

Pathology can determine the pathophysiology of liver fibrosis for GSD-IX

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Abstract

Introduction : The mechanism of hepatic fibrosis in glycogen storage diseases type IX (GSD-IX) remains unclear. Therefore, we aimed to clarify the pathophysiology of liver fibrosis in patients with GSD-IX through pathological examination.

Methods : Six boys with GSD-IX aged 4 months to 4 years were enrolled in the study. Four boys underwent histological analysis (including electron microscopy); of these, 3 cases were immunohistochemically compared with a control group of 59 cases that underwent liver biopsy.

Results : All 4 patients with GSD-IX showed liver fibrosis; however, no apparent bridging fibrosis was observed. Glycogen storage was particularly prominent around the center of hepatocytes, and loss of mitochondrial cristae was observed in electron microscopy. In the immunohistochemical analysis of pathological tissues (3 out of 4 cases analyzed), tissues from 3 cases yielded negative results for matrix metalloproteinase-13 (MMP13) and platelet-derived growth factor (PDGF). In contrast, endothelin-1 (ET-1) was positive in 2 cases of GSD-IX. These were found to be statistically significant compared to the control group ($P<0.05$).

Discussion : Fibrosis without PDGF expression was considered reversible, which is consistent with the characteristics of GSD-IX that is less prone to cirrhosis. In addition, fibrosis due to ET-1 occurred occasionally. Taken together, mild fibrosis in many patients with GSD-IX might be correlated with the presence of ET-1 and lack of vascular factors such as PDGF.

Introduction

Liver fibrosis is often observed in various liver diseases and is also comorbid to various conditions. Liver fibrosis is characterized by the deposition of type I collagen fibers as a reactive change to various hepatic disorders. Liver cirrhosis is a terminal stage of chronic liver disease due to fibrosis. However, the mechanism of hepatic fibrosis in children remains unclear, and details of how it contributes to disease state and prognosis have not been known, especially in children with varied metabolic disorders^{1,2)}.

In particular, glycogen storage diseases (GSDs) pro-

mote glycogen accumulation in hepatocytes, resulting in fibrosis, and may lead to liver cirrhosis. Depending on the type, there are complications such as tumors and neutropenia³⁾. Generally, type IX GSD (GSD-IX) patients have a good prognosis and are unlikely to develop liver cirrhosis or require liver transplantation⁴⁾, although there are more severe cases depending on the subtype⁵⁾.

To date, there are a few studies about hepatic fibrosis in GSD-IX. Therefore, in the present study, we aimed to clarify the pathophysiology of liver fibrosis in patients with GSD-IX, by comparing it with various diseases that cause liver fibrosis.

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Subjects and Methods

This retrospective observational study was conducted at Tokyo Medical University. Pediatric patients diagnosed with GSD at our hospital between February 2002 and September 2015 were included in the study. The pathological evaluation was compared with 59 control patients who underwent liver biopsy between March 29, 2002 and August 27, 2015. All patients had liver dysfunction and underwent liver biopsy. The control group age ranged from 1 month to 21 years, with a median of 6 years and a mean of 6.75 years. The male to female ratio was 39 : 20. Immunostaining was performed in 3 cases (Case 1, 3, 4) of GSD and 59 cases in the control group.

〈GSD cases〉

Six boys aged 4 months to 4 years who were diagnosed with GSD-IX participated in the study. Diagnosis was based on hepatic dysfunction and clinical symptoms, isoenzyme and Fernandez tests. Of the 6 boys, 4 underwent liver biopsy; the characteristics of the 6 boys are shown in Table 1. The boys in case 1 and 2 were brothers, aged 4 years and 8 months and 1 year and 2 months, respectively. The younger brother (case 2) was delivered at 40 weeks and 4 days, and there was no remarkable history that prompted the suspicion of hypoglycemia. At 5 months of age, he developed acute upper respiratory inflammation. At that time, blood tests showed transaminase elevation. Since the transaminase elevation was prolonged for more than 6 months, he underwent liver biopsy. The elder brother (case 1) had prominent abdominal distension and hepatomegaly confirmed 8 cm below the ribs beneath the right mid-clavicular line. Both brothers showed developmental delay. Case 3 was a boy delivered at 39 weeks and 6 days. His birth weight was 3,600 g, and his Apgar score was 8-9. He was admitted to the neonatal intensive care unit (NICU) because of hypoglycemia, hypercalcemia, and feeding disorder. At 4 months of age, he had fever, cough, and diarrhea for 2 weeks and showed apparent life-threatening event (ALTE)-like consciousness disturbance during sleep. After resuscitation, he was urgently admitted due to suspected sudden infant death syndrome. Although there was no disturbance of consciousness after hospitalization, alanine and aspartate aminotransferases (ALT and AST, respectively) were elevated. Cases 4, 5, and 6 were suspected of having GSD due to hepatic dysfunction and were diagnosed with GSD-IX based on clinical symptoms, isoenzyme test, and Fernandes test. Serum AST, ALT, cholesterol, and triglyceride (TG) levels of each case were 64-1,287, 29-898, 137-165, and 122-686 U/L, respectively. Three cases (1, 2, and 6) showed increased absorbance in abdominal computed tomography (CT). Phosphory-

lase-B kinase levels in erythrocytes were low in 3 cases (1, 2, and 6). In Case 5, phosphorylase-B kinase levels in erythrocytes were normal; however, in his liver tissue phosphorylase-B kinase levels were lower than that of control subjects. Case 3 and 4 were not measured for isoenzyme test in liver tissue, but it is suggested that they may have decreased as well. The clinical data of GSD cases included at the time of histological evaluation.

