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Hepatic Fibrinogen Storage Disease in a Patient with Hypofibrinogenemia: Report of a Case with a Missense Mutation of the *FGA* Gene

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Abstract

We report a 9-year-old patient with abnormal liver tests found incidentally during routine bloodwork as part of a preoperative evaluation for excision of a benign cyst. A liver biopsy demonstrated hepatocytes to have pale and expanded cytoplasm that contained multiple vague globular eosinophilic inclusions. Electron microscopy showed fingerprint-like structures in the dilated cisternae of the rough endoplasmic reticulum, characteristic of fibrinogen. Whole exome sequencing identified a heterozygous missense mutation at codon 35 of the fibrinogen α (*FGA*) gene. No mutation was identified in the β or γ chains. His plasma fibrinogen levels were found to be decreased to 85 mg/dL (normal range 215–464). His family history was pertinent for his mother and maternal grandfather with hypofibrinogenemia. He had not had any significant bleeding episodes except for minor bruising over the shins. This case illustrates a rare etiology of storage disease that causes abnormal liver function tests.

Keywords

- hepatic fibrinogen storage disease
- hypofibrinogenemia
- liver biopsy

Fibrinogen is a glycoprotein that is synthesized in the liver and plays an important role in platelet aggregation, clot formation, and fibrinolysis.¹ The final step of the coagulation cascade converts fibrinogen to fibrin. It activates the fibrinolytic system by exposing thrombin (antithrombin I) binding sites on fibrin to promote thrombolysis in a feedback cycle and binds with plasminogen, tissue plasminogen activator, and α 2-antiplasmin.^{2,3} It also binds to vascular endothelial cells, fibronectin, glycosaminoglycans, and peptide growth factors and plays a key role in covalent cross-linking by acting as a substrate for factor XIII and other transglutaminases.⁴

Case History

The patient was a 9-year-old boy who was born full-term following an uneventful pregnancy. He presented for follow-up of abnormal liver function tests incidentally discovered as

part of the preoperative evaluation for excision of a benign cyst behind his ear at age 2. His medical history was otherwise unremarkable. He had been growing and gaining body weight appropriately. He had a circumcision shortly after birth without any bleeding complications. There was no prior recollection of scleral icterus, jaundice, epistaxis, significant bruising, prolonged bleeding, or altered mental status. He had been taking multivitamins and using ketoconazole shampoo.

On physical examination, his liver was palpable 1 cm below the right costal margin. Minor bruising was noted over the shins. No ascites or other signs of chronic liver disease were appreciated. Abdominal ultrasound confirmed mild hepatomegaly. His laboratory values included a white blood cell count = $9.24 \times 10^3/\mu\text{L}$ (normal range 3.28–9.29), hemoglobin = 12.9 g/dL (12.3–16.3), hematocrit = 37.1% (37.4–47.0), mean corpuscular volume = 78.9 fL (79.0–95.0), platelet count = $348 \times 10^3/\mu\text{L}$ (143–398), partial

thromboplastin time (PTT) = 28.7 seconds (24.4–32.0), prothrombin time (PT) = 13.1 seconds (9.7–11.2), international normalized ratio = 1.3 (0.79–1.20), total protein = 6.8 g/dL (6.2–8.3), albumin = 4.7 g/dL (3.7–5.1), alanine aminotransferase = 57 U/L (4–45), aspartate aminotransferase = 42 U/L (7–36), alkaline phosphatase = 166 U/L (31–103), total bilirubin = 0.3 mg/dL (0.2–1.1), and conjugated bilirubin = 0.1 mg/dL (0.0–0.2). Serologic markers for Epstein-Barr virus, cytomegalovirus, hepatitis B virus, hepatitis C virus, anti-smooth muscle antibody, and anti-liver/kidney microsomal antibody were negative. His antinuclear antibody titer was 1:40 (< 1:40) and serum IgG level was 941 mg/dL (690–1660). His plasma fibrinogen level was decreased to 85 mg/dL (215–464). Further investigation revealed that his mother and his maternal grandfather had a history of hypofibrinogenemia.

