000 bp) was clearly observed in this patient.
B. DNA sequencing data using the Sanger method.
Sequencing analysis of the shorter DNA fragment (over 2000 bp) indicated breakpoints in the intron 7 (c.1063–592) and in the exon 10 (c.1263) in the *GYS2* gene.

Table 1
Summary of findings in patients with GSD 0a.

Patients	Sex	Mode of presentaiton	Age at		Nonspecific	Seizure	Mental	Short statue
			symptoms onset	diagnosis	fasting symptoms		retardation	
1 [9,14]	Male	Mental retardation	0.7	2.2	+	+	+	+
2 [9,14]	Male	Seizure	0.6	2.2	+	+	+	+
3 [9,14]	Female	Family history	−	0.8	−	−	−	NA
4 [9,14]	Male	Family history	−	3	−	−	−	NA
5 [1,10,19]	Female	Seizure	0–1	9	+	+	+	+
6 [1,16]	Male	Family history	0–3	13	+	−	−	−
7 [11]	Male	Morning lethargy	1.8	3.6	+	−	−	NA
8 [11]	Male	Family history	−	2.8	−	−	−	−
9 [1,12]	Female	Morning lethargy	3.5	4.4	+	−	−	NA
10 [1,12]	Male	Incidental findings	2	3.5	+	−	−	−
11 [1,12]	Male	Family history	−	4.7	−	−	−	−
12 [1]	Male	Short statue	0–4	7	−	−	−	+
13 [1]	Female	Incidental findings	NA	5	−	−	+	NA
14 [1]	Male	Family history	−	3	−	−	−	NA
15 [1]	Female	Family history	−	1	−	−	−	NA
16 [13]	Female	Seizure	1.3	6	−	+	−	−
17 [18]	Male	Incidental findings	−	5	−	−	−	−
18 [18]	Female	Incidental findings	−	9	−	−	−	−
19 [7]	Female	Incidental findings	3	7	+	−	−	−
20 [15]	Male	Seizure	0.7	0.7	−	+	−	NA
21 [15]	Female	Incidental findings	−	1.2	−	−	−	NA
22 [24]	Female	Morning lethargy	0.5	6	+	−	−	−
23 [28]	Male	Seizure	2.5	5	−	+	−	−
24 [25]	Female	Fasting irritability	1	7	+	−	−	−
25 [17]	Male	Incidental findings	−	7	−	−	−	−
26 [6]	Female	Seizure	0–4	5	NA	+	−	+
27 [6]	Male	Incidental findings	−	1.8	−	−	−	−
28 [6]	Female	Incidental findings	−	6.5	−	−	−	NA
29 [29]	NA	NA	NA	1–11	NA	NA	+	NA
30 [26]	Male	Incidental findings	−	4	−	−	−	NA
31 [8]	Female	Short statue	NA	5	−	−	−	+
32 [30]	Female	Morning lethargy	1.1	1.1–2	+	−	−	−
32 [*]	Male	Incidental findings	3	4.5	+	−	−	−
Total 33	M: F:NA 17:15:1	Symptomatic: 59% (onset age 0–4) Asymptomatic: 41% -		median 5 (0.7–13) Median 3 (0.8–9)	39% (12/31)	22% (7/32)	15% (5/33)	29% (6/21)

