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Glycogen storage disease type VI can progress to cirrhosis: ten Chinese patients with GSD VI and a literature review

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Abstract

Objectives: The aim of our study is to systematically describe the genotypic and phenotypic spectrum of Glycogen storage disease type VI (GSD VI), especially in Chinses population.

Methods: We retrospectively analyzed ten Chinese children diagnosed as having GSD VI confirmed by next generation sequencing in Children's Hospital of Fudan University and Jinshan Hospital of Fudan University. We described the genotypic and phenotypic spectrum of GSD VI through the clinical and genetic data we collected. Moreover, we conducted a literature review, and we compared the genotypic and phenotypic spectrum of GSD VI between Chinese population and non Chinese population.

Results: For the first time, we found that four Chinese patients showed cirrhosis in liver biopsy characterized by the formation of regenerative nodules. In addition, c.772+1G>A and c.1900G>C, p.(Asp634His) were recurrent in three Chinese families and four European families respectively indicating that the genotypic spectrum of *PYGL* gene may vary among the population. Furthermore, we identified seven novel variants in *PYGL* gene.

Conclusions: Our study enriched the genotypic and phenotypic spectrum of GSD VI, and provided a new clue for management of GSD VI.

Keywords: Chinese; cirrhosis; glycogen storage disease types VI; hepatic glycogen phosphorylase; *PYGL* gene.

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Introduction

Glycogen storage disease type VI (GSD VI; OMIM #232700) is an autosomal recessive genetic disease caused by the deficiency of hepatic glycogen phosphorylase. *PYGL* gene containing 20 exons is the only gene responsible for encoding hepatic glycogen phosphorylase. The best estimation for the incidence of GSD VI is 1 of 100,000; however, it is generally believed that the true incidence is underestimated because of nonspecific and variable phenotypes [1]. Patients with GSD VI often have disease onset during infancy or childhood, with common phenotypes including hepatomegaly, poor growth, elevated hepatic transaminases, hyperlipidemia, ketosis, and hypoglycemia, and rare cases of severe phenotypes including preprandial and postprandial lactic acidosis, recurrent hypoglycemia, or marked hepatomegaly have been reported [2, 3]. GSD VI usually has a benign disease course, with most children having intact intellectual development. Clinical and biochemical abnormalities have a tendency to improve with age, and there is no consensus whether patients with GSD VI should be treated with uncooked cornstarch and high-protein diet [4]. In recent years, fibrosis, cardiomyopathy, focal nodular hyperplasia, and hepatocellular carcinoma have been reported in some patients [4–7]. However, cirrhosis has not been reported in GSD VI. Molecular genetic testing can accurately diagnose GSD VI, and invasive liver biopsy may be avoided [2]. Although a GSD VI murine model recapitulating the phenotypes of human GSD VI was generated, there is a paucity of patients with GSD VI confirmed by molecular genetic testing in the literature including only one patient in Hong Kong, China [8, 9]. Considering the full genotypic and phenotypic spectrum of GSD VI continue to evolve, we reported 10 Chinese patients with GSD VI confirmed by next-generation sequencing (NGS) and reviewed literature to give a systematic description of patients with GSD VI in Chinese population. We believe that our study supports an expanding genotypic and phenotypic spectrum of GSD VI.

Methods

Subjects and genetic testing

We performed a retrospective analysis for patients diagnosed as having GSD VI in Children's Hospital of Fudan University and Jinshan Hospital from 2015 through 2019. All patients clinically suspected as having glycogen storage diseases (GSDs) were confirmed to carry biallelic pathogenic variants in *PYGL* gene by NGS. Live biopsy was complementary to NGS to diagnose GSD VI [10, 11]. NGS was conducted in Molecular Genetic Diagnosis Center, Children's Hospital of Fudan University or commercial companies, and NM_002863 is used for reference sequence. Known disease-causing variants such as nonsense, canonical splice site, and frameshift variants were regarded as pathogenic. The pathogenicity of novel missense variants was assessed *in silico* using tools such as PolyPhen-2 [12], Sorting Intolerant From Tolerant (SIFT) [13], and combined annotation-dependent depletion (CADD) score [14] and public database including the Exome Aggregation Consortium Browser (ExAC, http://exac.broadinstitute.org) and 1000 Genomes Project (1000G, http://www.internationalgenome.org/data). Medical records were reviewed for clinical symptoms, laboratory reports, imaging reports, liver biopsy, and NGS. This study was approved by the ethics committees of both Children's Hospital of Fudan University and Jinshan Hospital and was performed in compliance with the Declaration of Helsinki.

Statistical analysis

The age of initial symptom and liver biopsy was expressed in terms of mean ± standard deviation. The patients who underwent liver biopsy were classified into two groups. Group 1 stood for patients without hepatic fibrosis and cirrhosis, and group 2 stood for patients with hepatic fibrosis and cirrhosis. The age of liver biopsy in two groups was analyzed by using the Mann–Whitney *U*-test of IBM SPSS Statistics, version 23. A two-sided p value of <0.05 was considered as statistically significant.

