

# Hepatic fibrinogen storage disease and hypofibrinogenemia caused by fibrinogen Aguadilla mutation: a case report

Leilei Gu<sup>1,\*</sup> , Bin Wang<sup>2,3,\*</sup>, Lu Liu<sup>4</sup>,  
Qiaorong Gan<sup>4</sup>, Xiaolong Liu<sup>4</sup>, Lihong Chen<sup>2,3</sup>  
and Li Chen<sup>1</sup>

## Abstract

Hepatic fibrinogen storage disease is a rare autosomal dominant genetic disorder characterized by hypofibrinogenemia, as well as the retention of variant fibrinogen within the hepatocellular endoplasmic reticulum. Here, we describe an asymptomatic 4-year-old boy with abnormal liver function test results and unexpected hypofibrinogenemia. Liver biopsy showed circular eosinophil inclusion bodies in the hepato-cytoplasm. Immunostaining results of eosinophil inclusion bodies were positive for fibrinogen. Following pretreatment with diastase, the inclusion bodies failed to stain with the periodic acid–Schiff technique; moreover, immunostaining results were positive for fibrinogen, but negative for alpha-1-antitrypsin. Genetic analysis identified a heterozygous missense mutation c.1201C > T (p. Arg401Trp) within the fibrinogen  $\gamma$ -chain (*FGG*) gene and an additional single nucleotide polymorphism c.-58 A > G within the 5'-untranslated region of the fibrinogen A $\alpha$ -chain (*FGA*) gene. Thus, the patient was diagnosed with hepatic fibrinogen storage disease. Our results indicate that, for patients who exhibit chronic liver disease with unexpected hypofibrinogenemia, hepatic fibrinogen storage disease should be considered in the differential diagnosis. Moreover, our findings emphasize the importance of molecular diagnosis in patients with cryptogenic liver disease.

<sup>1</sup>Department of Gastroenterology, Ruijin Hospital North, Shanghai JiaoTong University, School of Medicine, Shanghai, China

<sup>2</sup>Department of Pathology, School of Basic Medical Sciences of Fujian Medical University, Fuzhou, China

<sup>3</sup>Department of Pathology, Mengchao Hepatobiliary Hospital, Fujian Medical University, Fuzhou, China

<sup>4</sup>Department of Hepatology, Mengchao Hepatobiliary Hospital, Fujian Medical University, Fuzhou, Fujian, China

\*These authors contributed equally to this work.

## Corresponding author:

Li Chen, Department of Gastroenterology, Ruijin Hospital North, Shanghai JiaoTong University, School of Medicine, No. 999 Xiwang Road, Shanghai 201801, China.  
Email: [drchenli@163.com](mailto:drchenli@163.com)



## Keywords

Hepatic endoplasmic reticulum storage disease, genetic diagnosis, pathology, afibrinogenemia, fibrinogen, single nucleotide polymorphism, differential diagnosis, inclusion bodies

Date received: 15 August 2019; accepted: 10 December 2019

## Introduction

Fibrinogen is a soluble protein in blood that plays an important role in the hemostatic cascade, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, and angiogenesis. Fibrinogen comprises two sets of three polypeptide chains— $A\alpha$ ,  $B\beta$ , and  $\gamma$ —that are joined by disulfide bridging within the N-terminal E domain.<sup>1,2</sup> Mutations in  $A\alpha$ ,  $B\beta$ , and  $\gamma$  fibrinogen-chain genes (*FGA*, *FGB*, *FGG*) are reportedly responsible for fibrinogen deficiencies and coagulation disorders.<sup>3</sup> Rarely, patients with hypofibrinogenemia can present with comorbid liver disease, known as hepatic fibrinogen storage disease (HFSD). This disease is a rare autosomal dominant genetic disorder, characterized by the retention of variant fibrinogen within the hepatocellular endoplasmic reticulum (ER) and hypofibrinogenemia.<sup>3</sup> HFSD and another well-known disease, congenital alpha-1-antitrypsin deficiency, are ER storage diseases that share similar molecular mechanisms, due to gene mutations of the corresponding proteins that result in hepatocellular protein storage, defective secretion, and plasma deficiency.<sup>4</sup> Here, we describe a Chinese patient with newly diagnosed HFSD, which was confirmed by genetic and pathological analyses.