Morphologic Findings

Histologic examination of a liver core biopsy showed unremarkable portal tracts. There was no significant portal or lobular inflammation. Bile ducts were unremarkable and ductular reaction was absent. There was no appreciable cholestasis, steatosis, or fibrosis. The hepatocytes exhibited pale and expanded cytoplasm, which contained multiple vague globular eosinophilic inclusions. These cytoplasmic

inclusions were better appreciated on periodic acid–Schiff (PAS) stain with diastase and phosphotungstic acid–hematoxylin (PTAH) stain (►Fig. 1). Electron microscopic examination of the liver biopsy showed dilated cisternae of the rough endoplasmic reticulum that contained densely packed curved tubular electron dense structures arranged in a fingerprint-like pattern (►Fig. 2). No viral particles or abnormal mitochondria were identified.

Molecular Findings with Whole Exome Sequencing

DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) liver biopsy tissue with the RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Life Technologies). The DNA library was prepared with the Ovation Ultralow Library Systems (Nugen Technologies), followed by exome enrichment using the Roche NimbleGen SeqCap EZ v3.0 kit. Sequencing was performed on the Illumina HiSeq. 2500 and analyzed using the NextGene software (SoftGenetics). Data analysis identified a heterozygous missense mutation (c.103C > T) at codon 35 (amino acid change: R35C) of the fibrinogen α (*FGA*) gene (►Fig. 3). This mutation was predicted to be probably damaging with a score of 1 by the Polymorphism Phenotyping v2 (PolyPhen-2) software. No

Fig. 1 Histologic examination of the liver biopsy showing hepatocytes with pale and expanded cytoplasm containing multiple eosinophilic globular inclusions (A, Hematoxylin and eosin stain, original magnification $\times 400$). The cytoplasmic inclusions were highlighted by periodic acid–Schiff stain with diastase (B, original magnification $\times 400$), and phosphotungstic acid–hematoxylin stain (C, original magnification $\times 400$).

mutations were identified in the *FGB* and *FGG* genes that encode the fibrinogen β and γ chains.

Discussion

Congenital fibrinogen disorders are caused by mutations that affect the synthesis and secretion of fibrinogen. These mutations cause a quantitative defect (afibrinogenemia or hypofibrinogenemia) or a qualitative defect (dysfibrinogenemia).² The prevalence of hypofibrinogenemia is extremely low, affecting 1 to 2 per million people. The inheritance pattern is either autosomal recessive or dominant.⁵ There are three genes which code for fibrinogen chains: α (*FGA*), β (*FGB*), and γ (*FGG*).^{6–8} These genes are located on chromosome 4q and are regulated and expressed together as a group. The protein is composed of two heterodimers, each with a single α , β , and γ chain, linked by disulfide bonds.⁹ Single heterozygous mutations of the *FGB* and *FGG* genes are most common in patients with hypofibrinogenemia.^{10,11} The Fibrinogen Database (www.geht.org/databaseang/fibrinogen) is an online database documenting all the mutations that have been discovered to date.

Therefore, fibrinogen disorders can be divided into three categories: afibrinogenemia, hypofibrinogenemia and dysfibrinogenemia. Afibrinogenemia is seen in homozygotes or compound heterozygotes with severe mutations. Mutations can occur in all three genes, but most are found in *FGA* as deletion, frameshift, nonsense or splicing mutations. These mutations have effects at the DNA, RNA, or protein level by affecting protein synthesis, assembly, or secretion.^{12,13} The mutations that commonly cause afibrinogenemia are also known to cause hypofibrinogenemia. The phenotypic and clinical manifestations are dependent on the homozygosity or heterozygosity of the mutation. The mutations in the *FGB* and *FGG* genes are predominantly missense mutations in the C-terminal domains.¹⁴ Interestingly, most cases of afibrinogenemia and hypofibrinogenemia do not have an accumulation of a mutant fibrinogen in hepatocytes. A notable exception is missense mutations of the *FGG* gene that frequently lead to the formation of cytoplasmic inclusions in hepatocytes.^{15,16}

Most dysfibrinogenemic patients are heterozygous for missense mutations. This causes abnormal fibrin polymerization or delayed or absent release of fibrinogen. Most of these mutations occur in the *FGA* gene.¹² Our case study demonstrated a missense mutation at codon 35 (R35C) of the *FGA* gene. This mutation has been previously documented to be associated with dysfibrinogenemia,¹⁷ but not with hypofibrinogenemia.

