CASE REPORT

A newly detected c.180 + 1G > A variant causes a decrease of *FGA* transcription in patients with congenital hypo-dysfibrinogenemia

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Abstract

Congenital hypo-dysfibrinogenemia is a rare autosomal dominant or co-dominant genetic disorder. This study was designed to analyze the clinical phenotype and genetic mutations of a patient with congenital hypo-dysfibrinogenemia. Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and *D*-dimer were measured using an automated coagulation analyzer. Fibrinogen (Fg) was assessed by the Clauss and PT-Fg methods. Sanger sequencing was conducted to identify the mutations in *FGA*, *FGB*, and *FGG* genes. The proband and proband's mother, maternal uncle, and maternal grandfather exhibited a prolonged TT, decreased Clauss Fg and PT-Fg, and normal PT, APTT, and *D*-dimer without abnormalities in liver and kidney function. The ratio of Clauss Fg to PT-Fg was less than 0.7. A novel *c.180 + 1G > A* mutation was detected in *FGA* gene by Sanger sequencing in the proband and proband's mother, maternal uncle, and maternal grandfather. *FGA* c.180 + 1G > A variant leads to decreased transcription of *FGA*. The patient was diagnosed with congenital hypo-dysfibrinogenemia presenting a novel *c.180 + 1G > A* mutation in *FGA*, causing a decrease in *FGA* transcription in proband's peripheral blood.

Keywords Congenital hypo-dysfibrinogenemia · *FGA* gene · Fibrinogen · Sanger sequencing

Introduction

Congenital dysfibrinogenemia (MIM: 616,004) is a rare autosomal dominant or co-dominant genetic disorder caused by mutations in *FGA*, *FGB*, and *FGG* that encode fibrinogen proteins [1–3]. Hypo-fibrinogenemia is a subcategory of the disease that is mostly autosomal dominant and a few autosomal recessive [4]. The mutation *FGA* Arg35 (Arg16) in exon 2 and the mutation *FGA* Arg301 (Arg275) in exon 8 are the two mutation "hot spots" of major significance in screening for abnormal fibrinogenemia, but other mutations are also common in the surrounding residues [5]. In this study, we

reported a 4-year-old boy with a prolonged thrombin time (TT) and decreased fibrinogen activity (Fg:Ac) and PT-Fg, without abnormalities in the liver and kidney function. *FGA* c.180 + 1G > A variant was sequenced in the proband and the proband's mother, maternal uncle, and maternal grandfather, which caused a decreased mRNA expression of *FGA*. The proband and the proband's mother, maternal uncle, and maternal grandfather were then diagnosed with congenital.

Case presentation

The proband of interest was a 4-year-old boy who presented with recurrent abdominal pain for 6 months. Three generations of the family were investigated, including the proband and the proband's elder sister, mother, father, maternal uncle, maternal grandfather, and grandmother. The mother of the proband described that she had difficulty in stopping the bleeding during the delivery of her second child (the proband). In addition, the proband, his maternal grandfather, and maternal uncle showed a slightly long duration for hemostasis after injury. Other family members showed no spontaneous bleeding and thrombosis, as well as other

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abnormalities. The abdominal pain symptoms were due to inflammation of the mesenteric lymph nodes and were not associated with hypo-fibrinogenemia.

Peripheral venous blood specimens (5 mL) were collected from each participant. The partial collection of blood samples was carried out with sodium citrate as anticoagulation and was used for coagulation tests (2 mL), RNA extraction (500 μL), and DNA extraction (500 μL). The other samples (2 mL) were used for liver and kidney function determination. For coagulation tests, blood specimens were centrifuged at 3500 r/min for 15 min, and the supernatant was utilized for determination of prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and *D*-dimer on an Sysmex CS-5100 analyzer (Sysmex, Kobe, Japan). Fibrinogen (Fg) was measured by the Clauss and PT-Fg methods. Serum liver and kidney functions were evaluated using a Beckman-Coulter AU-5800 auto analyzer (Beckman, Brea, CA, USA). As shown in Table 1, the proband exhibited a prolonged TT (26.8 s; reference interval: 15.0–22.0 s) and decreased Fg (0.5 g/L; Clauss method) and PT-Fg (1.55 g/L; PT-derived method) (reference interval: 1.8–4.0 g/L). The proband showed normal PT (12.2 s; reference interval: 9.0–14.0), APTT (23.8 s; reference interval: 23.0–38.0 s), and *D*-dimer (0.1 μg/mL; reference interval: < 0.55). The proband's grandmother, maternal grandmother, father, and sister showed a normal coagulation phenotype. However, the proband's mother, maternal uncle, and maternal grandfather showed increased TT and decreased Fg:Ac and PT-Fg, which was similar to that of the proband (Table 1). Routine blood examination revealed no abnormalities. No abnormalities were detected in the liver or kidney function of each participant.