## Case report

A 4-year-old boy had an incidental finding of abnormal liver function during a routine examination. The patient was asymptomatic; notably, he did not exhibit jaundice, loss

of appetite, or fever. He had no history of epistaxis, skin ecchymosis, bleeding, or mental disorder. On physical examination, his liver was palpable 2 cm below the right costal margin, but no signs of splenomegaly were observed. The patient showed no ascites or other noticeable signs of chronic liver disease. Clinical biochemistry tests revealed elevated serum alanine aminotransferase (122 IU/L; normal range: 9–50 IU/L), aspartate aminotransferase (119 IU/L; normal range: 15–40 IU/L), and lactate dehydrogenase (299 IU/L; normal range: 109–245 IU/L). Other biochemical factors (e.g., total bilirubin,  $\gamma$ -glutamyl transpeptidase, albumin, globin, plasma ammonia, and lactic acid) were within the respective normal ranges. Serology findings for hepatitis A, B, C, D, and E were negative. Autoantibody and immunoglobulin (IgG, IgM, IgE, and IgA) results were negative. Notably, levels of ceruloplasmin and ferritin were within the respective normal ranges, and complete blood count results were normal. Abdominal ultrasonography showed mild hepatomegaly. To protect the patient's privacy, other clinical details have been de-identified.

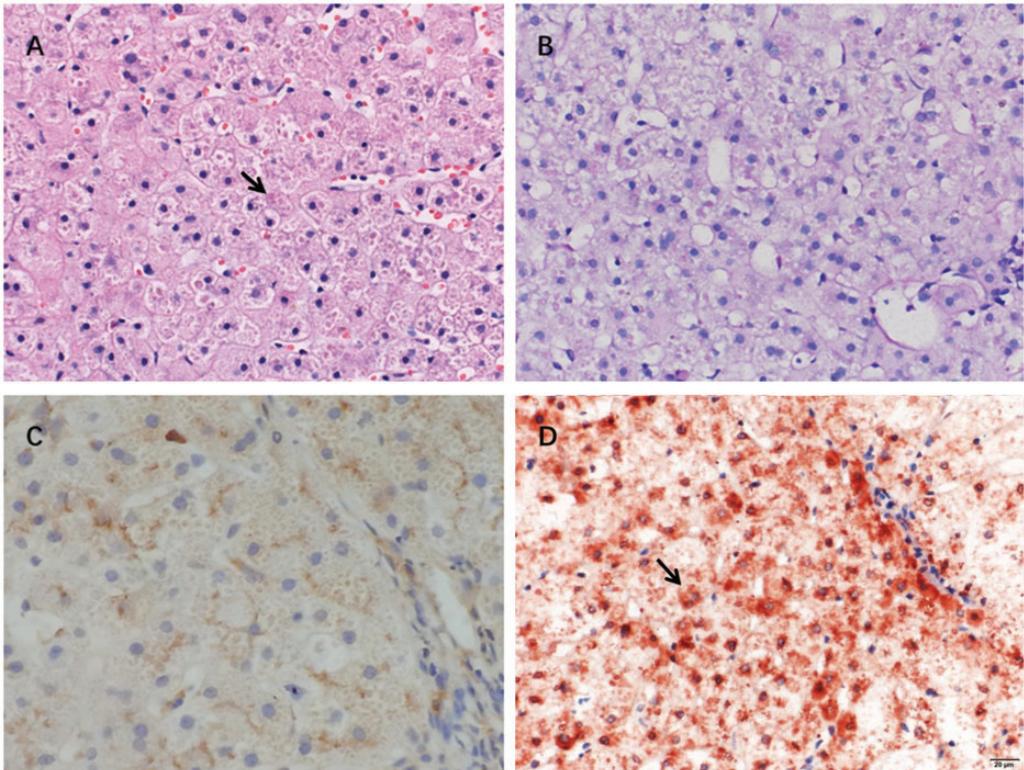
Coagulation tests showed remarkable abnormal results that were inconsistent with the severity of liver disease. The prothrombin time (17.1 seconds; normal range: 11–14.5 seconds), activated partial thromboplastin time (43 seconds; normal range: 26–40 seconds), and international normalization ratio (1.38; normal range: 0.72–1.2) were elevated, relative to the respective normal ranges. Moderate hypofibrinogenemia<sup>5</sup> (0.64 g/L; normal range: 2–4 g/L) was