Literature review

The literature review was conducted in December 2019. Search terms such as "GSD VI," "GSDs," "glycogen phosphorylase," and "*PYGL* gene" were used in various combinations and permutations to identify relevant literature in PubMed databases. Patients with GSD VI only confirmed by molecular genetic testing (at least one variant was identified in *PYGL* gene) were included in our literature review.

Results

Clinical features of Chinese patients with GSD VI at diagnosis

When we reviewed the literature, we found that a Chinese asymptomatic boy (Hong Kong, China) presented with isolated hepatomegaly [9]. To give a better description in Chinese population, we included him in our cohort (patient 11). The clinical features of 11 Chinese patients with GSD VI, 10 males and one female, from 11 unrelated families at diagnosis are summarized in Table 1. Except patient 11 who was a child of a consanguineous couple of first cousins, all patients had no consanguineous family history. The mean age of initial presentation was 20 ± 16 months (range: 5–48 months). Seven (64%) patients initially presented with elevated transaminase, while three (27%) patients and one (9%) patient initially presented with abdominal distention and hepatomegaly, respectively. Two (18%) patients presented with short stature (defined as a height less than the third percentile). Patient 2 was diagnosed as having hypothyroidism at a local hospital.

Biochemical features and hepatic ultrasound results at diagnosis

Hepatic ultrasound results and biochemical features including transaminase, hypoglycemia, cholesterol, triglycerides, and lactate are summarized in Table 1. An elevated transaminase level was presented in 10 (91%) patients, hypoglycemia was presented in six (55%) patients, hyperlipidemia was presented in three (27%) patients, and serum lactate was slightly elevated within or more than the boundary value in four (36%) patients. Hepatic ultrasound revealed hepatomegaly in all patients without echogenicity and splenomegaly.

Liver biopsy

The age of liver biopsy and histological features of our nine patients are summarized in Table 2. The age of liver biopsy was 30 ± 21 months (range: 13–73 months). The liver biopsy of patient 11 showed an extensive deposition of glycogen in the hepatocytes, with no cirrhosis or amylopectin found [9]. Excepted patient 6, all patients underwent liver biopsy and four patients (patients 7–10) underwent electron microscopy examination (Figure 1). Of the nine patients who underwent liver biopsy, all showed swollen hepatocytes (Figure 1A), and electron microscope examinations of four patients (patients 7–10) showed an extensive deposition of glycogen and lipid droplets in the swollen hepatocytes, which confirmed the diagnosis of GSDs (Figure 1B). Periodic Acid-Schiff (PAS) staining and Diastase-Periodic Acid-Schiff (D-PAS) staining showed liver sections stained with PAS-positive material and then subjected to

Table 1: Clinical phenotypes of 11 Chinese patients with GSD VI at diagnosis and most recent follow-up.

Patient	1	2	3	4	5	6	7	8	9	10	11[b]
Onset age, (months)	48	6	17	12	5	6	6	40	42	16	24
Initial symptoms	ET	ET	ET	ET	AD	ET	AD	ET	ET	AD	Hepatomegaly
Sex	M	M	M	M	F	M	M	M	M	M	M
Hepatomegaly, (mm below costal)	55	55	38	80	90	54	100	50	50	40	NA
Height	3rd	28th	6th	3rd	<1st	<1st	38th	46th	16th	76th	NA
Weight	40th	11th	10th	21st	39th	3rd	55th	62nd	79th	48th	NA
Hypoglycemia	+	+	–	+	+	+	–	–	–	+	–
ALT[a]/AST[c]	250/273	610/273	579/469	484/541	1143/655	182/264	823/478	109/105	238/213	654/373	–
CHOL[d]/TG[e]	4.12/1.43	3.33/1.03	3.85/1.65	6.18/4.14	3.11/2.93	4.9/1.13	4.4/0.94	4.32/0.95	5.16/1.58	5.88/2.93	–
Serum lactate[f]	2.2	1.3	1.9	2.1	1.6	0.69	2.2	1.9	2.2	2.3	–
Liver biopsy	F	F	G	G	G	NA	C	C	C	C	G
Status at the most recent follow-up											
Age, (months)	58	60	51	52	34	NA	NA	NA	113	NA	NA
UCS, (g/kg/day)	2	1.5–2	2	2	2	NA	NA	NA	1.5	NA	NA
Height	26th	<1st	12th	14th	<1st	NA	NA	NA	81st	NA	NA
Weight	25th	28th	7th	11th	43rd	NA	NA	NA	89th	NA	NA
Hepatomegaly, (mm below costal)	25	40	25	40	60	NA	NA	NA	40	NA	NA
ALT/AST	39/48	31/34	12/30	133/397	242/127	NA	NA	NA	63/46	NA	NA
Serum lactate	1.8	1.4	1.5	1.3	1.3	NA	NA	NA	2.1	NA	NA

+, positive result; –, negative result; NA, not available; ALT, alanine transaminase; AST, aspartate aminotransferase; CHOL, cholesterol; TG, triglyceride; ET, elevated transaminase; AD, abdominal distention; M, male; F, female; G, glycogenosis; F, fibrosis; C, cirrhosis; UCS, uncooked cornstarch.