Genomic DNA was extracted from peripheral venous blood samples for DNA sequence analysis. Sanger sequencing revealed *FGA* c.180 + 1G > A variant in the proband, and *FGA* c.180 + 1G > A variant caused the splice site mutation (Fig. 1). *FGA* c.180 + 1G > A variant was identified in the proband's mother, maternal uncle, and maternal grandfather. However, no variant was identified in the proband's father, sister, grandmother, and maternal grandmother. These results suggested that the proband inherited *FGA* c.180 + 1G > A variant from the proband's mother (Fig. 2). The proband's mother and maternal uncle inherited the variant from the proband's maternal grandfather (Fig. 2).

Then *FGA* mRNA expression was compared between the proband and his sister. Initially, total RNA was extracted from the peripheral blood samples using the RNA extraction kit (Liferiver, Shanghai, China). The cDNA was reversely transcribed, and then PCR amplification was carried out in a total volume of 25 μL containing 2 μL cDNA, 0.5 μL each primer, 9.5 μL double-distilled water, and 12.5 μL TB green (TAKARA, Japan). Amplification was performed in a 7500 real-time PCR system (ABI, USA). The reaction included 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and 95 °C for 15 s. PCR primers were *FGA* (forward 5′-CTCGCCCTGTCAGAGGTATC-3′ and reverse 5′-CTGCCCCCAAGGAACTTAC-3′) and *GAPDH* (forward 5′-AGATCCCTCCAAAATCAAGTGG-3′ and reverse 5′-GGCAGAGATGATGACCCTTTT-3′). *FGA* mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method. GAPDH was used as the housekeeping gene. As presented in Fig. 3, the mRNA expression of *FGA* was significantly decreased in the proband compared to the proband's sister ($P < 0.05$).

## Discussion

Fibrinogen protein consists of two groups of Aα, Bβ, and γ-chains bridged by disulfide bonds and are an extended 45-nm structure [6]. Fibrinogen is synthesized by hepatocytes and secreted into the circulation, playing a crucial

Table 1 Coagulation function test

Subjects	PT (s)	APTT (s)	TT (s)	Clauss Fg (g/L)	PT-Fg (g/L)	*D*-dimer (μg/mL)	Clauss Fg/PT-Fg
Proband	12.2	23.8	26.8#	0.50#	1.55#	0.10	0.32
Sister	11.1	29.5	16.0	2.99	2.20	0.20	1.36
Mother	13.3	26.2	25.3#	0.39#	1.55#	0.12	0.25
Father	10.5	31.5	15.8	3.88	2.72	0.16	1.43
Maternal uncle	13.4	24.8	24.4#	0.48#	1.57#	0.13	0.31
Maternal grandfather	12.3	28.0	23.8#	0.40#	1.57#	0.08	0.25
Maternal grandmother	12.1	25.5	15.9	3.35	2.57	0.79	1.30
Grandmother	12.0	24.6	16.0	3.80	2.72	0.65	1.40
Reference interval	9.0–14.0	23.0–38.0	15.0–22.0	1.8–4.0	1.8–4.0	< 0.55	

