Novel SLC37A4 Mutations in Korean Patients With Glycogen Storage Disease Ib

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Background: Molecular techniques are fundamental for establishing an accurate diagnosis and therapeutic approach of glycogen storage diseases (GSDs). We aimed to evaluate SLC37A4 mutation spectrum in Korean GSD Ib patients.

Methods: Nine Korean patients from eight unrelated families with GSD Ib were included. SLC37A4 mutations were detected in all patients with direct sequencing using a PCR method and/or whole-exome sequencing. A comprehensive review of previously reported SLC37A4 mutations was also conducted.

Results: Nine different pathogenic SLC37A4 mutations were identified in the nine patients with GSD Ib. Among them, four novel mutations were identified: c.148G>A (p.Gly50Arg), c.320G>A (p.Trp107*), c.412T>C (p.Trp138Arg), and c.818G>A (p.Gly273Asp). The most common mutation type was missense mutations (66.7%, 6/9), followed by nonsense mutations (22.2%, 2/9) and small deletion mutations (11.1%, 1/9). The most common mutation identified in the Korean population was c.443C>T (p.Ala148Val), which comprised 39.9% (7/18) of all tested alleles. This mutation has not been reported in GSD Ib patients in other ethnic populations.

Conclusions: This study expands knowledge of the SLC37A4 mutation spectrum in Korean patients with GSD Ib.

Key Words: Glycogen storage disease, GSD Ib, Korean population, mutation, SLC37A4

INTRODUCTION

Glycogen storage disease type I (GSD I) is a group of rare autosomal recessive disorders caused by deficiencies in the activities of glucose-6-phosphatase-α (G6Pase-α)/glucose-6-phosphate transporter (G6PT) complexes. The disease has an overall incidence of approximately 1 in 100,000 individuals [1, 2]. In this complex, G6Pase-α and G6PT are functionally coupled; G6PT transports G6P from the cytoplasm into the lumen of the endoplasmic reticulum, where it is hydrolyzed to glucose and inorganic phosphate by G6Pase-α [3]. A functional G6Pase-α/G6PT complex maintains interprandial glucose homeostasis. Specifically, the complex serves as a catalyst in the hydrolysis of intracellular G6P to glucose in the terminal step of gluconeogen-
esis and glycogenolysis in the liver, kidney, and intestine [2, 3]. Mutations in the G6PC gene, which encodes G6Pase-α, are responsible for approximately 80% of all GSD I cases, classified as GSD Ia. Mutations in the SLC37A4 gene, which encodes G6PT, are responsible for the remaining ~20% of GSD I cases, and are classified as GSD Ib [4].

GSD is a clinically and genetically heterogeneous group of diseases that differs according to the site of abnormal glycogen metabolism (i.e., the liver, muscle, heart, or brain) [5]. Different types of GSDs can be clinically indistinguishable. For example, patients with GSD I, GSD III, GSD O, and GSD XI present with hepatomegaly and/or hypoglycemia, and manifest as hepatic GSDs [5]. Patients with GSD Ib have symptoms similar to those of patients with GSD Ia; however, those with GSD Ib also have neutropenia and inflammatory bowel disease, which require different therapeutic options for management [4, 6]. The molecular diagnosis of GSD avoids the need for invasive liver biopsies [7]. Furthermore, the molecular diagnosis of GSD is important for establishing appropriate therapeutic and monitoring plans [8]. A recent clinical practice guideline recommended that the diagnosis of GSD I should be confirmed by using full-gene sequencing of the G6PC (GSD Ia) and SLC37A4 (GSD Ib) genes [4]. The guideline also mentioned that although full-gene sequencing of both genes is available for clinical testing, targeted mutation analysis would be helpful for some ethnic groups. Testing for specific, common mutations can identify up to 100% of affected individuals, depending on the ethnic group [4]. In this context, identifying the mutation spectrum in specific ethnic populations is important for patient care [4, 8].

Since the GSD enzyme activity tests were first introduced in Korea, there have only been a few case reports of SLC37A4 mutations in Korean patients with GSD Ib [9-12]. Therefore, the aim of this study was to evaluate the mutation spectrum in Korean patients with GSD Ib for the first time, and to further compare the spectrum to previously reported mutation spectra reported for other ethnic populations.