[a]Reference range for ALT: 0–40 IU/L.

[b]Literature review.

[c]Reference range for AST: 0–40 IU/L.

[d]Reference range for CHOL: 2.3–5.7 mmoL/L.

[e]Reference range for TG: 0.34–1.71 mmoL/L.

[f]Reference range for lactate : 0.6–2.2 mmoL/L.

Table 2: Histological features of nine Chinese patients with GSD VI.

Patient	Group	Age	Genotype	Microscopy				Electron microscope	
				Enlarged hepatocyte	Glycogen condensation	Inflammation	Conclusion	Glycogen deposition	Lipid droplet
1	2	50 months	c.772+1G>A/c.1289C>A, p.(Ser430Tyr)	+	+	–	Fibrosis	NA	NA
2	2	27 months	c.697G>A, p.(Gly233Ser) (hom)	+	+	+	Fibrosis	NA	NA
3	1	18 months	c.772+1G>A (hom)	+	+	+	Glycogenosis	NA	NA
4	1	13 months	c.1768+1G>A (hom)	+	+	+	Glycogenosis	NA	NA
5	1	16 months	c.1768+1G>A/c.107T>G, p.(Leu36Arg)	+	+	+	Glycogenosis	NA	NA
7	2	17 months	c.244–1G>A/c.730C>T, p.(Leu244Phe)	+	+	–	Cirrhosis	+	+
8	2	42 months	c.772+1G>A/c.2467C>T, p.(Gln823Ter)	+	+	–	Cirrhosis	+	+
9	2	73 months	c.1366G>A, p.(Val456Met)/ c.2417_2418delTA, p.(Ieu806Serfs*9)	+	+	–	Cirrhosis	+	+
10	2	17 months	c.1519–1G>C/c.1475G>A, p.(Trp492Ter)	+	+	–	Cirrhosis	+	+

+, positive result; –, negative result; NA, not available.

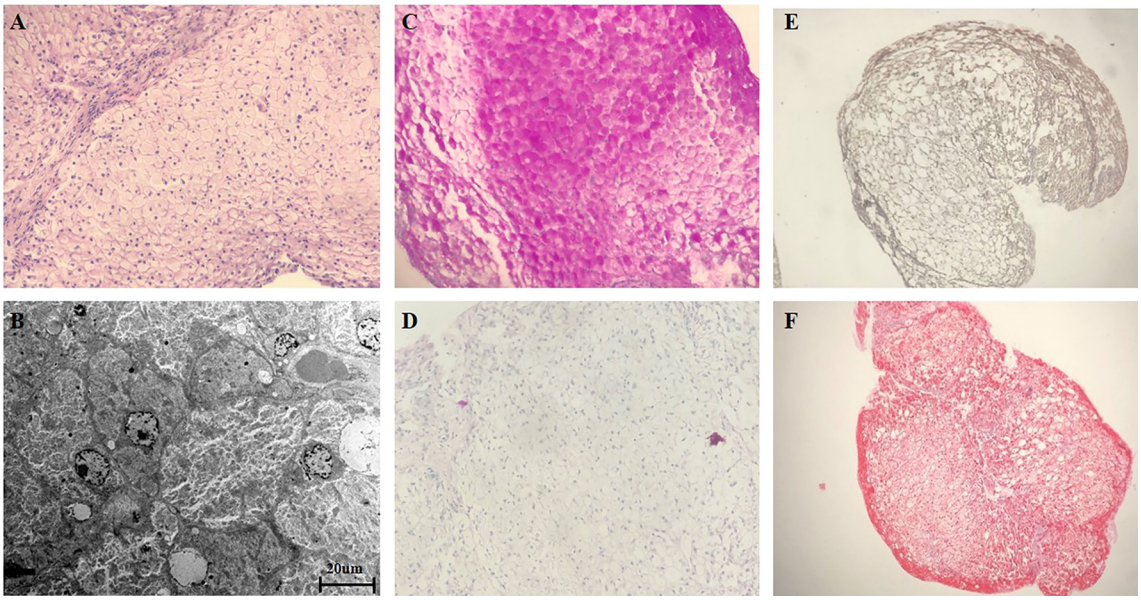

Figure 1: Histological findings in four patients with GSD VI and cirrhosis. (A) Hematoxylin-eosin staining showed diffuse swollen hepatocytes resembling a lake with infiltration of inflammatory cells (200×); (B) Electron microscopy showed accumulation of glycogen and lipid droplets in swollen hepatocytes (scale bar 20 μm); (C and D) PAS staining and D-PAS staining showed liver sections stained with PAS-positive material and then subjected to diastase digestion no longer contained PAS-positive material (200x); (E and F) Reticular and Masson staining revealed that extensive fibrosis encircled regenerative nodule indicating cirrhosis (100×).