Patients with fibrinogen disorders display a wide variety of clinical manifestations depending on the molecular defect. Severe symptoms include joint, muscle, and intracranial hemorrhage. Patients with hypofibrinogenemia often have a milder course of disease with bleeding episodes following a major trauma or invasive medical procedure.² Spontaneous bleeding episodes are not as common compared with afibrinogenemic patients.¹³ The more common clinical manifestations include menorrhagia, muscle hematoma, and gastrointestinal bleeding.¹⁸

Because the conversion of fibrinogen to fibrin is the final step of the coagulation cascade, patients with severe fibrinogen disorders typically have an abnormal PT, PTT, and thrombin time. However, patients with a milder clinical phenotype of hypofibrinogenemia may or may not have prolonged PT and PTT. Plasma fibrinogen levels can be quantified by using an immunoassay with antifibrinogen antibodies. If the quantitative and activity levels are not compatible, a diagnosis of dysfibrinogenemia is suggested. It should be noted that the fibrinogen levels may vary depending on periods of stress because it is an acute phase reactant, as well as age, gender, race, smoking, obesity, and pregnancy.²

One of the earliest documented examples of hepatic fibrinogen storage disease was by Pfeifer et al in 1981. In that case, proteinaceous cytoplasmic inclusions were strongly reactive with antihuman fibrinogen IgG, but nonreactive with antibodies against human α 1-antitrypsin and albumin.¹⁹ In 1986, Cella and colleagues also described these inclusions within liver cells. They felt that the histologic features closely resembled the ground glass inclusions of hepatitis B surface antigen (HBsAg), but they were negative for HBsAg and PAS stains.²⁰ Both small and large inclusions have been described. The small inclusions are irregular and the large inclusions are more spherical and vacuolated. These inclusions typically show positivity by PTAH stain and weak positivity by PAS stain,^{19,21} and can be highlighted by immunohistochemistry. Under electron microscopy, these proteinaceous inclusions consisted of densely packed, irregularly arranged tubules of 40 nm in diameter,¹⁹ located in dilated cisternae of the rough endoplasmic reticulum. The arrangement of the tubules gives rise to the unique and characteristic appearance of a fingerprint.^{19,21}

It should be noted that rare cases of acquired hepatic fibrinogen storage disease have been reported in the literature.^{22,23} For example, Simsek et al described a case that occurred in a 40-year-old woman secondary to estrogen therapy.²² The patient presented with elevated serum liver enzyme levels, but her fibrinogen level was within normal limits. Her PT and PTT were also within normal limits and the patient did not have a history of prolonged bleeding episodes, indicating no evidence of dysfibrinogenemia. It should also be mentioned that chronic hepatitis, fibrosis, and even cirrhosis have been described in patients with hepatic fibrinogen storage disease.^{15,22}

In summary, this report highlights the characteristic histologic and electron microscopic features of hepatic fibrinogen storage disease that is associated with hypofibrinogenemia in a young patient. Molecular studies demonstrated a missense mutation of the *FGA* gene, which has previously been reported in patients with dysfibrinogenemia, but not with hypofibrinogenemia or afibrinogenemia.

Abbreviations

FGA	fibrinogen α
FGB	fibrinogen β
FGG	fibrinogen γ

PAS	periodic acid–Schiff
PTAH	phosphotungstic acid–hematoxylin
FFPE	formalin-fixed, paraffin-embedded
PT	prothrombin time
PTT	partial thromboplastin time
HBsAg	hepatitis B surface antigen

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