identified, but an elevated D-Dimer level was absent (0.05 mg/L; normal range: 0–0.55 mg/L). We assessed fibrinogen levels by the Clauss method and did not measure fibrinogen antigen because we did not have access to suitable equipment.

The patient underwent liver core-needle biopsy, following the provision of informed consent by his parents. The biopsy specimen was fixed in phosphate-buffered formalin and embedded in paraffin, then sectioned for staining (hematoxylin-eosin; periodic acid–Schiff technique, with and without diastase predigestion) and immunostaining with antibodies against alpha-1-antitrypsin. Electron microscopy analysis

was not performed because we did not have access to suitable equipment. Liver pathology showed normal hepatic lobule structure with spotty and focal necrosis scattering in hepatic lobule and interlobular fibrous septal formation. Circular eosinophil inclusion bodies of varying sizes were observed in the hepato-cytoplasm. Following pretreatment with diastase, the inclusion bodies failed to stain with the periodic acid-Schiff technique; moreover, immunostaining results were positive for fibrinogen, but negative for alpha-1-antitrypsin (Figure 1).

HFSD was suspected based on the pathology findings. Thus, blood samples were collected from the patient, his older



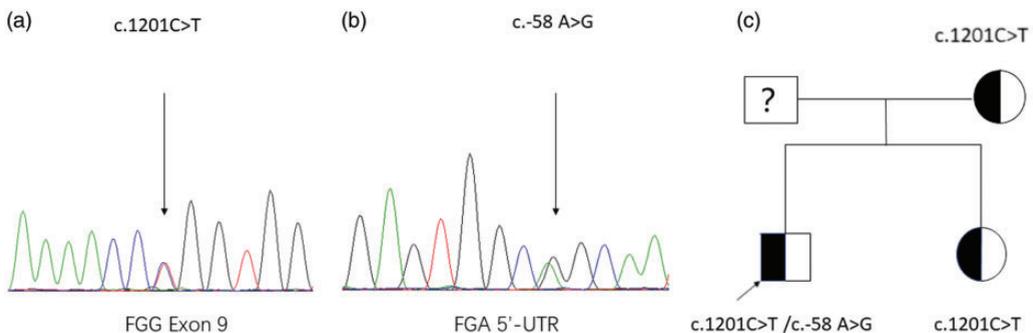
**Figure 1.** Liver pathology revealed eosinophilic intracytoplasmic inclusion bodies with hematoxylin-eosin staining (arrow, a), which failed to stain using the periodic acid-Schiff method (b). On immunostaining, the inclusion bodies were negative for alpha-1-antitrypsin antibody (c) and positive for fibrinogen antibody (arrow, d).

sister, and his mother, following the provision of informed consent by the patient's parents. A genetic analysis study and the preparation of this case report were approved by the ethical committee of Mengchao Hepatobiliary Hospital, Fujian Medical University. Biochemical and coagulation tests were also performed for the patient's sister and mother. Genomic DNA was extracted in accordance with the kit manufacturer's instructions (Qiagen Inc., Valencia, CA, USA). The *FGA*, *FGB*, and *FGG* genes were amplified using specific primers for each gene (Supplemental Table 1). The PCR products were directly sequenced using an ABI automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Mutation nomenclature followed the guidelines of the Human Genome Variation Society. Genetic analysis of the patient revealed a heterozygous missense mutation of the *FGG* gene (c.1201C>T, p.Arg401Trp, p.Arg375Trp in the mature alpha-chain lacking the signal peptide) and a single nucleotide polymorphism (SNP) within the 5'-untranslated region of the *FGA* gene (c.-58 A>G, rs2070011). Data from the 1000 Genomes Project Phase 3 showed that the frequency of this SNP in the East Asian population was 0.5. According to ClinVar

(www.ncbi.nlm.nih.gov/clinvar/), the interpretation record of this SNP was benign. Further pathogenicity prediction for this SNP, using online software Mutation Taster (www.mutationtaster.org), suggested the presence of a polymorphism (i.e., probably harmless). No mutations were found within the *FGB* gene. The same Arg401Trp mutation was detected in the patient's sister and mother (Figure 2). However, their liver function and coagulation tests revealed normal findings.