Fibrinogen (Fg) was examined by the Clauss and PT-Fg methods. PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time. #, Abnormal data.
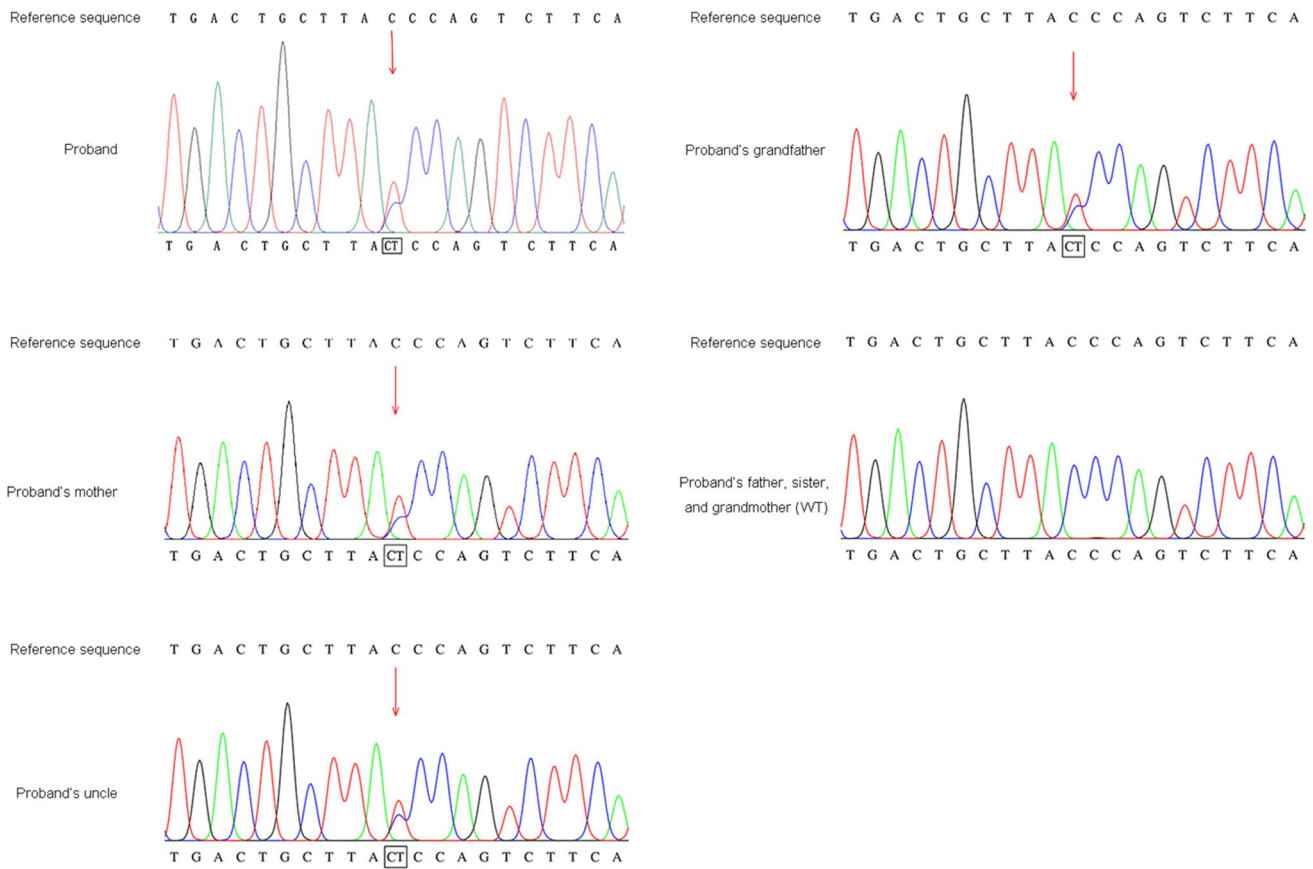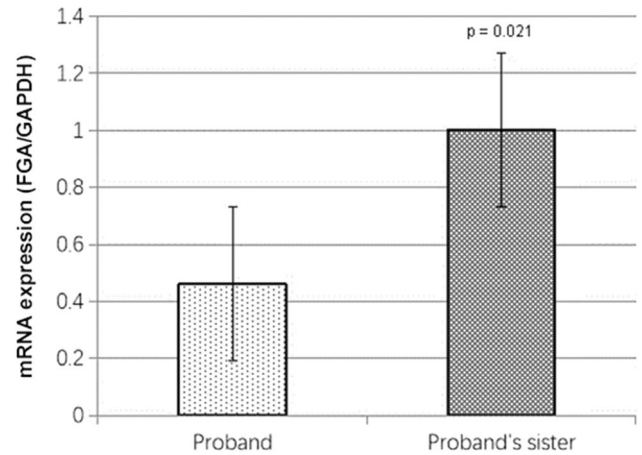
Fig. 1 Sanger sequencing map
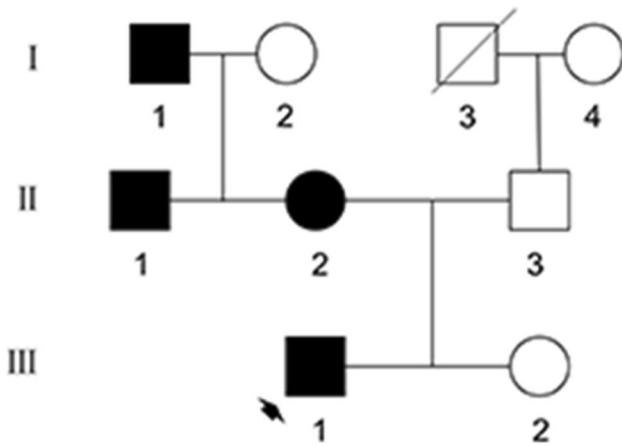
Fig. 2 Pedigree of the family