〈Evaluation of pathology〉

We pathologically compared these GSD-IX cases with the control group of 59 patients who underwent liver biopsy and whose samples remained at our hospital. Conditions in the control group included 6 cases of cholestasis (2 cases of Alagille syndrome, 3 cases of biliary atresia, and 1 case of congenital biliary dilatation), 19 cases of nonalcoholic steatohepatitis (NASH) or nonalcoholic fatty liver disease (NAFLD), 2 cases of drug-induced liver damage, 5 cases of metabolic disorders (hemochromatosis, protoporphyria, and mitochondrial disease), 6 cases of viral hepatitis, 12 cases of chronic hepatitis, 1 case of common variable immunodeficiency (CVID), 1 case of anencephaly, 1 case of hepatitis-related total parenteral nutrition-induced liver injury (TPN), and 6 other cases (sudden death, malignant tumor, immunodeficiency, etc.).

In addition, immunostaining was performed to evaluate liver fibrosis and regeneration. For immunohistochemistry, paraffin sections were dewaxed in xylene and rehydrated in decreasing concentrations of ethanol. In brief, the sections were deparaffinized and treated with a solution of H₂O₂ in methanol for 20 min at room temperature to block endogenous peroxidase activity. The sections were antigen retrieved by autoclaving for 20 minutes at 105°C and subsequently incubated with the respective dilutions of primary antibodies (Anti-MMP13 IgG rabbit polyclonal, 1 : 150, ABCAM; Anti-PDGF B IgG rabbit polyclonal, 1 : 500, ABCAM; Anti-Endothelin 1 IgG rabbit polyclonal, 1 : 2,000, ABCAM). Negative controls with no primary antibody were used to assess nonspecific staining. The secondary antibodies used included horseradish peroxidase-conjugated goat anti-rabbit IgG (Nichirei) and goat anti-mouse IgG (Nichirei).

The resulting sections were stained and analyzed using a microscope by more than two doctors, including pathologists Shigeo Nishimata and Hisashi Kawasima. For immunostaining, GSD-IX and controls were compared using chi-square [χ^2] test. Data were analyzed with IBM SPSS Statistics 27.

Results

Fibrosis marker (serum type IV collagen) levels were high in 2 out of 3 cases [Table 1].

Liver pathology findings obtained from all 4 patients

Table 1 GSD case profiles

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age	4 y	1 y 7 m	4 m	1 y 2 m	1 y 0 m	4 y
Gender	M	M	M	M	M	M
Symptoms	Abdominal distension	Abdominal distension	ALTE	Liver dysfunction	Liver dysfunction	Liver dysfunction
Hepatomegaly	+	+	+	—	+	+
Hypoglycemia	—	—	+	—	—	—
Family history	Younger brother (case 2) has GSD	Elder brother (case 1) has GSD	—	Grandmother : Liver dysfunction	—	—
Height	— 1.6 SD	— 2.3 SD	N.D.	N.D.	— 1.5 SD	— 1.2 SD
AST/ALT (U/L)	428/311	1,287/898	64/29	119/74	350/270	222/180
γ -GTP (U/L)	40	202	N.D.	8	49	42
T-Bil/D-Bil (mg/dL)	0.62/0.04	0.53/0.08	0.48	0.44/0.22	0.58/0.1	1.0/0.7
TG/T-cholesterol	189/154	686/165	N.D.	N.D.	N.D.	122/137
IV type collagen (<137)	136 ng/ml	N.D.	N.D.	356	150	N.D.
P-III-P (<0.8)	0.66 U/ml	N.D.	N.D.	N.D.	N.D.	N.D.
CT (Liver)	Hepatomegaly, CT high value	Hepatomegaly, CT high value	N.D.	N.D.	Normal	Hepatomegaly, CT high value
Phosphorylase B kinase (nmol/min/gHb) (control)	0.3↓ (9.7–12)	0.8↓ (9.7–12)	15.9→ (8.2–8.4)	14.3→ (8.2–8.4)	47.8→ (24.8–25.5) Liver 24.7↓ (89.5–109.4)	0.6↓ (NR)
Diagnosis	GSD-IX	GSD-IX	GSD-IX	GSD-IX	GSD-IX	GSD-IX