## Discussion

Congenital fibrinogen defects are conventionally classified as quantitative (type I) or qualitative (type II) deficiencies based on the plasma concentration. Quantitative deficiencies include afibrinogenemia/severe hypofibrinogenemia and hypofibrinogenemia, while qualitative deficiencies comprise dysfibrinogenemia and hypodysfibrinogenemia.<sup>2</sup> Analysis of population-based exome- and genome-sequencing data have suggested that the global prevalence of dominantly inherited fibrinogen deficiency is approximately 1 in every 91 individuals.<sup>2</sup> Nevertheless, the global prevalence of HFSD remains unclear. HFSD was first described in German and Italian families, then confirmed by a mutation in the



**Figure 2.** Genetic analysis showed a heterozygous missense mutation (c.1201C>T/p. Arg401Trp) within the *FGG* gene (a) and an additional SNP (c.-58 A>G) within the 5'-untranslated region of the *FGA* gene (b). Pedigree of the family screened for *FGG* and *FGA* genes (c).

fibrinogen gamma gene.<sup>6</sup> Thus far, only 27 affected individuals from 17 families have been reported worldwide.<sup>7–18</sup> Most mutations have been located within the *FGG* gene and are categorized as Aguadilla, Al du Pont, Angers, Beograd, Brescia, Ankara, and Pisa, based on the cities in which the probands lived.<sup>11,13–15,18,19</sup> The Arg35Cys mutation within the *FGA* gene was reportedly responsible for hypofibrinogenemia and HFSD in a patient;<sup>9</sup> this is a well-known mutation leading to congenital dysfibrinogenemia, but not hypofibrinogenemia.<sup>20</sup> Here, we described a patient with a heterozygous Aguadilla mutation within the *FGG* gene. To the best of our knowledge, this is the second patient with the Aguadilla mutation in China, although Aguadilla is the most common causative mutation for HFSD.<sup>7,8,10,12,16–18,21</sup> The Aguadilla mutation was *de novo* in the first histopathologically and genetically documented Chinese patient with HFSD.<sup>7</sup> In contrast, we have described a Chinese patient who inherited the Aguadilla mutation from his mother.

There are many clinical manifestations of HFSD; in particular, affected patients may be asymptomatic, have moderate to severe hepatitis, or have liver cirrhosis.<sup>17</sup> HFSD can affect children, adults, and older people.<sup>16</sup> Although the  $\gamma$  module is important for maintenance of the normal function of fibrinogen, most of the patients described thus far have not experienced extensive bleeding events. Therefore, the target organ affected by the mutation within the *FGG* gene may be the liver; coagulation or bleeding disorders may not occur in affected patients. HFSD is presumably an underdiagnosed condition and its prevalence may be underestimated.

Notably, the patient's sister and mother had normal findings in liver function and coagulation tests, although they harbored the same heterozygous mutation within the *FGG* gene. This phenomenon has also

been observed in another family with HFSD<sup>8</sup> and in a family with a similar disease, alpha-1-antitrypsin deficiency.<sup>22</sup> Long-term follow-up studies have shown that more than 85% of children with the homozygous mutation p.Glu342Lys (PiZZ phenotype) in the *AAT* gene have persistent normal serum transaminase levels and do not exhibit liver dysfunction.<sup>22</sup> These clinical observations suggest that other factors (e.g., additional inherited traits or environmental factors) might be responsible for the pathogenesis characteristics of HFSD.