Fig. 3 mRNA expression of *FGA* in peripheral blood of the proband and healthy individuals

role in coagulation, fibrinolysis, cellular and matrix interactions, inflammatory responses, wound healing, and biological activity of tumor [7]. In this study, a novel splicing mutation *FGA* c.180 + 1G > A occurring in exon 2 and intron 2 boundaries was identified in a 4-year-old boy with congenital hypo-dysfibrinogenemia. There is no record of this mutation in *the Fg gen*e mutation database (http://www.geht.org/databaseang/fibrinogen), so the mutation is considered to be an unreported mutation. The mutation caused a decrease in *FGA* transcription in the peripheral blood in the proband.

Mutations in the gene encoding fibrinopeptides, such as large deletions, promoter, splice site, frameshift, nonsense, and missense mutations, are reported to result in structural and functional changes in the fibrinogen [8]. Structural and functional abnormalities impede the release of fibrin peptide A/B, polymerization of fibrin monomers, or factor XIIIa-mediated fibrin cross-linking [9]. In this study, Sanger sequencing identified a splice site mutation of *FGA* c.180 + 1G > A in the proband. The proband's mother, maternal uncle, and maternal grandfather had the *FGA* c.180 + 1G > A mutation, which was not detected in the proband's father, sister, and maternal grandmother. Therefore, it was concluded that the *FGA* c.180 + 1G > A mutation in the proband was derived from the proband's mother. Besides, the patient's mother inherited the *FGA* c.180 + 1G > A mutation from the proband's maternal grandfather. *FGA* c.180 + 1G > A mutation has not been annotated in either ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) or HGMD (http://www.hgmd.cf.ac.uk/ac/index.php).

Similar splicing mutations of *FGA* have been reported in several other literatures. Site-specific mutations in intron 1 (c.IVS1 + 1G > A and c.IVS1 + 3A > G) and in intron 3 (c.IVS3 + 1_ + 4delGTAA) affect the consensus sequence of the conserved donor splice site [10–13]. In addition, studies revealed three mutations in the same splice site GT (c.180 + 1G > C, c.180 + 1G > T, and c.180 + 2 T > C) that have been reported to be associated with hypofibrinogenemia [14, 15]. Further, a mutation site (*FGA* c.169_180 + 2del) was reported by Zhou et al. [16]. Unlike the present case this variant was due to deletion disrupting the splice consensus sequence GT at the junction of exon 2 and intron 2, resulting in disruption of normal processing of mRNA transcripts and impaired coagulation activity [16]. However, the expression and secretion of fibrinogen were not hindered [16]. Hypo-abnormal fibrinogenemia can be caused by different molecular mechanisms. Heterozygosity for a single mutation leads to the synthesis of an abnormal fibrinogen chain, which is secreted less efficiently than normal fibrinogen. Compound heterozygous mutation results in fibrinogen deficiency and abnormal molecular function [8]. More studies are required to investigate the pathogenicity and molecular pathogenic mechanisms of *FGA* c.180 + 1G > A.