diastase digestion no longer contained PAS-positive material (Figure 1C,D). Two patients (patient 1 and patient 2) revealed hepatic fibrosis. Four patients (patients 7–10) showed that the structure of normal liver lobules disappeared, and Reticular and Masson staining confirmed that extensive fibrosis encircled regenerative nodule (Figure 1E,F), indicating stage 4 (cirrhosis) [15]. For all these four patients with cirrhosis, serum albumin, prothrombin time, and international normalized ratio were normal and splenomegaly was not found under hepatic ultrasound. Except patient 7 who showed marked elevated transaminase and hepatomegaly under hepatic ultrasound (100 mm below costal), there was no significant difference from other patients. Group 1 included three patients (patients 3–5), and group 2 included six patients (patients 1–2 and patients 7–10). The age of liver biopsy in group 1 was significantly earlier than group 2 (p=0.048).

Follow-up and prognosis

The current age, transaminase, growth, lactate, and hepatic ultrasound during the last visit and treatment for individuals are summarized in Table 1. Once the diagnosis was established, therapy with uncooked cornstarch was initiated. The dose of uncooked cornstarch varied from 0.5 to 2 g/kg/day. We also recommended a high-protein diet (2 g/kg/day) for all patients. Six patients (patients 1–5 and patient 9) had follow-up data, and four patients (patients 6–8 and patient 10) were lost to follow-up. No information about follow-up was recorded in the literature for patient 11. Elevated transaminases and hepatomegaly improved in all patients. Growth was improved in patients 1, 3, 4, and 9. However, the height of patient 2 deteriorated from 28th centile to less than first centile, and the height of patient 5 still remained under the first centile. Two biochemical hypoglycemic episodes were recorded in patient 1 (minimal glucose value, 2.6 mmoL/L), and he was treated with uncooked cornstarch (2 g/kg/day). Patient 2 with no episodes of hypoglycemia recorded was treated with levothyroxine sodium and uncooked cornstarch, and the dose of uncooked cornstarch was increased from 1.5 to 2 g/kg/day. One biochemical hypoglycemic episode was recorded in patient 3 (glucose value, 3.84 mmoL/L), patient 4 (glucose value, 3.3 mmoL/L), and patient 5 (glucose value, 3.0 mmoL/L); they were treated with uncooked cornstarch (2 g/kg/day). Patient 9 was 9 years old at the last visit, he experienced no hypoglycemic episode either clinically or biochemically, and he was treated with uncooked cornstarch (0.5 g/kg/day).

Molecular genetic analysis

Of the 11 Chinese patients, five were homozygous and six were compound heterozygous. Patient 11 was homozygous for c.698G>A, p. (Gly233Asp), which originated from the father and mother, respectively, and this variant was not identified in our cohort [9]. Totally, we identified 13 *PYGL* variants in 11 unrelated Chinese patients including six missense variants, two nonsense variants, four canonical splice-site variants, and one frameshift variant (Table 3). We identified seven novel variants including one nonsense variant: c.1475G>A, (Trp492Ter); two canonical splice-site variants: 244-1G>A, 1519-1G>C; one frameshift variant: c.2417_2418delTA, p.(Ile806Serfs*9), and three missense variants: c.107T>G, p.(Leu36Arg), c.730C>T, p.(Leu244-Phe), and c.1289C>A, p.(Ser430Tyr). All seven novel variants were not found in public database including ClinVar, ExAC, and 1000G, and all three novel missense variants were predicted to be deleterious in *in silico* analysis. Thus, we considered all seven novel variants as pathogenic. Of note, c.772+1G>A was recurrent in three unrelated families, and c.1768+1G>A, c.2467C>T, and p.(Gln823Ter) were recurrent in two unrelated families.

Literature review

Totally, we found 41 patients from the literature review [3, 4, 7, 16–22] (Table 4). One patient in Hong Kong, China, was included in our cohort as mentioned previously [9]. With available data, the average age of initial presentation of 40 non-Chinese patients from 36 families, 18 males and 22 females, was 20 ± 12 months (range: 1–54 months). The initial symptoms included hepatomegaly in 16 (73%) patients, distended abdomen in four (18%) patients, fasting miserable in one (4.5%) patient, and feeding difficulty in one (4.5%) patient. The clinical phenotypes were dominated by hepatomegaly (100%) and elevated transaminase (90%). Hyperlipidemia (82%) and short stature (74%) were common, and hypoglycemia (43%) was less common. The liver biopsy was performed in 15 patients, and six (40%) patients showed hepatic fibrosis. The genotypic spectrum of *PYGL* gene in Chinese and non-Chinese population is shown in Figure 2. Except one patient who carried only one *PYGL* gene variant, c.1900G>C, p.(Asp634His) [3], all patients carried biallelic *PYGL* gene variants. Totally, we identified 43 variants in non-Chinese population including 27 (63%) missense variants, eight (19%) splice variants, four (9%) nonsense variants, two (5%) large insertion and deletion variants, one (2%) synonymous variant, and one (2%) in-frame deletion variant. c.1900G>C, p.(Asp634His)

Table 3: The *in silico* assessment of *PYGL* variants identified in 11 Chinese patients.