*ALTE : apparent life-threatening event ; ND : not done ; AST : aspartate aminotransferase ; ALT : alanine aminotransferase ; GSD : glycogen storage disease ; GTP : glutamyl transferase ; Bil : bilirubin ; CT : computed tomography ; TG : triglycerides ; NR : denotes data not reported

showed a large number of enlarged hepatocytes with clear cytoplasm in hepatic lobules, strong positive periodic acid-Schiff staining (PAS), and negative PAS-diastase staining, which was confirmed as an accumulation of glycogen. Hepatocyte wall thickening and plant-like cell morphology were found. Pericellular fibrosis and inflammatory cell invasion were also observed [Fig. 1a, b, c, d, e, f], and the New Inuyama Classification of liver fibrosis was F1A1 in all 4 cases [Table 2]. Although liver fibrosis was detected in all cases, severe fibrosis with apparent bridging fibrosis was not observed [Fig. 1f, g]. Most of the controls were A1F0 to A2F2 according to the new Inuyama Classification, and 32 out of 59 cases were A1F1. Only one case of Anencephaly had strong fibrosis, F2F3.

In case 1 (that also presented with hepatomegaly), fatty degeneration was seen in hepatocytes and a mild inflammatory infiltration mainly composed of lymphocytes was found in the portal region. Electron microscopy of tissue samples obtained from case 1 showed that glycogen retention was prominent at the periphery but not at the center of hepatocytes, and mitochondrial cristae loss was observed. Collagenous fibers were intense around the cells, glycogen was incorporated into lysosomes, and small to big droplets were observed in the

cells [Fig. 1h].

⟨immunohistochemical studies⟩

In immunohistochemical studies [Table 3], 3 cases of GSD were negative for MMP-13 [Fig. 1i] and PDGF [Fig. 1k]. On the contrary, MMP-13 positivity was noted in 8 of 59 cases in the control group (1 case [hemochromatosis] was strongly positive [Fig. 1j], 5 cases [CVID, drug-induced liver injury, mitochondrial disorder, sudden death, anencephaly] were moderately positive, and 2 cases [Hirschsprung disease/TPN, and NAFLD] were weakly positive) (chi-square $[\chi^2]$ test $P<0.05$).

PDGF positivity was noted in 8 cases. Of these, 5 cases (hemochromatosis, CVID, drug-induced liver injury, mitochondrial disorder, and anencephaly) were strongly positive [Fig. 1l] and 3 cases (two chronic and one viral hepatitis) were weakly positive. PDGF was negative in all NASH and NAFLD cases (chi-square $[\chi^2]$ test $P<0.05$).

On the other hand, ET-1 was positive in 2 out of 3 GSD cases (Fig. 1m), negative in 7 out of 19 NASH and NAFLD cases, and positive in all other disease groups (Fig. 1n) (chi-square $[\chi^2]$ test $P<0.05$).