Imbalanced homeostasis, caused by excessive aggregation of variant fibrinogen in the ER, is regarded as the central event in the pathogenesis of HFSD. Because fibrinogen is an acute-phase protein synthesized in hepatocytes, its levels increase as an inflammatory response to acute infection. However, variant fibrinogen deposited within the ER can be degraded by autophagy.<sup>23</sup> Therefore, concomitant defects in protein degradation pathways (e.g., the autophagic or proteasomal systems) may also contribute to the pathogenesis of ER storage diseases. In addition, a potential synergistic effect of the SNP within 5'-untranslated region of the *FGA* gene in patients with the Aguadilla mutation cannot be excluded. Previous studies have shown that this SNP may influence fibrinogen  $\gamma'$  levels.<sup>24,25</sup> The fibrinogen  $\gamma'$  chain is an important  $\gamma$  chain variant that contributes to downregulation of potential plasma XIII-mediated cross-linking activity.<sup>26</sup> Importantly, the SNP was absent from the patient's sister and mother.

The autophagy enhancer carbamazepine has been shown to alleviate aggregate-related toxicity in patients with HFSD when combined with ursodeoxycholic acid treatment.<sup>13</sup> However, the patient's parents refused carbamazepine treatment for the patient; the patient's mother also refused carbamazepine treatment. Therefore, follow-up clinical data were not collected.

In conclusion, we have described a patient with HFSD, characterized by classical pathological changes; he harbored the Aguadilla mutation within the *FGG* gene and had an additional SNP within the 5'-untranslated region of the *FGA* gene. The discrepancy we observed between phenotype and genotype in mutation carriers implies that defects in protein degradation pathways may be involved in the pathogenesis of HFSD. Increasing numbers of new patients with HFSD and novel treatments may improve the broader clinical understanding of this rare inherited disease.

### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

### Funding

This study was funded by the National Natural Science Foundation of China (Grant No. 81770590).

### ORCID iD

Leilei Gu  <https://orcid.org/0000-0002-3878-7262>

### Supplemental material

Supplemental material for this article is available online.

### References

- Mosesson MW, Siebenlist KR and Meh DA. The structure and biological features of fibrinogen and fibrin. *Ann N Y Acad Sci* 2001; 936: 11–30.
- Paraboschi EM, Duga S and Asselta R. Fibrinogen as a pleiotropic protein causing human diseases: the mutational burden of Aalpha, Bbeta, and gamma chains. *Int J Mol Sci* 2017; 18: pii: E2711.
- Medicina D, Fabbretti G, Brennan SO, et al. Genetic and immunological characterization of fibrinogen inclusion bodies in patients with hepatic fibrinogen storage and liver disease. *Ann N Y Acad Sci* 2001; 936: 522–525.
- Callea F, Brisigotti M, Fabbretti G, et al. Hepatic endoplasmic reticulum storage diseases. *Liver* 1992; 12: 357–362.
- Casini A, Undas A, Palla R, et al. Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. *J Thromb Haemost* 2018; 16: 1887–1890.
- Brennan SO, Wyatt J, Medicina D, et al. Fibrinogen brescia: hepatic endoplasmic reticulum storage and hypofibrinogenemia because of a gamma284 Gly→Arg mutation. *Am J Pathol* 2000; 157: 189–196.
- Zhang MH, Knisely AS, Wang NL, et al. Fibrinogen storage disease in a Chinese boy with de novo fibrinogen Aguadilla mutation: incomplete response to carbamazepine and ursodeoxycholic acid. *BMC Gastroenterol* 2016; 16: 92.
- Sari S, Yilmaz G, Gonul II, et al. Fibrinogen storage disease and cirrhosis associated with hypobetalipoproteinemia owing to fibrinogen Aguadilla in a Turkish child. *Liver Int* 2015; 35: 2501–2505.
- Lee MJ, Venick R, Bhuta S, et al. Hepatic fibrinogen storage disease in a patient with hypofibrinogenemia: report of a case with a missense mutation of the FGA gene. *Semin Liver Dis* 2015; 35: 439–443.
- Casini A, Sokollik C, Lukowski SW, et al. Hypofibrinogenemia and liver disease: a new case of Aguadilla fibrinogen and review of the literature. *Haemophilia* 2015; 21: 820–827.
- Asselta R, Robusto M, Braidotti P, et al. Hepatic fibrinogen storage disease: identification of two novel mutations (p.Asp316Asn, fibrinogen Pisa and p.Gly366Ser, fibrinogen Beograd) impacting on the fibrinogen gamma-module. *J Thromb Haemost* 2015; 13: 1459–1467.
- Al-Hussaini A, Altalhi A, El Hag I, et al. Hepatic fibrinogen storage disease due to the fibrinogen gamma375 Arg → Trp mutation “fibrinogen Aguadilla” is present in Arabs. *Saudi J Gastroenterol* 2014; 20: 255–261.
- Puls F, Goldschmidt I, Bantel H, et al. Autophagy-enhancing drug carbamazepine diminishes hepatocellular death in