Patient	cDNA (NM_002863)	Protein change	*In silico* pathogenicity predictions			Allele frequency in the public database		
			SIFT	PolyPhen-2	CADD[b]	ExAC	ExAC (East Asian)	1000G
1	c.772+1G>A				21.8	0.000008244	0.0001156	0
	c.1289C>A[a]	p.Ser430Tyr	D	PD	31	0	0	0
2	c.697G>A (hom)	p.Gly233Ser	D	PD	23.2	0.00004119	0.0001156	0
3	c.772+1G>A (hom)				21.8	0.000008244	0.0001156	0
4	c.1768+1G>A (hom)				22.5	0.00002471	0.0001156	0
5	c.1768+1G>A				22.5	0.00002471	0.0001156	0
	c.107T>G[a]	p.Leu36Arg	D	PD	30	0	0	0
6	c.2467C>T (hom)	p.Gln823Ter			40	0.00005766	0.0008089	0
7	c.244-1G>A[a]				27.2	0	0	0
	c.730C>T[a]	p.Leu244Phe	D	PD	35	0	0	0
8	c.772+1G>A				21.8	0.000008244	0.0001156	0
	c.2467C>T	p.Gln823Ter			30	0.00005766	0.0008089	0
9	c.1366G>A	p.Val456Met	D	PD	32	0.00003295	0	0
	c.2417_2418delTA[a]	p.Ieu806Serfs*9			–	0	0	0
10	c.1475G>A[a]	p.Trp492Ter			41	0	0	0
	c.1519–1G>C[a]				22.7	0	0	0
11	c.698G>A(hom)	p.Gly233Asp			20.3	0	0	0

D, damaging; PD, probably damaging; ExAC, Exome Aggregation Consortium; 1000G, 1000 Genomes Project.

[a]Novel variants.

[b]The threshold of pathogenicity for CADD, >20.

Table 4: The phenotype and genotype of patients with GSD VI published in the literature.

Patient	Onset age, (month)	Race	Sex	Onset symptoms	Hepatomegaly	Short stature	Hypoglycemia	Elevated liver transaminase	Hyperlipidemia	Liver biopsy	Genotype
[16]	22	Mennonite kindred	Female	NA	+	+	–	NA	NA	Glycogenosis	c.1620+1G>A (hom)
Subject 1 [17]	24	Israeli Arab	Male	Hepatomegaly	+	+	–	+	+	NA	c.1768+1G>A (hom))
Subject 2 [17]	24	Suriname Hindustani	Male	Hepatomegaly	+	+	+	+	–	Glycogenosis	c.529–1G>C /c.1016A>G, p.(Asn339Ser)
Subject 3 [17]	12	Turkish	Female	Hepatomegaly	+	–	–	+	+	Glycogenosis	c.1131C>G, p.(Asn377Lys) (hom)
Subject 1 [3]	16	British Asian	Female	Fasting miserable	+	+	+	+	+	NA	c.2042A>C, p.(Lys 681Thr) (hom)
Subject 2 [3]	5	British	Female	Hepatomegaly	+	–	–	+	+	NA	c.1471C>T, p.(Arg491Cys) /c.1964_ 1969inv6; c.1969 + 1_ +4delGTAC
Subject 3[a] [3]	14	French	Male	Hepatomegaly	+	+	+	+	+	NA	c.1366G>A, p.(Val456Met) /c.2024C>T, p.(Ser675Leu)
Subject 4[a] [3]	27	French	Male	Hepatomegaly	+	+	+	+	+	NA	c.1366G>A, p.(Val456Met) /c.2024C>T, p.(Ser675Leu)
Subject 5 [3]	25	Polish	Male	Abdominal distension	+	+	–	+	+	Glycogenosis	c.1895A>T, p.(Asn632Ile) /c.2023T>A, p.(Ser675Thr)
Subject 6 [3]	42	Polish	Male	Abdominal distension	+	–	–	+	–	Glycogenosis	c.38A>C, p.(Gln13Pro) /c.1900G>C, p.(Asp634His)
Subject 7 [3]	27	Polish	Female	Abdominal distension	+	+	–	+	+	Glycogenosis	c.1195C>T, p.(Arg399Ter)