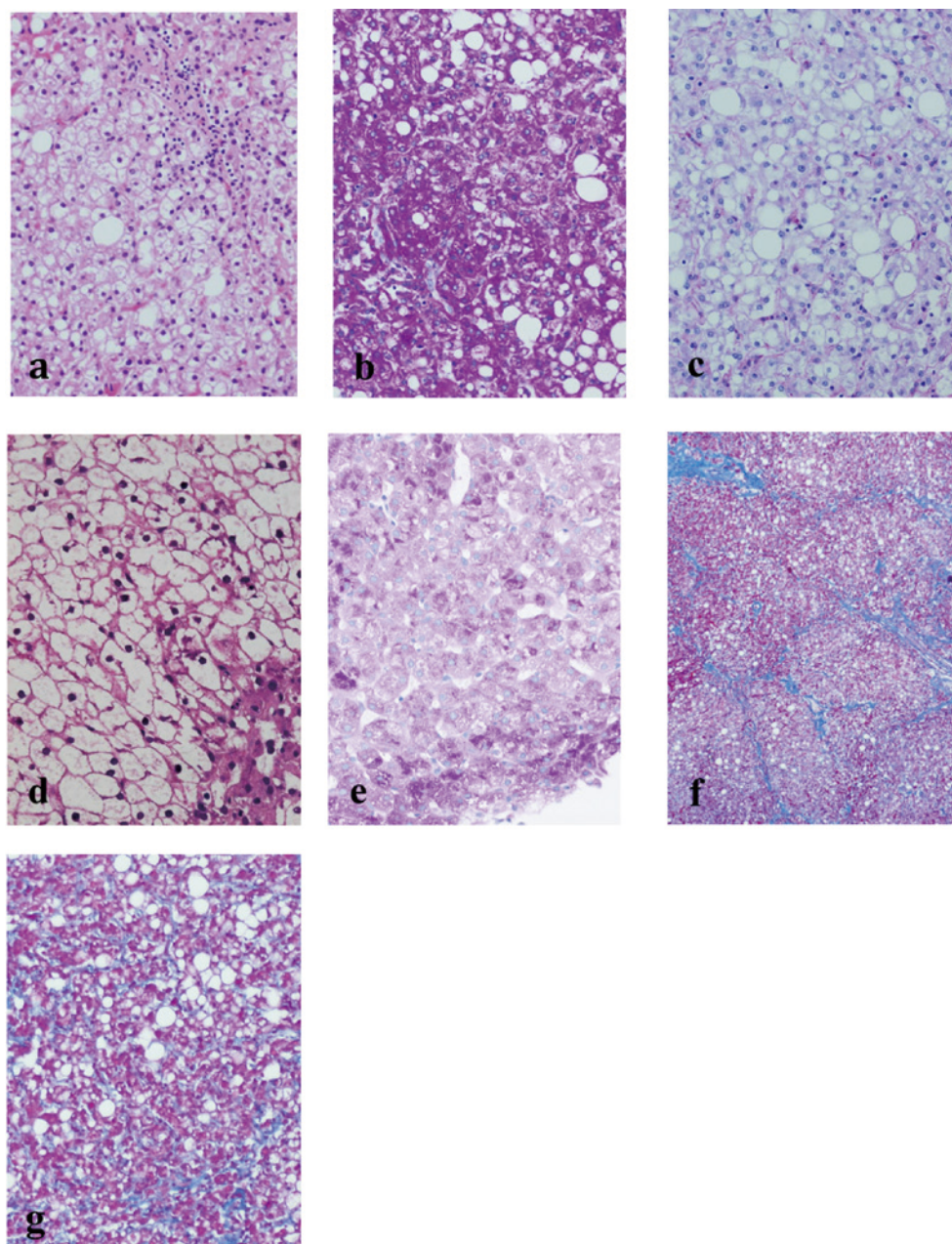


Fig. 1 HE staining $\times 200$ [a], PAS staining $\times 200$ [b], and digestive PAS staining $\times 200$ [c] of case 1. HE staining $\times 200$ [d] and PAS staining $\times 200$ [e] of case 5. [f] Azan staining $\times 50$, [g] Azan staining $\times 200$ of case 1 with liver fibrosis.

A large number of enlarged hepatocytes with clear cytoplasm in hepatic lobules, strong positive periodic acid-Schiff staining (PAS), and negative PAS-diastase staining, which was confirmed as an accumulation of glycogen. Hepatocyte wall thickening and plant-like cell morphology invasion were found. Pericellular fibrosis and inflammatory cell invasion were found. Electron microscopy of liver cells obtained from case 1[h] (left : remarkable accommodation of glycogen [G], middle : fat droplets [F], right : glycogen in lysosome [L]). The accumulation of glycogen in hepatocytes was not central to the cells, but was prominent around the cells, and there was a loss of mitochondrial cristae and cell structure due to remarkable glycogen storage.

MMP-13 immunostaining of case 1 (negative) [i] and hemochromatosis (positive) [j].

PDGF immunostaining of case 1 (negative) [k] and hemochromatosis (positive) [l].

ET-1 immunostaining of case 1 (positive) [m] and hemochromatosis (positive) [n].

Discussion

Although all cases were diagnosed as GSD-IX no bridging fibrosis or cases leading to liver cirrhosis were

found, although liver fibrosis may be seen at different ages. It is therefore suggested that GSD-IX may be associated with reversible liver fibrosis.