Patients	Fasting state			Postprandial state		Glucosuria	Glucagon response	
	Hypoglycemia	Ketonemia/Ketonuria	Hypoalaninemia	Hyperglycemia	Hyperlactatemia		Fasting	Postprandial
1 [9,14]	+	+	NA	+	+	+	poor	normal
2 [9,14]	+	+	NA	+	+	NA	poor	normal
3 [9,14]	+	NA	NA	+	+	NA	normal	normal
4 [9,14]	+	NA	NA	+	+	NA	normal	normal
5 [1,10,19]	+	+	+	+	+	+	poor	normal
6 [1,16]	+	+	+	+	+	NA	NA	NA
7 [11]	+	+	+	+	+	−	poor	normal
8 [11]	+	+	NA	+	+	NA	NA	NA
9 [1,12]	+	+	+	+	+	NA	NA	NA
10 [1,12]	+	+	+	+	+	NA	NA	NA
11 [1,12]	+	+	+	+	+	NA	NA	NA
12 [1]	+	+	−	+	+	NA	NA	NA
13 [1]	+	NA	NA	+	NA	NA	NA	NA
14 [1]	+	NA	NA	+	NA	NA	NA	NA
15 [1]	+	NA	NA	+	NA	NA	NA	NA
16 [13]	+	+	NA	+	+	NA	poor	normal
17 [18]	+	+	NA	+	+	+	normal	NA
18 [18]	+	+	NA	+	+	+	NA	NA
19 [7]	+	+	+	+	+	NA	normal	NA
20 [15]	+	+	NA	+	+	NA	poor	normal
21 [15]	+	NA	NA	+	NA	NA	poor	poor*
22 [24]	+	+	NA	+	+	NA	poor	NA
23 [28]	+	+	NA	+	+	NA	NA	NA
24 [25]	+	+	NA	+	+	NA	NA	NA
25 [17]	+	+	+	+	+	NA	poor	NA
26 [6]	+	+	NA	+	+	NA	NA	NA
27 [6]	+	+	NA	+	+	NA	NA	NA
28 [6]	+	+	NA	+	+	NA	NA	NA
29 [29]	+	+	NA	NA	NA	NA	NA	NA
30 [26]	+	+	NA	+	+	NA	poor	NA
31 [8]	+	+	NA	+	+	NA	normal	NA
32 [30]	+	+	NA	+	NA	NA	NA	NA

(*continued on next page*)

Table 1 (*continued*)

Patients	Fasting state			Postprandial state		Glucosuria	Glucagon response	
	Hypoglycemia	Ketonemia/Ketonuria	Hypoalaninemia	Hyperglycemia	Hyperlactatemia		Fasting	Postprandial
32 [*]	+	+	+	+	+	−	poor	NA
Total 33	100% (33/33)	100% (27/27)	90% (9/10)	100% (32/32)	100% (27/27)	67% (4/6)	69% (11/16)	11% (1/9)

Patients	Hepatomegaly	Hepatic transaminase	Hyperlipidemia	Hepatic steatosis	Liver biopsy	
					Glygogen content	Glycogen synthase activity
1 [9,14]	−	NA	NA	+	Reduced	Reduced
2 [9,14]	+	NA	NA	NA	NA	NA
3 [9,14]	−	NA	NA	NA	NA	NA
4 [9,14]	−	NA	NA	NA	NA	NA
5 [1,10,19]	+	NA	NA	NA	Reduced	Reduced
6 [1,16]	−	NA	NA	NA	NA	NA
7 [11]	+	Mild elevation	−	+	Reduced	Reduced
8 [11]	−	NA	NA	NA	NA	NA
9 [1,12]	−	NA	NA	+	Reduced	Reduced
10 [1,12]	−	NA	NA	+	Reduced	Reduced
11 [1,12]	−	NA	NA	NA	NA	NA
12 [1]	NA	NA	NA	NA	Normal	Reduced
13 [1]	NA	NA	NA	NA	Reduced	NA
14 [1]	NA	NA	NA	NA	NA	NA
15 [1]	NA	NA	NA	NA	NA	NA
16 [13]	−	NA	NA	−	Reduced	Reduced
17 [18]	−	NA	NA	NA	NA	NA
18 [18]	−	NA	NA	NA	NA	NA
19 [7]	−	NA	+	+	Reduced	Reduced
20 [15]	−	NA	NA	NA	NA	NA
21 [15]	−	NA	NA	NA	NA	NA
22 [24]	−	NA	NA	NA	NA	NA
23 [28]	–	NA	+	–	NA	NA
24 [25]	−	NA	NA	NA	NA	NA
25 [17]	NA	normal	−	NA	NA	NA
26 [6]	−	normal	+	+	NA	NA
27 [6]	−	normal	+	+	NA	NA
28 [6]	−	normal	+	+	NA	NA
29 [29]	NA	NA	NA	NA	NA	NA
30 [26]	−	NA	NA	NA	NA	NA
31 [8]	−	NA	−	NA	NA	NA
32 [30]	NA	Mild elevation	+	NA	NA	NA
32 [*]	−	normal	−	−	NA	NA
Total	12% (3/26)	29% (2/7)	60% (6/10)	73% (8/11)	89% (8/9)	100% (8/8)

NA, not available. The mutations identified in our patient are indicated by an asterisk. References are denoted in parentheses.