Table 4: (continued)

Patient	Onset age, (month)	Race	Sex	Onset symptoms	Hepatomegaly	Short stature	Hypoglycemia	Elevated liver transaminase	Hyperlipidemia	Liver biopsy	Genotype
											/c.2017G>A, p.(Glu673Lys)
Subject 8 [3]	1	Austrian	Female	Feeding difficulties	+	+	–	+	+	Glycogenosis	c.1900G>C, p.(Asp634His) /unknow
Subject 1 [18]	9	Algeria	Male	NA	+	NA	+	+	+	NA	c.501_502ins361b (hom)
Subject 2 [18]	10	Tunisia	Female	NA	+	NA	–	+	+	NA	c.1499 T>G, p.(Leu500Arg) (hom)
Subject 3 [18]	21	Europe	Male	NA	+	+	–	+	+	NA	c.2108-2110delAAG, p.(Glu703del) /c.528+2T>C
Subject 4 [18]	4	Europe	Female	NA	+	NA	–	+	+	NA	c.564C>A, p.(Asn188Lys) /c.2461T>C, p.(Tyr821His)
Subject 5 [18]	48	Turkey	Female	NA	+	+	+	+	+	NA	c.1131C>G, p.(Asn377Lys) (hom)
Subject 6 [18]	24	Europe	Male	NA	+	NA	–	+	+	NA	c.1145C>T, p.(Pro382Leu) /c.1472G>A, p.(Arg491His)
Subject 7 [18]	14	Europe	Male	NA	+	+	–	+	+	NA	c.1366G>A, p.(Val456Met) /c.2024C>T, p.(Ser675Leu)
Subject 8 [18]	NA	Indian	Female	NA	+	NA	–	+	N	NA	c.1620+1G>C (hom)
Subject 9 [18]	12	Algeria	Female	NA	+	NA	+	+	+	NA	c.682G>T, p.(Asp288Tyr) (hom)
Subject 10 [18]	10	Europe	Female	NA	+	+	NA	+	+	NA	c.2313-1G>T /c.1768+1G>A
Subject 11 [18]	26	Africa	Male	NA	+	+	+	+	+	NA	c.856-29_c.1518 + 614del (hom)
Subject 1[b] [4]	8	Canadian	Female	Hepatomegaly	+	–	NA	+	NA	Fibrosis	c.1729C>T, p.(Gln577Ter) /c.856–9G>A

Table 4: (continued)

Patient	Onset age, (month)	Race	Sex	Onset symptoms	Hepatomegaly	Short stature	Hypoglycemia	Elevated liver transaminase	Hyperlipidemia	Liver biopsy	Genotype
Subject 2[b] [4]	13	Canadian	Female	Hepatomegaly	+	+	NA	+	NA	Fibrosis	c.1729C>T, p.(Gln577Ter) /c.856−9G>A
Subject 3 [4]	22	Canadian	Male	Hepatomegaly	+	−	NA	+	NA	Fibrosis	c.1198C>T, p.(His400Tyr) (hom)
Subject 4 [4]	54	Canadian	Male	Hepatomegaly	+	−	NA	+	NA	Fibrosis	c.1518G>A, p.(Glu506Glu) (hom)
[19]	12	Indian	Female	Abdominal distension	+	+	+	+	+	Fibrosis	c.1297G>T, p.(Glu433Ter) (hom)
Subject 1[c] [7]	12	Caucasian	Female	Hepatomegaly	+	NA	NA	−	−	Glycogenosis	c.777T>A, p.(Asn259Lys) /c.1900G>C, p.(Asp634His)
Subject 2[c] [7]	36	Caucasian	Male	Hepatomegaly	+	NA	NA	+	−	NA	c.777T>A, p.(Asn259Lys) /c.1900G>C, p. (Asp634His)
Subject 3[c] [7]	13	Caucasian	Female	Hepatomegaly	+	NA	NA	−	+	NA	c.777T>A, p.(Asn259Lys) /c.1900G>C, p.(Asp634His)
Subject 4 [7]	12	Caucasian	Female	Hepatomegaly	+	+	NA	+	+	NA	c.43A>G, p.(Ser15Gly) (hom)
Subject 5 [7]	28	Caucasian	Female	Hepatomegaly	+	NA	NA	+	−	Hepatic fibrosis	c.1901A>G, p.(Asp634Gly) /c.2017G>A, p.(Glu673Tyr)
Subject 6 [7]	16	Caucasian	Female	Hepatomegaly	+	NA	NA	−	+	Glycogenosis	c.2435T>C, p.(Phe812Ser) /c.2446C>T, p.(Arg816Ter)
Subject 1 [20]	NA	NA	Male	NA	NA	NA	NA	NA	NA	NA	c.385G>A p.(Asp129Asn) /c.2446C>T, p.(Arg816Ter)
Subject 2 [20]	NA	NA	Male	NA	NA	NA	NA	NA	NA	NA	c.418C>G, p.(Leu140Val)/ c.1366G>A, p.(Val456Met)
Subject 3 [20]	NA	NA	Male	NA	NA	NA	NA	NA	NA	NA	c.131G>A, p.(Arg44His)/ c.1900G>C, p.(Asp634His)
[21]	38	NA	Male	NA	NA	NA	+	NA	NA	NA	c.1727G>A, p.(Arg576Gln) (hom)
Subject 1 [22]	NA	Brazilian	Female	NA	NA	NA	NA	NA	NA	NA	c.697G>A, p.(Gly233Ser) (hom)
Subject 2 [22]	NA	Brazilian	Female	NA	NA	NA	NA	NA	NA	NA	c.131G>A, p.(Arg44His) (hom)