Maire et al. reported that GSD-IX is the most frequent

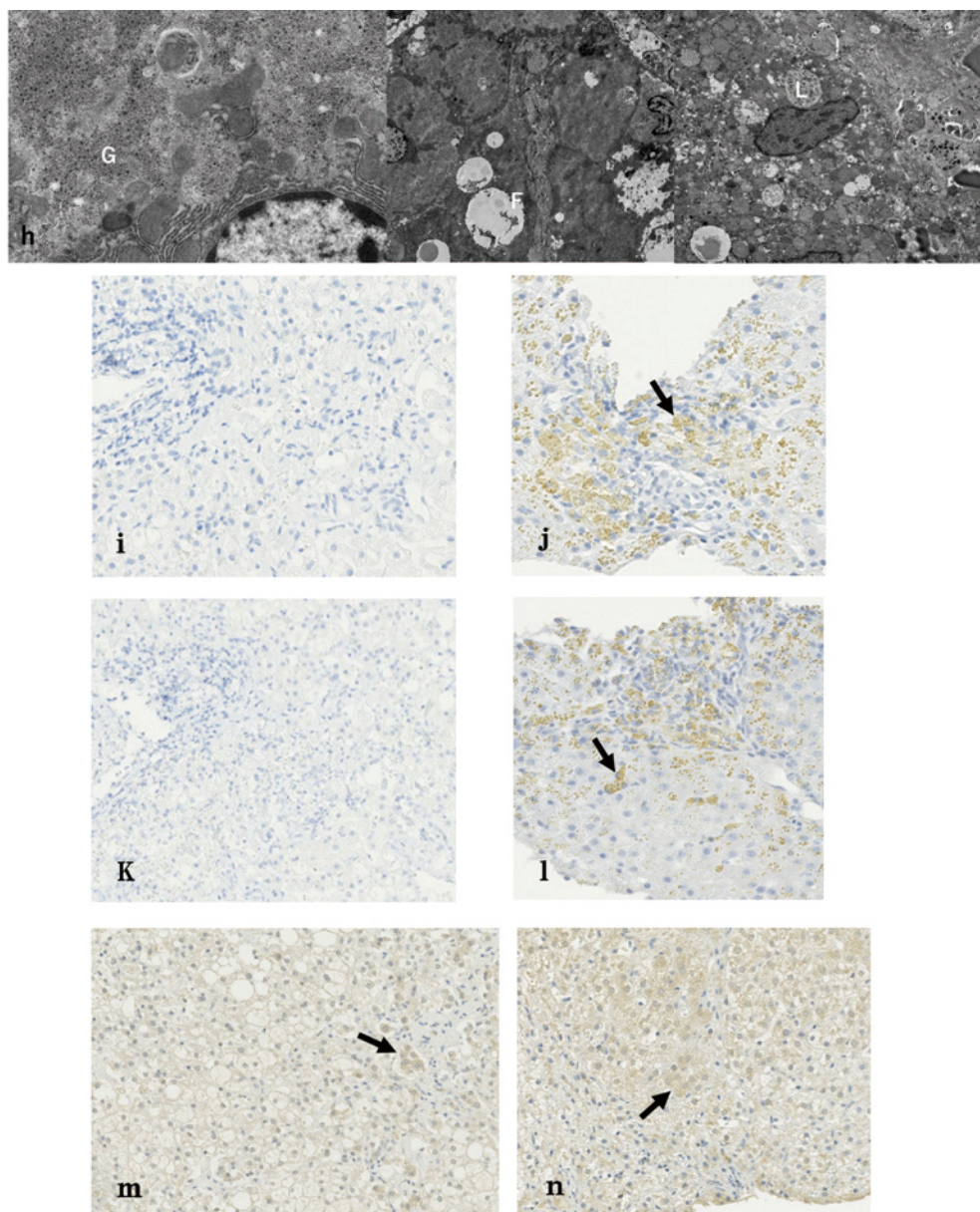


Fig. 1 Continued

Table 2 Liver pathology findings obtained from 4 GSD cases

Liver pathology	Case 1	Case 3	Case 4	Case 5
Accumulation of glycogen	+	+	+	+
Liver fibrosis	+	+	+	+
Invasion of inflammatory cells	+	+	+	+
Enlarged hepatocytes with clear cytoplasm	+	+	+	+
New Inuyama Classification	F1A1	F1A1	F1A1	F1A1

form of GSD, accounting for 25% of cases. GSD-IX (liver phosphorylase kinase deficiency) is divided into subtypes a-f, and subtypes d, e, and f are accompanied by myopathy and other muscle symptoms. In GSD-IX the activation of phosphorylase b to phosphorylase a is disturbed and active phosphorylase is not generated, ultimately impairing glycogenolysis.

Phosphorylase kinase is composed of four kinds of subunits (α , β , γ , and δ) and a tissue-specific isozyme⁶⁾. Among these subunits, α and β are regulatory subunits, γ is a catalytic subunit, and δ is a Ca-binding calmodulin. The α subunit abnormality causes the most frequent GSD-IXa through X-linked recessive inheritance, the β subunit abnormality causes GSD-IXb, the γ subunit abnormality causes GSD-IXc, and the δ subunit abnormality causes GSD-IXd⁷⁾. All GSD cases in this study did not show any muscle symptoms and were classified as GSD-IXa. The affected organ in GSD-IXa is primarily the liver, and the symptoms are mainly hepatomegaly and growth disorders. Since symptoms improve from school age, GSD-IXa is regarded as the mildest form of GSD. Most GSD-IX forms are considered to have good prognosis, rarely lead-

Table 3 Liver pathological tissue immunostaining (MMP-13, PDGF, and ET-1)

	Disease (number)	MMP-13	PDGF	ET-1
	GSD (3)	—	—	+ (2)
Accumulation disease	Hemochromatosis	++	+	+
	Protoporphyrria	—	—	+
	Mitochondrial disorder (3)	+ (1)	+ (1)	+ (3)
Bile stasis	Alagille syndrome (2)	—	—	+ (2)
	Biliary atresia (3)	—	—	+ (3)
Abnormal secretion	CVID	+	+	+
	Anencephaly	+	+	+
Absorption disorder	Hirschsprung disease/ TPN-induced liver injury	±	—	+
Metabolic	NASH/NAFLD (19)	± (1)	—	+ (10) ± (2)
Chronic	Chronic hepatitis (13)	—	± (2)	+ (9) ± (4)
Infection	Viral hepatitis (5)	—	± (1)	+ (4) ± (1)
Drug induced	Drug-induced liver injury (2)	+ (1)	+ (1)	+ (1) ± (1)
	Other condition* (6)	+ (1)	—	+ (3) ± (3)

*one B lymphoblastic leukemia, one X-linked agammaglobulinemia, two Reye syndrome, one sudden death, and one Patent Ductus Venosus

MMP-13 : matrix metalloproteinase 13 ; PDGF : platelet-derived growth factor ; ET-1 : endothelin 1 ; CVID : common variable immunodeficiency ; TPN : Total parenteral nutrition ; NASH : nonalcoholic steatohepatitis ; NAFLD : nonalcoholic fatty liver disease

— : negative ; ± : weakly positive ; + : moderately positive ; ++ : strongly positive

ing to liver cirrhosis. According to Hers et al., 3 cases of liver adenomas and 2 cases of liver cirrhosis were observed in 205 cases of GSD-IX, but genetic studies were not conducted⁸⁾. Genetic analysis was not performed in this study either, but it may be worthwhile to perform genetic analysis because the course of the disease may differ depending on the subtype.