Biochemical findings can help diagnose GSD 0a (Table 1). Impaired GS in the liver causes insufficient glycogen accumulation, leading to inadequate glycogenolysis, resulting in fasting hypoglycemia. Ketonemia and ketonuria also develop with fasting because fatty acid oxidation is intact. Postprandial hyperglycemia results from excess glucose as a consequence of the inability to store glucose as glycogen. Hyperglycemia leads to overloading of the glycolytic pathway and can cause hyperlactatemia and hyperlipidemia. The combination of fasting ketotic hypoglycemia and postprandial hyperglycemia/hyperlactatemia strongly suggests this diagnosis. Glycemic response to glucagon in the fasting state was often poor, but normal in some cases. Previous studies have presumed that glycogen stores were not completely depleted despite the decrease in GS activity, and pharmacological amounts of glucagon were able to stimulate their breakdown [7,8]. A small amount of glycogen was observed in liver biopsy [1,7,9–13], and there was also a case with normal glycogen content [1]. In the fed state, response to glucagon was almost normal [9–11,13–15]. Although only one case showed a poor response after a meal [15], this was probably due to her high blood glucose level of 215 mg/dL before glucagon administration.

5.2. Long-term management and treatment

In our review, most symptoms (nonspecific fasting symptoms, short stature, seizures, and mental retardation) were related to hypoglycemia. Previous reports have shown that the goal of treatment is to prevent hypoglycemia by dietary management [5,6,8,16,17]. In addition, it has been recognized that the management of GSD 0a is straightforward. Some reports assumed that patients with GSD 0a suffer relatively little brain damage even when exposed to severe hypoglycemia and usually have normal intellectual outcomes [5,7,12,18]. Seizures and mental retardation have been described in only 20% and 13% of patients in our review. Even in our case, although severe hypoglycemia (30–40 mg/dL) was common in the morning before treatment, his intelligence was normal without seizures or mental retardation. Increased plasma ketones formed from fatty acid oxidation provide alternative energy sources and may offer some protection to the brain during hypoglycemia. It is also known that tolerance to fasting improves with age in GSD 0a patients [7,8,19].

However, we propose that clinicians manage patients with GSD 0a for hypoglycemia and glucose toxicity due to hyperglycemia. It is generally accepted that impaired glucose tolerance has a high risk of type 2 diabetes mellitus, cardiovascular disease, neuropathies, and collateral damage to other organs [20,21]. Patients with GSD 0a instinctively or as a treatment tend to compensate for their hypoglycemia with frequent meals, resulting in excessive postprandial blood-glucose exposure. When pancreatic β-cells are chronically exposed to hyperglycemia, their insulin secretory function gradually declines [22]. In the only adult patient, circulating insulin levels were suppressed even during pregnancy, particularly during hyperglycemia [19]. In addition, genome-wide association study of 26,676 diabetic cases and 132,532 controls suggested that the single nucleotide variant rs10841855 at the *GYS2* locus was associated with type 2 diabetes mellitus [23].