METHODS

1. Study population
Between April 2003 and September 2015, nine Korean children from eight unrelated families who have been identified as having SLC37A4 variants were included in this study. Two of the patients were previously reported (cases 1 and 2 in Table 1) [9, 10]. All of these patients were identified as having SLC37A4 mutations at Samsung Medical Center, Seoul, Korea. Written informed consent was obtained from all subjects and/or their parents. This study was conducted according to the guidelines of the Declaration of Helsinki. All procedures involving human subjects were approved by the Institutional Review Board of Samsung Medical Center.

2. SLC37A4 mutation analysis
Human genomic DNA was prepared from peripheral blood sam-
### Table 1. Continued

| Case No. | Neutropenia | IBD/Enterocolitis | G-CSF treatment | Delayed puberty | Renal disease | Neuro-cognitive effects | Liver histology | Liver Glycogen | RBC Glycogen | Allele 1 | Allele 2 | Ref. |
|----------|-------------|-------------------|-----------------|----------------|---------------|------------------------|---------------|---------------|--------------|----------|---------|------|
| 1        | Yes         | No                | NA              | NA             | NA            | NA                     | c/w GSD       | NA            | NA          | c.443C>T  | p.Ala148Val | [10] |
| 2        | Yes         | Yes               | NA              | NA             | NA            | NA                     | c/w GSD       | 12.30%        | NA          | c.443C>T  | p.Ala148Val | [10] |
| 3        | Yes         | No                | 6               | Yes            | No            | Normal                 | c/w GSD       | NA            | NA          | c.83G>A   | p.Arg28His  | [9]  |
| 4        | Yes         | Yes               | 6               | No             | NA            | Developmental delay    | c/w GSD       | 13.20%        | 2.00%       | c.1496G>A | p.Gly506Gl   | This study |
| 5        | NA          | NA                | NA              | NA             | NA            | NA                     | c/w GSD       | NA            | NA          | c.1486G>A | p.Gly506Arg | This study |
| 6**       | Yes         | No                | 3               | Yes            | N.a           | Asymmetric widening of right temporal horn on sella MRI | c/w GSD       | 16.72%        | 4.05%       | c.1179G>A | p.Trp393*  | This study |
| 7††       | Yes         | No                | 1               | Yes            | Yes           | Normal                 | c/w GSD       | NA            | NA          | c.443C>T  | p.Ala148Val | This study |
| 8††       | Yes         | No                | 0               | No             | No            | Normal                 | c/w GSD       | NA            | NA          | c.443C>T  | p.Ala148Val | This study |
| 9††       | NA          | NA                | 6               | No             | Yes           | Normal                 | c/w GSD       | NA            | NA          | c.412T>C   | p.Trp393Arg | This study |

Cases 1, 2, 7, 8, and 9 were confirmed to have variant alleles located in trans in the family DNA analysis.

1 | Proteinuria, renal stones, nephrocalcinosis, or altered creatinine clearance; 2 | Absolute neutrophil count <1.5 × 10⁹/L; 3 | Infection frequency requiring hospitalization after diagnosis; 4 | Swollen hepatocytes with periodic acid–Schiff positivity and increased accumulation of glycogen in the cytoplasm on electron microscopy (consistent with glycogen storage disease); 5 | Reference range 1–6% /wet liver weight; 6 | Reference range <10%/packed red blood cell weight; 7 | c/w GSD, compatible with; 8 | IBD, inflammatory bowel disease; 9 | TG, triglycerides; 10 | NA, not available (the information was not submitted); 11 | Not applicable because of patient's age or sex; 12 | Developmental delay; 13 | This variant was also identified in his asymptomatic female sibling, who was a heterozygote. She did not carry the other mutation of c.1042_1043del.

Abbreviations: GSD, glycogen storage disease; c/w, compatible with; FBS, fasting blood sugar (glucose); F/U, follow-up; G-CSF, granulocyte-colony stimulating factor; HA, hepatic adenoma; HCC, hepatocellular carcinoma; IBD, inflammatory bowel disease; TG, triglycerides; NA, not available (the information was not submitted); N.a, not applicable because of patient's age or sex; mo, months; hos, hospitalization; RBC, red blood cell.