- fibrinogen storage disease. *J Hepatol* 2013; 59: 626–630.
14. Brennan SO, Davis RL, Conard K, et al. Novel fibrinogen mutation gamma314 Thr→Pro (fibrinogen AI duPont) associated with hepatic fibrinogen storage disease and hypofibrinogenemia. *Liver Int* 2010; 30: 1541–1547.
  15. Dib N, Quelin F, Ternisien C, et al. Fibrinogen angers with a new deletion (gamma GVYYQ 346-350) causes hypofibrinogenemia with hepatic storage. *J Thromb Haemost* 2007; 5: 1999–2005.
  16. Rubbia-Brandt L, Neerman-Arbez M, Rougemont AL, et al. Fibrinogen gamma375 arg→trp mutation (fibrinogen aguadilla) causes hereditary hypofibrinogenemia, hepatic endoplasmic reticulum storage disease and cirrhosis. *Am J Surg Pathol* 2006; 30: 906–911.
  17. Francalanci P, Santorelli FM, Talini I, et al. Severe liver disease in early childhood due to fibrinogen storage and de novo gamma 375Arg→Trp gene mutation. *J Pediatr* 2006; 148: 396–398.
  18. Brennan SO, Maghzal G, Shneider BL, et al. Novel fibrinogen gamma375 Arg→Trp mutation (fibrinogen aguadilla) causes hepatic endoplasmic reticulum storage and hypofibrinogenemia. *Hepatology* 2002; 36: 652–658.
  19. Callea F, Giovannoni I, Sari S, et al. A novel fibrinogen gamma chain mutation (c.1096C>G; p.His340Asp), fibrinogen Ankara, causing hypofibrinogenemia and hepatic storage. *Pathology* 2017; 49: 534–537.
  20. Casini A, Neerman-Arbez M, Ariens RA, et al. Dysfibrinogenemia: from molecular anomalies to clinical manifestations and management. *J Thromb Haemost* 2015; 13: 909–919.
  21. Sogo T, Nagasaka H, Komatsu H, et al. Fibrinogen storage disease caused by Aguadilla mutation presenting with hypobeta-lipoproteinemia and considerable liver disease. *J Pediatr Gastroenterol Nutr* 2009; 49: 133–136.
  22. Sveger T. The natural history of liver disease in alpha 1-antitrypsin deficient children. *Acta Paediatr Scand* 1988; 77: 847–851.
  23. Kruse KB, Dear A, Kaltenbrun ER, et al. Mutant fibrinogen cleared from the endoplasmic reticulum via endoplasmic reticulum-associated protein degradation and autophagy: an explanation for liver disease. *Am J Pathol* 2006; 168: 1299–1308; quiz 1404-1295.
  24. Kotze RC, Nienaber-Rousseau C, De Lange Z, et al. Genetic polymorphisms influencing total and gamma' fibrinogen levels and fibrin clot properties in Africans. *Br J Haematol* 2015; 168: 102–112.
  25. Jobse A, Pieters M, Nienaber-Rousseau C, et al. The contribution of genetic and environmental factors to changes in total and gamma' fibrinogen over 5 years. *Thromb Res* 2015; 135: 703–709.
  26. Mosesson MW. Fibrinogen gamma chain functions. *J Thromb Haemost* 2003; 1: 231–238.