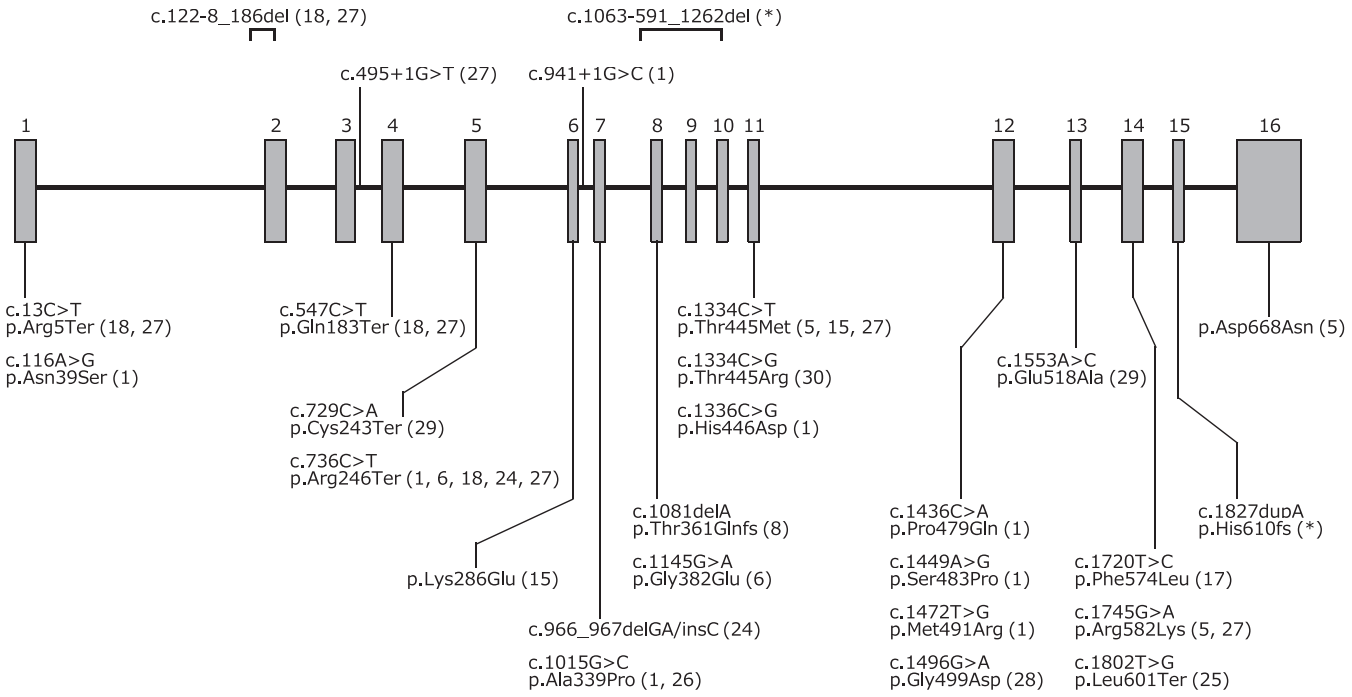

Fig. 3. Organization of the GYS2 gene, and disease-associated mutations hitherto reported.
The number above each box indicates the exon number. References are denoted in parentheses. The mutations identified in our patient are indicated by an asterisk [27].

We also need to be aware of fatty liver in these patients. Our review showed that 73% of patients had hepatic steatosis, and 60% had hyperlipidemia. In addition, although the absence of hepatomegaly is characteristic of GSD type 0, we noted hepatomegaly in 12% of patients (patients 2, 5, 7). Patient 7 had moderate hepatomegaly (2.5 cm below the right costal margin) and a mild elevation of serum transaminases (AST 90–145 IU/L and ALT 60–134 IU/L). A liver biopsy of this patient revealed hepatic steatosis with the accumulation of hepatocellular lipid droplets.

There are few reports of adolescent and adult patients with GSD0a, and there is little evidence that mutations in the *GYS2* gene cause diabetes or fatty liver in the future. However, they cannot convert excess glucose after a meal into glycogen, and the surplus causes hyperglycemia, glucosuria, and hypertriglyceridemia, which theoretically lead to diabetes mellitus and fatty liver.

5.3. Genotype-phenotype correlation

Thus far, although 27 different mutations have been identified, including our case, there were no genotype-phenotype relationship analyses. We reviewed disease-associated mutations hitherto reported (Fig. 3), and propose that there is no genotype-phenotype correlation in patients with GSD 0a. There were asymptomatic patients with obvious loss-of-function mutations, and patients with the same mutations had different symptoms. For example, one patient (patient 17 in Table 1) with compound heterozygous nonsense mutations (p.Arg5Ter in exon 1 and p.Gln183Ter in exon 4) had diagnosed GSD 0a at 5 years old, presenting with glucosuria. He was asymptomatic and had neither mental retardation nor seizures [18]. The other three patients (12, 26, and 27) with the same homozygous mutation (p.Arg246Ter in exon 5) had different phenotypes. Only patient 26 had recurrent hypoglycemic convulsive seizures, and the other two were asymptomatic [1,6]. In siblings (patients 5 and 6), the younger sister had mental retardation and repeated morning convulsions from 7 years old to 9 years old when she started dietary treatment; her older brother became less responsive and drowsy in the morning until the first meal of the day, his symptoms occurred in early childhood and improved by 3 years old without any intervention [1,10,16]. It is crucial to screen a patient's siblings even if they are asymptomatic because patients with the same mutations have different phenotypes.