References: [1] Proteinuria, renal stones, nephrocalcinosis, or altered creatinine clearance; [2] Absolute neutrophil count <1.5 × 10⁹/L; [3] Infection frequency requiring hospitalization after diagnosis; [4] Swollen hepatocytes with periodic acid–Schiff positivity and increased accumulation of glycogen in the cytoplasm on electron microscopy (consistent with glycogen storage disease); [5] Reference range 1–6% /wet liver weight; [6] Reference range <10%/packed red blood cell weight; [7] c/w GSD, compatible with; [8] IBD, inflammatory bowel disease; [9] TG, triglycerides; [10] NA, not available (the information was not submitted); [11] Not applicable because of patient's age or sex; [12] Developmental delay; [13] This variant was also identified in his asymptomatic female sibling, who was a heterozygote. She did not carry the other mutation of c.1042_1043del. 

In addition, a comprehensive review of the literature was performed using public algorithms such as Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/).
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RESULTS

Nine patients from eight unrelated families, including two previously reported Korean patients, were identified to have SLC37A4 mutations. Among the nine patients, 77.8% were male. The clinical information and specific SLC37A4 mutations identified in the nine Korean GSD Ib patients are shown in Table 1. The median age of onset was 14.5 months (range 3 months to 10.9 yr). The median age at molecular diagnostic work-up was 10.4 yr (range 6 months to 19 yr). All patients presented with hepatomegaly and elevated AST and ALT levels as the first clinical signs. All of the patients also had neutropenia (absolute neutrophil count <1.5×10^9/L). Because of limited clinical information, the genotype–phenotype correlation could not be assessed.

Among 18 mutant alleles, nine different SLC37A4 mutations were identified (Table 2). These mutations were distributed among all of the coding exons of SLC37A4, except for exon 11. Among the nine mutations, four were novel variants: c.148G>A (p.Gly50Arg), c.412T>C (p.Trp138Arg), and c.818G>A (p.Gly273Asp). The remaining five mutations were previously reported: c.83G>A (p.Arg28His), c.149G>A (p.Gly50Glu), c.443C>T (p.Ala148Val), c.1042_1043del (p.Leu348Valfs*53), and c.1179G>A (p.Trp393*) [1, 15-21]. Two of the novel variants were classified as “pathogenic”, including c.148G>A (p.Gly50Arg) and c.320G>A (p.Trp107*). The other two novel variants, c.412T>C (p.Trp138Arg) and c.818G>A (p.Gly273Asp), were classified as “likely pathogenic” according to the ACMG sequence variants interpretation guidelines [14]. Among the nine mutations, the most common were missense mutations (66.7%, 6/9) followed by nonsense mutations (22.2%, 2/9) and small deletion mutations (11.1%, 1/9).

Among the 18 tested alleles, the variants c.443C>T (p.Ala148Val), c.1042_1043del (p.Leu348Valfs*53), and c.1179G>A (p.Trp393*) were repeatedly identified in different individuals (7 times, 3 times, and 2 times, respectively). Notably, the most common mutation identified in Korean patients was c.443C>T (p.Ala148Val), accounting for 55.6% (5/9 patients) of all GSD Ib patients and 38.9% of the tested alleles (7/18 alleles). WES followed by Sanger sequencing was used to confirm that one patient (case 6) carried the known homozygous pathogenic mutation c.1179G>A (p.Trp393*). Cases 7 and 8 are siblings from the same family; case 7 is the proband of the family and case 8 is her brother.

DISCUSSION

To our knowledge, this is the first study to summarize the clinical characteristics of Korean patients with GSD Ib. The SLC37A4 mutation spectrum is known to be distributed widely across the
SLC37A4 gene. In this study, the most common mutations were missense mutations, which is consistent with the data in the HGMD database.