The clinical diagnosis of GSD-IX is determined by hypoglycemia ; hepatosplenomegaly ; short stature ; developmental retardation ; easy fatigability ; and increased levels of lactic acid, uric acid, and lipids ; however, there is a considerable number of atypical cases⁹⁾. According to recent genetic studies, 15 cases of GSD-IX were caused by mutations in PHKA2 (10 cases), PHKG2 (2 cases), and PHKB (3 cases). The phenotype of PHKA2 mutation is varied, but the phenotypes of PHKB mutation are mild¹⁰⁾. There is also a study reporting liver cirrhosis with PHKA2 mutation¹¹⁾. A recessive mutation in *PHKG2* caused an abnormality in the γ subunit and cirrhosis in 12 out of 17 cases¹²⁾. The case of liver fibrosis in this study is clinically considered to be GSD-IXa, but it cannot be determined. In the future, genomic studies will be needed to for performance analysis? in order to estimate the prognosis of liver fibrosis.

Hepatic fibrosis is caused by reactive changes in several liver diseases and is characterized by the accumulation of type I collagen fibers. Liver cirrhosis is the final stage, in which extreme fibrosis is present. The pattern

of fibrosis is divided into lobular and portal regions. Both fibrosis patterns progress and finally lead to liver cirrhosis through bridging fibrosis between the portal area and portal or central vein regions.

In this study, 1 of the 4 GSD-IX cases showed mild fibrosis and inflammation in the portal region. The other 3 cases showed pericellular fibrosis, predominantly in the central vein region.

Hepatic stellate cells (HSCs) play a central role in the mechanism of liver fibrosis. Liver fibrosis is a state in which the balance between accumulation and decomposition of hepatic ECM is biased toward accumulation. HSCs are responsible for ECM production and are involved with factors such as MMP-13, PDGF, ET-1, and TGF- β . In a fibrotic state, type 1 collagen is produced by HSCs due to ET-1 stimulation. HSCs themselves are activated by various cytokines (PDGF, transforming growth factor- β 1 [TGF- β 1], and interleukin-1) and changes in the extracellular matrix (ECM). When this transformation occurs, myofibroblasts are formed and collagen synthesis is accelerated. At the same time, the production of MMP, a proteolytic enzyme, is also enhanced, but it is thought that fibrillogenesis is enhanced by the significant production of metalloproteinase tissue inhibitor, which is an MMP¹³⁾. In various types of chronic hepatitis, various cytokines and growth factors related to inflammation are produced by affected hepatocytes and other cells, and HSCs are activated to perform a paracrine function (Fig. 2). HSCs also pro-

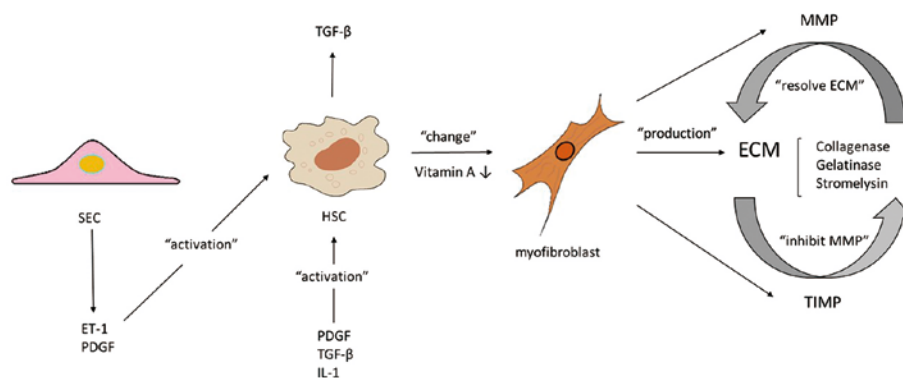


Fig. 2 Hypothesis of the pathophysiology of hepatic fibrosis.

HSC : hepatic stellate cell ECM : extracellular matrix SEC : sinusoidal endothelial cell MMP : matrix metalloproteinase ET-1 : endothelin 1 PDGF : platelet-derived growth factor TGF- β : transforming growth factor- β IL-1 : interleukin 1

duce various cytokines and growth factors and promote the proliferation and activation of other HSCs in a paracrine manner¹⁴. PDGF, a type of cytokine, is secreted from sinusoidal endothelial cells, platelets, and activated HSCs. Although it does not promote the synthesis of ECM, it promotes the proliferation of HSCs, especially activated HSCs¹⁵.

In this study, immunohistochemical findings indicated that GSD-IX patients were negative for PDGF and MMP; however, some other cases of severe fibrosis were positive for PDGF and MMP (5 out of 59 cases). Fibrosis without PDGF or MMP expression is not strong in the least, suggesting that it may be reversible and reflects the characteristics of GSD-IX, which rarely leads to cirrhosis because of the repair process that occurs.