Laboratory tests for PT, APTT, TT, and fibrinogen and genetic analysis are essential for the diagnosis of congenital hypo-dysfibrinogenemia. However, patients with congenital hypo-dysfibrinogenemia showed normal PT and APTT. TT is the primary screening indicator for the disease. Abnormal fibrinogen inhibits the release of fibrinopeptides A/B or impedes fibrin monomer polymerization, resulting in prolonged TT in patients [4]. However, a case of shortened TT due to abnormal fibrinogen has been reported previously [17]. The diagnosis of congenital hypo-dysfibrinogenemia can be further verified by the fibrinogen activity/antigen ratio. Clauss method and PT-Fg methods are the main methods to determine fibrinogen. The Clauss method was used to determine the functional activity of fibrinogen, while the PT-Fg method was used to determine the fibrinogen concentration indirectly through the reaction curve of PT. When Clauss Fg/PT-Fg was less than 0.7 or more than 1.43, the sensitivity and specificity for the diagnosis of dysfibrinogenemia was 100% [18, 19]. Clauss fibrinogen levels of the proband and the proband's mother, maternal uncle, and maternal grandfather were less than 1.8 g/L. The PT-Fg tests yielded higher values than the Clauss results, and the degree of the disparity may be related to the calibrator and the turbidity of the assayed samples. The diagnosis of abnormal blood fibrinogen was then confirmed by excluding abnormal liver function. Confirmation of the diagnosis of congenital hypo-dysfibrinogenemia requires sequencing of the fibrinogen-encoding gene in patients and families. The results of RT-PCR showed that the mRNA expression of *FGA* in the peripheral blood of the proband was significantly reduced compared with that the proband's sister, verifying that *FGA* c.180 + 1G > A mutation affected *FGA* transcription. This splicing site mutation causes a low level of FGA protein in the proband.

Mutant fibrinogen molecules are present in heterozygous forms in patients as homodimers (i.e., both chains are mutant) and heterodimers (i.e., one chain is mutant), resulting in differences in the ratio of homodimers to heterodimers and leading to clinical heterogeneity of dysfibrinogenemia [20]. Analysis of 102 cases of dysfibrinogenemia by Zhou et al. revealed that 68.6% of the patients displayed no clinical symptoms, 27.5% had bleeding manifestations, and 3.9% developed thrombosis [21]. Bleeding manifestations include nose bleeding, easy bruising, excessive menstruation, joint hematomas, postoperative bleeding, postpartum bleeding, antepartum bleeding, and delayed wound healing. Defective binding of abnormal fibrinogen to thrombin leads to elevated thrombin levels, and abnormal fibrinogen can form a fibrin clot that resists degradation by fibrinolytic enzymes, which may be the molecular mechanism that leads to thrombosis [15]. Therefore, female patients with dysfibrinogenemia are of particular clinical concern because the disease may lead to excessive menstrual bleeding, obstetric bleeding, and postpartum thrombosis.

Conclusions

Congenital dysfibrinogenemia is a clinical asymptomatic and rare disease, which may be likely to be misdiagnosed. In this study, the proband and the proband's mother, maternal uncle, and maternal grandfather exhibited a prolonged TT and decreased Clauss Fg and PT-Fg, without abnormalities

in the liver and kidney function. Sanger sequencing detected a novel mutation *FGA* c.180 + 1G > A in the proband and the proband's mother, maternal uncle, and maternal grandfather. *FGA* c.180 + 1G > A variant caused the decreased mRNA expression of *FGA*. Clinical should conduct genealogical survey and genetic analysis to diagnose congenital hypo-dysfibrinogenemia.

Author contribution Jintu Lou contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Zhe Li, Wei Li, and Lin Chen. The first draft of the manuscript was written by Zhe Li and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Children's Hospital, Zhejiang University School of Medicine.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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