[a, b, c]: Siblings in each family.

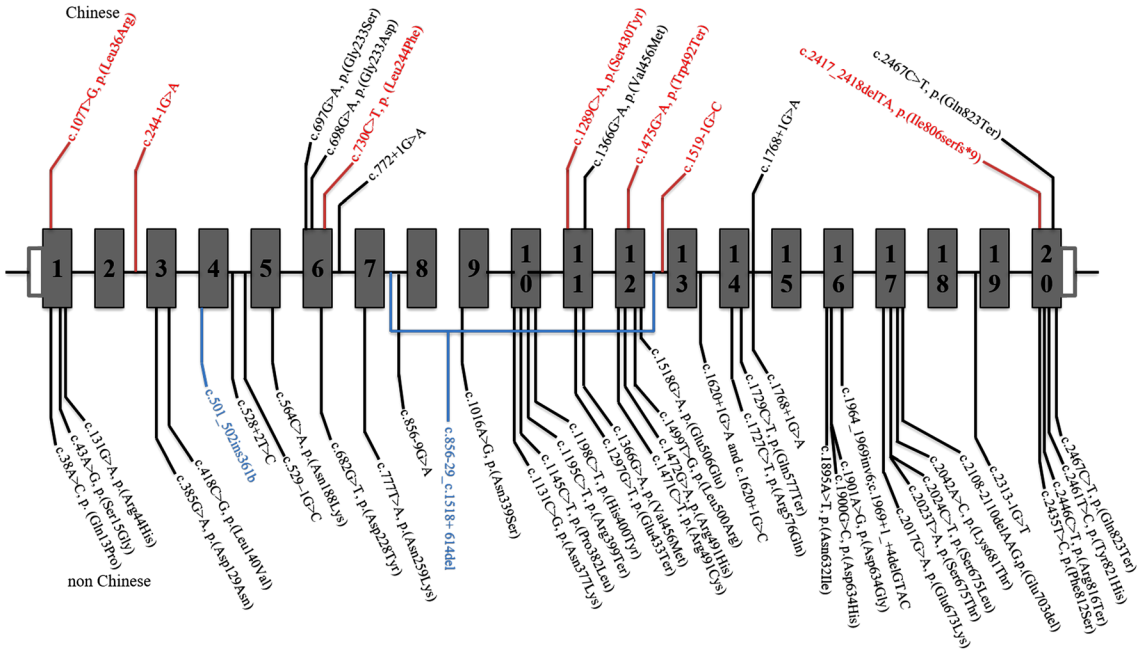

Figure 2: Genotypic spectrum of *PYGL* gene in Chinese and non-Chinese population. Gray box: exons of *PYGL* gene (exons were not drawn in the scale); the variants above exons are *PYGL* gene variants identified in Chinese population; the variants below exons are variants identified in *PYGL* gene in non-Chinese population; red variants: novel *PYGL* gene variants identified in our study; blue variants: large *PYGL* gene variants of insertion and deletion.

and c.1366G>A, p.(Val456Met) were recurrent in four and three unrelated families, respectively. When we conducted a closer scrutiny to c.1900G>C, p.(Asp634His), we found that it carried an allele frequency of 0.003509 in general population and an allele frequency of 0.005469 in European population in the ExAC database, and it was predicted to be deleterious by using PolyPhen-2, SIFT, and CADD. All variants distributed over *PYGL* gene with no variant was found on exon 2, exon 13, exon 15, exon 18, and exon 19, so far.