ET-1 is also a vascular regulator derived from the endothelium and plays a central role in regulating vascular tone. It is a potent vasoconstrictor peptide mainly secreted by sinusoidal endothelial cells and contracts HSCs. Additionally, it has been reported that ET-1 is involved in cell differentiation and proliferation during the fibrosis process, and ET-1 and its receptors are increased in the fibrotic liver¹⁶. On the other hand, HSCs have receptors for ET-1, and activated HSCs produce ET-1 and are correlated with fibrosis development¹⁷. MMP-13 is elevated in the early stage of hepatic fibrosis amelioration and is thought to be a hepatic fibrosis-improving factor (Fig. 2).

There are a few reports about electron microscopy in GSD. Pears et al. reported that intracellular glycogen storage is particularly remarkable in GSD⁹. However, there are no studies about liver fibrosis in GSD-IX in particular. HSCs are in a stationary phase in the normal liver; the cells are occupied by lipid droplets and contain a large amount of vitamin A. They are also responsible for forming the three-dimensional structure of the liver sinuses by fixing the contacting position with the hepatic parenchyma cells and retaining the inner endothe-

lial cells. On the other hand, in liver cirrhosis, HSCs are activated and lose the lipid droplets containing vitamin A. Protein synthesis ability, especially ECM production, is remarkably enhanced and plays a major role in liver fibrosis¹⁴. In this study, electron microscopy showed that the accumulation of glycogen in hepatocytes was not central to the cells, but was prominent around the cells, and there was a loss of mitochondrial cristae and cell structure due to remarkable glycogen storage (Fig. 1h). Moreover, these are some of the few structures that may show autophagy. From these findings, it was presumed that cellular function was impaired and that there was an accumulation of lipid droplets.

On the other hand, fibrosis may occur in GSD-IX cases due to the action of ET-1 in the portal area. A previous study of GSD-VI and IX cases followed up for an average of 8.4 years showed myocardial hypertrophy and liver adenoma. Additionally, 48% of GSD-IV and GSD-IX cases had liver fibrosis, and less than 5% had cirrhosis¹⁸. Collectively, the findings of other studies and the present study indicate that careful observation and diet management are necessary even for GSD-IX cases whose prognosis is considered to be favorable. This is evident from the fact that fatty liver was also observed in 1 case of GSD-IX. Smit et al. performed a 10-year follow-up of 139 cases of GSD and reported that the growth pattern improved in adult age; nevertheless, hepatomegaly and hypercholesterolemia persisted in half of the cases¹⁹. In conclusion, it may not be necessary to perform frequent liver biopsy to determine the extent of liver fibrosis in GSD-IX, and if the degree of hepatic fibrosis is mild, a positive impact in the quality life of GSD-IX patients can be achieved.

Limitations

The study has several limitations. First, this study did not evaluate liver fibrosis by GSD-IX subtype, as no genetic studies were performed in this study. Second,

the pathological assessment was estimated only by two researchers, which may have introduced bias. In addition, the timing and age of liver pathology evaluation varies from case to case, and the disease status may be inconsistent. Another limitation is that we did not have data of tissues obtained consecutively from the same patient. Since fibrosis in patients with GSD-IX was mild, additional studies should be performed to investigate other severe GSD types to determine the pathophysiology of liver fibrosis in GSD. Future prospective studies may provide a more accurate assessment.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical considerations

The authors declare that all experiments were carried out in compliance with relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki. Ethical approval for this study was granted on 6 March 2020 by the Tokyo Medical University REC (T2019-0226).

Author contribution

H.T. and S.N. designed and performed experiments the study ; H.T., S.N., H.K. collected and analyzed data ; H. T. wrote the manuscript ; N.T., M.S. and Y.K. gave technical support and conceptual advice. All authors read and approved the final manuscript.