In addition, we surmised haploinsufficiency for *GYS2*. There have been many reports of impaired glucose tolerance in the families of GSD0a patients. The original description by Lewis et al. demonstrated that the patient's father was investigated for glucosuria when 8 years old and showed reduced glucose tolerance [9]. A parent who carried a heterozygous mutation c.941 + 1G > C developed hypoglycemia during prolonged fasting [1]. Another parent who carried a heterozygous mutation 966_967 delGAinsC experienced some episodes of hypoglycemia (45–49 mg/dL) after moderate alcohol ingestion and was treated by glucose infusion during adolescence [24].

However, there is a limitation to our analysis of the genotype-phenotype relationship due to racial bias. Most of the GSD 0a patients have been reported from Europe and the United States; therefore, our Japanese case is valuable data on GSD 0a. Further studies are needed to confirm whether the regional bias in patients is due to racial differences.

6. Conclusion

We report a Japanese GSD 0a patient with novel *GYS2* mutations. As hepatic steatosis and hepatomegaly may occur secondarily due to postprandial hyperglycemia, the treatment's ultimate goal is to prevent both hypoglycemia and hyperglycemia.

Declaration of Competing Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments

We thank Dr. Hideo Sasai (Department of Pediatrics, Graduate School of Medicine, Gifu University), Mina Nakama (Clinical Genetics Center, Gifu University Hospital), and Dr. Osamu Ohara (Department of Applied Genomics Kazusa DNA Research Institute) for the interpretation of and comments about mutation analysis.

This research was partially supported by the Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development, AMED (Grant Number JP19ek0109276/JP20ek0109482).

References

[1] M. Orho, N.U. Bosshard, N.R. Buist, R. Gitzelmann, A. Aynsley-Green, P. Blümel, M.C. Gannon, F.Q. Nuttall, L.C. Groop, Mutations in the liver glycogen synthase gene in children with hypoglycemia due to glycogen storage disease type 0, J. Clin. Invest. 102 (1998) 507–515, https://doi.org/10.1172/JCI2890.

[2] A. Buschiazzo, J.E. Ugalde, M.E. Guerin, W. Shepard, R.A. Ugalde, P.M. Alzari, Crystal structure of glycogen synthase: homologous enzymes catalyze glycogen synthesis and degradation, EMBO J. 23 (2004) 3196–3205, https://doi.org/10.1038/sj.emboj.7600324.

[3] Y. Ago, H. Otsuka, H. Sasai, E. Abdelkreem, M. Nakama, Y. Aoyama, H. Matsumoto, R. Fujiki, O. Ohara, K. Akiyama, K. Fukui, Y. Watanabe, Y. Nakajima, H. Ohnishi, T. Ito, T. Fukao, Japanese patients with mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency: in vitro functional analysis of five novel HMGCS2 mutations, Exp. Ther. Med. 20 (2020), https://doi.org/10.3892/etm.2020.9166.

[4] R. Fujiki, M. Ikeda, A. Yoshida, A. Maeda, Y. Yao, M. Nishimura, K. Matsushita, T. Ichikawa, T. Tanaka, H. Morisaki, T. Morisaki, O. Ohara, Assessing the accuracy of variant detection in cost-effective gene panel testing by next-generation sequencing, J. Mol. Diagn. 20 (2018) 572–582, https://doi.org/10.1016/j.jmoldx.2018.04.004.

[5] D.A. Weinstein, C.E. Correia, A.C. Saunders, J.I. Wolfsdorf, Hepatic glycogen synthase deficiency: an infrequently recognized cause of ketotic hypoglycemia, Mol. Genet. Metab. 87 (2006) 284–288, https://doi.org/10.1016/j.ymgme.2005.10.006.