We identified four novel pathogenic [c.148G>A (p.Gly50Arg) and c.320G>A (p.Trp107*)] or likely pathogenic [c.412T>C (p.Trp138Arg) and c.818G>A (p.Gly273Asp)] SLC37A4 mutations. Among them, c.148G>A has not been reported previously. In contrast, c.148G>C has been previously reported as a pathogenic mutation. The c.148G>C mutation results in the same amino acid change (p.Gly50Arg), abolishes the microsomal G6P uptake activity, and compromises G6PT stability [15]. The c.148G>A mutation could be categorized as a pathogenic variant according to the ACMG standards and guidelines for the interpretation of sequence variants [14].

Notably, the most common mutation identified in the Korean population was c.443C>T (p.Ala148Val), which was found in 55.6% of the GSD Ib patients and in 38.9% of the tested alleles. This mutation has not been reported in other ethnic patients with GSD Ib. It has only been reported in two alleles in East Asia, and was identified as heterozygous among 43,554 individuals (87,108 tested alleles) with an allele frequency of 2.296×10−5 in the ExAC database. However, this site is covered in <80% of the individuals in the ExAC database, which may indicate a low-quality site. These findings suggest that this variant might be an important marker for the diagnosis of GSD Ib in the East Asian population specifically. Considering that no Japanese or Chinese patients have been reported to have c.443C>T, it is possible that this represents a recurrent mutation specific to Koreans. Although this is a very rare single nucleotide variant (SNV) in the public database, this variation was detected in most cases simultaneously with another SNV that seems to have a greater impact on protein function. Therefore, further studies are needed to confirm the pathogenicity of this recurrent SNV.

The pathogenic variant of c.1042_1043del (p.Leu348Valfs*53) was the second most frequent mutation (33.3%, 3/9 of GSD Ib patients; 16.7%, 3/18 tested alleles). This mutation has been frequently reported in mixed Caucasian (27–31%) and German (32%) populations [4]. Other mutations that have been frequently reported in other ethnic populations have not been identified in the Korean population [4]. These include c.352T>C (p.Trp118Arg) identified in 37–50% of Japanese people, and c.1015G>T (p.Gly339Cys) identified in 19–21% of mixed Caucasians and in 29% of Germans. These results suggest that Korean SLC37A4 mutations differ from those of other ethnic populations owing to the genetic divergence of Homo sapiens. Furthermore, the c.443C>T mutation may be relatively new, as compared with c.1042_1043del [22], given that identification of a large proportion of rare alleles can be a signature of recent expansion, as mutations that have occurred since the expansion will not have had sufficient time to spread throughout the population [23].

In this study, cases 7 and 8, both of whom have homozygous c.443C>T mutant alleles, are siblings from the same family. These patients also showed similar clinical manifestations, including short stature and hepatic adenoma. Granulocyte-colony stimulating factor treatment was only used in case 7. However, both cases 7 and 8 tolerated dietary management (including corn starch) and only presented with minor infections (such as chronic otitis media or mucosal infection) that did not require hospitalization.

In this study, all patients with SLC37A4 mutations had neutropenia, except for two cases whose clinical information was not available. In the literature, no correlation could be established between the presence of “leaky” mutations and the absence of neutropenia, in both, homozygous and compound heterozygous patients [24]. Further studies are needed to take into account clinical and biochemical data, which are integral for assessing genotype-phenotype correlations in the Korean population. However, recent molecular diagnostic approaches based on mutation analysis for the disease-causing genes of each type of GSD provide the advantages of avoiding invasive liver biopsies or enzymatic studies. These latter methods are historical diagnostic methods that can potentially introduce ambiguity when differentiating between several types of GSD with similar clinical findings [4]. Despite its limitations, this study is valuable to improve understanding of the SLC37A4 mutation spectrum in the Korean population.

In conclusion, we identified four novel pathogenic and likely pathogenic SLC37A4 variations. The c.443C>T (p.Ala148Val) variant was novel and the most common mutation identified. The SLC37A4 mutation spectrum in Korean GSD Ib patients tends to differ from that of other ethnic populations. Direct sequence analysis of full-gene sequences is needed to provide accurate molecular diagnoses, and WES could be an effective approach in the diagnosis of GSD Ib.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.
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