Discussion

We reported genotypic and phenotypic spectrum of 10 patients with GSD VI in mainland China. Hepatomegaly is the most common symptom caused by increased glycogen storage in hepatocytes in Chinese patients with GSD VI, and hypoglycemia may not be present as gluconeogenesis is intact. The most common abnormal laboratory examination finding is elevated transaminase due to the damage of hepatocytes. Elevated transaminase can reflect the degree of hepatocyte damage to some extent because there are not enough hepatocytes to release transaminase in the later stage. Liver cirrhosis is the final usual outcome of various chronic liver diseases, with most patients progressing into decompensated stage silently. The prevalence of cirrhosis varies among the different types of GSDs with unclear pathogenesis. Patients with glycogen storage disease type I (GSD I) had the most severe metabolic phenotype, with only mildly elevated transaminase. However, hepatic fibrosis or cirrhosis was not observed in animal models and patients with GSD I [23, 24]. Transaminase levels elevated most obviously in glycogen storage disease type III (GSD III), and hepatic fibrosis is a common long-term complication of GSD III, with live biopsy showing the deposition of abnormally structured glycogen, resembling limit dextrin [1]. Although dietary treatment can prevent hypoglycemia and improve growth, it was found that the improvement of metabolism cannot prevent progressive hepatic fibrosis in animal models and patients with GSD III [25–27]. Patients with glycogen storage disease type IV showing the deposition of abnormally structured glycogen, resembling plant-like fibers of amylopectin in live biopsy, can rapidly progress to cirrhosis [1]. A high-protein diet and cornstarch cannot prevent progression of the liver disease, and liver transplantation is the primary treatment [28]. All these accumulations of abnormally structured glycogen may be a critical factor for hepatocyte damage and result in a rapid progression

to hepatic fibrosis and cirrhosis. Cirrhosis had a high frequency in glycogen storage disease type IXc (GSD IXc), with 40% of patients with GSD IXc affected by cirrhosis, and although information about treatment and prognosis was rare, it was found that some patients did not develop cirrhosis under treatment of uncooked cornstarch and protein supplementation [29]. Cirrhosis was also reported in some cases with glycogen storage disease type IXa, and a structured treatment regimen can improve metabolic control and cirrhosis [30, 31]. Hepatic fibrosis is caused by chronic liver injury because of viral infection, drugs, toxins, or metabolic disorders [32]. Activated hepatic stellate cells (HSCs) play an important role in pathogenesis of hepatic fibrosis [8, 32]. Under the stimulation of cytokines and chemokines released by immune cells and damaged hepatocytes, HSCs can be activated, which then differentiate into myofibroblasts that secrete various extracellular matrix proteins causing hepatic fibrosis [32]. Cirrhosis can always develop silently, and liver biopsy is an effective way to identify cirrhosis. Here, for the first time, we reported that four patients showed cirrhosis featured by the formation of regenerative nodule in liver biopsy, revealing a risk of progression to end-stage liver disease. Because most liver biopsies in literature review were conducted without exact time and visual confirmation, we cannot conduct a systematic analysis for hepatic histopathology. However, in our study, we analyzed the time of liver biopsy between two groups to explain cirrhosis. We found that the age of liver biopsy conducted in patients without hepatic fibrosis and cirrhosis was significantly earlier. Thus, we cannot rule out the possibility that hepatic fibrosis and even cirrhosis may present over time. A recently constructed GSD VI murine model also revealed that the damage, inflammation, and fibrosis of hepatocytes aggravated with aging [8]. So far, no cirrhosis was reported in patients with GSD VI, and this may be a result of the lower number of liver biopsy that has been investigated. Moreover, cirrhosis is a complicated process where genetic and metabolic factor may be involved in, and it has been demonstrated that cirrhosis was a pathway of hepatocellular carcinoma transformation [25]. Here, our study combined with published literature further confirms that GSD VI can also be in another extreme of the disease severity. Considering mounting evidence supported that long-term complications can be prevented by optimizing metabolic control, we recommend an aggressive therapy for GSD VI [30]. Systematic follow-up and specific biomarkers are also needed to monitor this silent course although the improvement of elevated transaminase was observed in most of our patients with GSD VI [15, 33].

Kobayashi et al. concluded that variants with an allele frequency higher than 0.01% in the ExAC database were generally already well characterized in the literature as known founder mutations [34]. When we reviewed the literature, we found that c.1900G>C, p.(Asp634His) was recurrent in four European families, and we speculated that this variant may be a founder mutation in European population. Notably, this variant contributed a large burden of disease with an allele frequency of 0.003509 in general population and an allele frequency of 0.005469 in European population in the ExAC database. Considering this variant alone can contribute to a world-wide prevalence of 1.2 of 100,000 in the genetic context based on the Hardy-Weinberg equilibrium (the prevalence is equal to the square of the minor allele frequency), the prevalence of GSD VI is further confirmed to be underestimated. Inconsistent with an autosomal recessive pattern, only one patient is female in our cohort; this may be due to boys getting more attention than girls in the family, and their parents have a stronger inclination to bring them to tertiary hospital in China. It is also suggested that some female patients are underdiagnosed or unreported in China. As c.772+1G>A was recurrent in three families in Chinese population, it is suggested that the genotypic spectrum of *PYGL* gene may vary among the population. Furthermore, we identified seven novel variants, bringing the total number of *PYGL* gene variants to 53, and we considered that all these novel variants were pathogenic based on the type of variant, *in silico* analysis, and allele frequency in the public database.

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