[6] Ç.S. Kasapkara, Z. Aycan, E. Açoğlu, S. Senel, M.M. Oguz, S. Ceylaner, The variable clinical phenotype of three patients with hepatic glycogen synthase deficiency, J. Pediatr. Endocrinol. Metab. 30 (2017) 459–462, https://doi.org/10.1515/jpem-2016-0317.

[7] A.M. Laberge, G.A. Mitchell, G. Werve, M. Lambert, Long-term follow-up of a new case of liver glycogen synthase deficiency, Am. J. Med. Genet. A 120A (2003) 19–22, https://doi.org/10.1002/ajmg.a.20110.

[8] B. Hacıhamdioğlu, G. Özgürhan, B. Çaran, E. Meydan-Aksanlı, E. Keskin, Glycogen storage disease type 0 due to a novel frameshift mutation in glycogen synthase 2 (GYS2) gene in a child presenting with fasting hypoglycemia and postprandial hyperglycemia, Turk. J. Pediatr. 60 (2018) 581–583, https://doi.org/10.24953/turkjped.2018.05.018.

[9] G.M. Lewis, J. Spencer-Peet, K.M. Stewart, Infantile hypoglycaemia due to inherited deficiency of glycogen synthetase in liver, Arch. Dis. Child. 38 (1963) 40–48, https://doi.org/10.1136/adc.38.197.40.

[10] A. Aynsley-Green, D.H. Williamson, R. Gitzelmann, Hepatic glycogen synthetase deficiency. Definition of syndrome from metabolic and enzyme studies on a 9-year-old girl, Arch. Dis. Child. 52 (1977) 573–579, https://doi.org/10.1136/adc.52.7.573.

[11] R.D. de Kremer, A.P. de Capra, C.D. de Boldini, E. Hliba, I. Givogri, Hepatic glycogen synthetase deficiency or glycogen storage disease-zero. Mild phenotype with partial enzymatic defect, Medicina 50 (1990) 299–309.

[12] R. Gitzelmann, M.A. Spycher, G. Feil, J. Müller, B. Seilnacht, M. Stahl, N. U. Bosshard, Liver glycogen synthase deficiency: a rarely diagnosed entity, Eur. J. Pediatr. 155 (1996) 561–567, https://doi.org/10.1007/BF01957905.

[13] S.L. Rutledge, J. Atchison, N.U. Bosshard, B. Steinmann, Case report: liver glycogen synthase deficiency—a cause of ketotic hypoglycemia, Pediatrics. 108 (2001) 495–497, https://doi.org/10.1542/peds.108.2.495.

[14] J.R. Dykes, J. Spencer-Peet, Hepatic glycogen synthetase deficiency. Further studies on a family, Arch. Dis. Child. 47 (1972) 558–563, https://doi.org/10.1136/adc.47.254.558.

[15] R. Spiegel, J. Mahamid, M. Orho-Melander, D. Miron, Y. Horovitz, The variable clinical phenotype of liver glycogen synthase deficiency, J. Pediatr. Endocrinol. Metab. 20 (2007) 1339–1342, https://doi.org/10.1515/jpem-2016-0317.

[16] A. Aynsley-Green, D.H. Williamson, R. Gitzelmann, Asymptomatic hepatic glycogen-synthetase deficiency, Lancet 1 (1978) 147–148, https://doi.org/10.1016/s0140-6736(78)90442-7.

[17] E. Szymańska, D. Rokicki, U. Wątrobinska, E. Ciara, P. Halat, R. Płoski, A. Tylki-Szymańka, Pediatric patient with hyperketotic hypoglycemia diagnosed with glycogen synthase deficiency due to the novel homozygous mutation in GYS2, Mol. Genet. Metab. Rep. 4 (2015) 83–86, https://doi.org/10.1016/j.ymgmr.2015.07.003.

[18] B.E. Bachrach, D.A. Weinstein, M. Orho-Melander, A. Burgess, J.I. Wolfsdorf, Glycogen synthase deficiency (glycogen storage disease type 0) presenting with hyperglycemia and glucosuria: report of three new mutations, J. Pediatr. 140 (2002) 781–783, https://doi.org/10.1067/mpd.2002.124317.