References

- 1) Fang YL, Chen H, Wang CL, Liang L : Pathogenesis of non-alcoholic fatty liver disease in children and adolescence : From “two hit theory” to “multiple hit model”. *World J Gastroenterol* **24**(27) : 2974-2983, 2018
- 2) Burda P, Hochuli M : Hepatic glycogen storage disorders : what have we learned in recent years? *Curr Opin Clin Nutr Metab Care* **18**(4) : 415-421, 2015
- 3) Hicks J, Wartchow E, Mierau G : Glycogen storage diseases : A brief review and update on clinical features, genetic abnormalities, pathologic features, and treatment. *Ultrastruct Pathol* **35** : 183-196, 2011
- 4) Schimke RN, Zakheim RM, Corder RC, Hug G : Glycogen storage disease type IX : Benign glycogenesis of liver and hepatic phosphorylase kinase deficiency. *J Pediatr* **83** : 1031-1034, 1973
- 5) Kagalwalla AF, Kagalwalla YA, al Ajaji S, Gorka W, Ali MA : Phosphorylase b kinase deficiency glycogenesis with cirrhosis of the liver. *J Pediatr* **127** : 602-605, 1995
- 6) Maire I, Baussan C, Moatti N, Mathieu M, Lemonnier A : Biochemical diagnosis of hepatic glycogen storage diseases : 20 years French experience. *Clin Biochem* **24** : 169-178, 1991
- 7) Kono N : Liver pathology in glycogen storage diseases. *Kan-tan-sui* **24** : 385-393, 1992 (in Japanese)
- 8) Hers HG, Hoof FV, Barsy T : Glycogen storage diseases. In : Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic Basis of Inherited Disease*. 6th edn. New York : McGraw-Hill, 425-451, 1989
- 9) Pears JS, Jung RT, Hopwood D, Waddell ID, Burchell A : Glycogen storage disease diagnosed in adults. *Q J Med* **82** : 207-222, 1992
- 10) Beauchamp NJ, Dalton A, Ramaswami U, Niinikoski H, Mention K, Kenny P, Kolho KL, Raiman J, Walter J, Treacy E, Tanner S, Sharrard M : Glycogen storage disease type IX : High variability in clinical phenotype. *Mol Genet Metab* **92** : 88-99, 2007
- 11) Johnson AO, Goldstein JL, Bali D : Glycogen storage disease type IX : Novel PHKA2 missense mutation and cirrhosis. *J Pediatr Gastroenterol Nutr* **55** : 90-92, 2012
- 12) Albash B, Imtiaz F, Al-Zaidan H, Al-Manea H, Banemai M, Allam R, Al-Suheel A, Al-Owain M : Novel PHKG2 mutation causing GSD IX with prominent liver disease : Report of three cases and review of literature. *Eur J Pediatr* **173** : 647-653, 2014
- 13) Okazaki I, Watanabe T, Tokozawa S : Molecular pathophysiology of liver fibrosis. *Gendai Iryo* **30** : 2109-2116, 1998
- 14) Okuno M, Adachi S, Akita K, Moriwaki H, Kojima S, Friedman SL : Liver fibrosis and hepatic stellate cells. *Connective Tissue* **32** : 401-406, 2000 (in Japanese)
- 15) Shubo T, Manda T : Pathophysiology and strategy of treatments for liver fibrosis. *Igaku no Ayumi Digestive diseases (Supple)* : 130-134, 1999 (in Japanese)
- 16) Pinzani M, Milani S, Franco RD, Grappone C, Caligiuri A, Gentilini A, Tosti-Guerra C, Maggi M, Failli P, Ruocco C, Gentilini P : Endothelin 1 is overexpressed in human cirrhotic liver and exerts multiple effects on activated hepatic stellate cells. *Gastroenterology* **110** : 534-548, 1996
- 17) Li J, Kuruba R, Wilson A, Gao X, Zhang Y, Li S : Inhibition of endothelin-1-mediated contraction of hepatic stellate cells by FXR ligand. *PLoS One* **5** : e13955, 2010
- 18) Roscher A, Patel J, Hewson S, Nagy L, Feigenbaum A, Kronick J, Raiman J, Schulze A, Siriwardena K, Mercimek-Mahmutoglu S : The natural history of glycogen storage disease types VI and IX : Long-term outcome from the largest metabolic center in Canada. *Mol Genet Metab* **113** : 171-176, 2014

- 19) Smit GP, Fernandes J, Leonard JV, Matthews EE, Moses SW, Odievre M, Ullrich K : The long-term

outcome of patients with glycogen storage diseases. J Inherit Metab Dis **13** : 411-418, 1990

Phosphorylase B kinase 欠損糖原病（糖原病 IX 型）における 小児期の肝病変—肝線維化機序の検討

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【要旨】 はじめに：糖原病 IX 型（GSD-IX）における肝線維化の機序は不明なままである。そこで、GSD-IX における肝線維化の病態生理を病理学的に明らかにすることを目的とした。

方法：対象は生後 4 ヶ月から 4 歳までの GSD-IX と診断された 6 例の男児である。そのうち 4 例は組織学的（電子顕微鏡検査を含む）に、うち 3 例は免疫組織学的解析を行い、肝生検を受けたその他疾患の 59 例（対照群）と比較した。

結果：組織学的に評価した GSD-IX の 4 例はすべて肝線維化を示したが、明らかな架橋形成は認めなかった。電子顕微鏡ではグリコーゲン貯蔵は肝細胞の中心付近で特に顕著であり、ミトコンドリアのクリステ消失を認めた。免疫組織学的解析（4 例中 3 例施行）では、MMP-13 と PDGF はすべての症例で染色されなかったが、ET-1 は 2 例で陽性であった。

考察：PDGF の発現を伴わない線維化は可逆性であることが考えられ、肝硬変になりにくい GSD-IX の特徴と一致していた。また一部の症例では、ET-1 を介して惹起される線維化が認められた。多くの GSD-IX は線維化が軽度であり、PDGF などの血管因子が発現していないことと相関している可能性がある。

〈キーワード〉 電子顕微鏡、糖原病 IX 型、免疫組織学、肝線維化、病理学