[19] B.M. Byrne, M.D. Gillmer, R.C. Turner, A. Aynsley-Green, Glucose homeostasis in adulthood and in pregnancy in a patient with hepatic glycogen synthetase deficiency, Br. J. Obstet. Gynaecol. 102 (1995) 931–933, https://doi.org/10.1111/j.1471-0528.1995.tb10886.x.

[20] A.G. Tabák, C. Herder, W. Rathmann, E.J. Brunner, M. Kivimäki, Prediabetes: a high-risk state for developing diabetes, Lancet 379 (2012) 2279–2290, https://doi.org/10.1016/S0140-6736(12)60283-9.

[21] M. Kabootari, M. Hasheminia, F. Azizi, M. Mirbolouk, F. Hadaegh, Change in glucose intolerance status and risk of incident cardiovascular disease: tehran lipid and glucose study, Cardiovasc. Diabetol. 19 (2020) 41, https://doi.org/10.1186/s12933-020-01017-4.

[22] H. Kaneto, Pancreatic β-cell glucose toxicity in type 2 diabetes mellitus, Curr. Diabetes Rev. 11 (2015) 2–6, https://doi.org/10.2174/1573399811666141216160217.

[23] R.A. Scott, L.J. Scott, R. Mägi, L. Marullo, K.J. Gaulton, M. Kaakinen, et al., An expanded genome-wide association study of type 2 diabetes in Europeans, Diabetes 66 (2017) 2888–2902, https://doi.org/10.2337/db16-1253. Epub 2017 May 31.

[24] A.P. Soggia, M.L. Correa-Giannella, M.A. Fortes, A.M. Luna, M.A. Pereira, A novel mutation in the glycogen synthase 2 gene in a child with glycogen storage disease type 0, BMC Med. Genet. 11 (2010) 3, https://doi.org/10.1186/1471-2350-11-3.

[25] A. Nessa, A. Kumaran, R. Kirk, A. Dalton, D. Ismail, K. Hussain, Mutational analysis of the GYS2 gene in patients diagnosed with ketotic hypoglycaemia, J. Pediatr. Endocrinol. Metab. 25 (2012) 963–967, https://doi.org/10.1515/jpem-2012-0165.

[26] T. Holsten, K. Tsiakas, U. Kordes, B. Bison, T. Pietsch, S. Rutkowski, R. Santer, U. Schüller, Group 3 medulloblastoma in a patient with a GYS2 germline mutation and glycogen storage disease 0a, Childs Nerv. Syst. 34 (2018) 581–584, https://doi.org/10.1007/s00381-017-3666-9.

[27] L.M. Brown, M.M. Corrado, R.M. van der Ende, T.G. Derks, M.A. Chen, S. Siegel, K. Hoyt, C.E. Correia, C. Lumpkin, T.B. Flanagan, C.T. Carreras, D.A. Weinstein, Evaluation of glycogen storage disease as a cause of ketotic hypoglycemia in children, J. Inherit. Metab. Dis. 38 (2015) 489–493, https://doi.org/10.1007/s10545-014-9744-1.

[28] I. Miwa, T. Taguchi, H. Asano, T. Murata, T. Yorifuji, H. Nagasaka, T. Takatani, Low level of fasting plasma mannose in a child with glycogen storage disease type 0 (liver glycogen synthase deficiency), Clin. Chim. Acta 411 (2010) 998–999, https://doi.org/10.1016/j.cca.2010.03.024.

[29] A. Ghosh, H. Schlecht, L.E. Heptinstall, J.K. Bassett, E. Cartwright, S.S. Bhaskar, et al., Diagnosing childhood-onset inborn errors of metabolism by next-generation sequencing, Arch. Dis. Child. 102 (2017) 1019–1029, https://doi.org/10.1136/archdischild-2017-312738.

[30] J.J. Arko, M. Debeljak, M.Z. Tansek, T. Battelino, U. Groselj, A patient with glycogen storage disease type 0 and a novel sequence variant in GYS2: a case report and literature review, J. Int. Med. Res. 48 (2020), https://doi.org/10.1177/0300060520936857.