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Letter to the Editors-in-Chief

A novel deletion in the fibrinogen beta chain (*FGB*) gene causing hypofibrinogenemia

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Congenital fibrinogen disorders are rare inherited bleeding disorders characterized by bleeding or thrombosis. They can be divided into type I deficiencies or quantitative defects (afibrinogenemia and hypofibrinogenemia) which are defined when those with fibrinogen levels are below 1.5 g/L and type II deficiencies or qualitative defects (dysfibrinogenemia and hypodysfibrinogenemia) [1]. The estimated prevalence of afibrinogenemia is about 1:1,000,000. Although dysfibrinogenemia and hypofibrinogenemia are more frequent, their accurate prevalence remains unknown as most cases are asymptomatic [1,2]. They do not present with spontaneous bleeding but may develop bleeding associated with trauma, surgery or postpartum condition. In addition to bleeding, patients with afibrinogenemia and severe hypofibrinogenemia may develop thrombotic complications [3,4]. Most of the cases with thrombosis are associated with dysfibrinogenemia due to defects in binding thrombin and forming fibrin clots resistant to degradation by plasmin [1].

Fibrinogen is a plasma glycoprotein, forming a symmetrical hexameric structure (Aα, Bβ, γ). It is encoded by three different fibrinogen genes: *FGA*, *FGB*, and *FGG*, respectively located on chromosome 4q28-q31 [4,5]. The disease-associated mutations are mostly identified in the *FGA* gene responsible for hypofibrinogenemia and afibrinogenemia, but less frequently found in the *FGB* gene [4]. At least 100 different variants scattered throughout the *FGB* gene have been described with the most common being missense and/or nonsense mutations (Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php), accessed in August 2019) [5]. The splice-junction alterations, nucleotide deletions or insertions have also been reported.

According to current guidelines, plasma-derived fibrinogen concentrate was suggested to prevent bleeding or treat patients with fibrinogen deficiency initially at 50–100 mg/kg once a week to maintain fibrinogen activity higher that 0.5 g/L [6]. Simurda et al. reported an afibrinogenemic patient who received regular administration of fibrinogen for secondary prophylaxis had improvement in quality of life without any thrombotic complications [7].

Here, we report a 6-year-old Thai girl presented with gum bleeding for three days after tooth extraction. There was no history of previous bleeding after trauma or surgery. Her physical examination showed bleeding from tooth pocket, several bruises at both legs with the largest size about 1 × 1.5 cm in diameter without hepatosplenomegaly. She was given oral tranexamic acid without blood transfusion. Her bleeding

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Table 1
Coagulation tests of the proband family with hypofibrinogenemia.

Family members	PT (sec)	INR	aPTT (sec)	Fibrinogen activity (mg/dL)	Fibrinogen antigen (mg/dL)
Proband	65	7.5	120	< 30	22
Younger sibling	110	11.2	125	< 30	36.5
Father	9.9	0.87	22.7	133	239.6
Mother	ND	ND	ND	ND	ND
Normal range	11.7–15.1	0.87–1.2	31.8–43.7	162–401	200–350

ND: not done.

score according to the International Society on Thrombosis and Haemostasis (ISTH)/Scientific and Standardization Committee assessment tool was 3 [8]. Initial laboratory results revealed normal numbers and size of platelets with Hb 12 g/dL, normal bleeding time, significantly prolonged prothrombin time (PT) of 65 s (normal range for 5–10 years old, 11.7–15.1 s), an international normalized ratio (INR) of 7.5 (normal range for age, 0.87–1.20) and activated partial thromboplastin time (aPTT) of 120 s (normal range for 5–10 years old, 31.8–43.7 s). She had a fibrinogen activity < 30 mg/dL (normal range, 162–401 mg/dL), by Clauss method and fibrinogen antigen 22 mg/dL (normal range, 200–350 mg/dL), by *in vitro* competition ELISA assay, Abcam, UK. She also had one younger sibling who was four years old with recurrent bruising at both extremities starting at 2 years of age following minor trauma. On physical examination, the younger sibling was noted to have multiple ecchymosis of both legs with the largest size about 2 × 2 cm. Her bleeding score according to the International Society on Thrombosis and Haemostasis (ISTH)/Scientific and Standardization Committee assessment tool was 1 [8]. She had prolonged PT of 110 s (normal range for age, 12.1–14.5 s), INR of 11.2 (normal range for age, 0.92–1.14), APTT of 125 s (normal range for 1–5 years old, 33.6–43.8 s). She had a fibrinogen activity < 30 mg/dL (normal range, 162–401 mg/dL) and fibrinogen antigen 36.5 mg/dL (normal range, 200–350 mg/dL). Their parents denied history of bleeding tendencies. After informed consent, genomic DNA was extracted from peripheral blood leukocytes obtained from the proband and family members using a Puregene blood kit (Qiagen, Hilden, Germany). Whole exome sequencing (WES) was performed using the Ilumina HiSeq 2000 sequencer at Macrogen Inc., Seoul, Korea. Sequence reads were mapped against UCSC hg19 using Burrows-Wheeler Alignment (BWA) software (http://bio-bwa.sourceforge.net/). The variants would be called novel if they were not listed in the ClinVar database (https://clinvarminer.genetics.utah.edu/) and the Exome Aggregation Consortium database (exac.broadinstitute.org/). WES revealed that the proband and her younger sibling were homozygous for a 15-bp deletion, c.745_759del GGTGAAACATCTGAA in the fibrinogen beta chain (*FGB*) gene. Both parents were heterozygous for the deletion. Based on clinical features, fibrinogen antigen/activity and molecular findings, both siblings were diagnosed with congenital hypofibrinogenemia. The complete results of coagulation assays are shown in Table 1.

In this study, we identified a novel in-frame deletion in the *FGB* gene in a Thai family with hypofibrinogenemia. The in-frame deletion, c.745_759delGGTGAAACATCTGAA is expected to result in deletion of five amino acids starting at position 249 (p.Gly249_Glu253del) within the carboxy-terminus of the beta-chain (the D domain of the fibrinogen protein). This variant was not found in our in-house database of unrelated 1876 Thai exomes and genome aggregation database (gnomAD). The proband and her sister were homozygous for the deletion and had mild bleeding tendency after minor trauma and dental procedures while their parents were heterozygous and remained asymptomatic (Fig. 1). A previously reported deletion, p.Asn167_Glu171del which is close to the novel p.Gly249_Glu253del was also identified in three family members with increased clotting time and low functional and physical fibrinogen concentrations. These patients were found to have hypodysfibrinogenemia [9].

The in-frame five amino acid deletion in the *FGB* gene is expected to result in a protein with five amino acid missing at the start of the highly conserved globular domain of the beta chain. Several studies have demonstrated the importance of structural integrity of this domain for fibrinogen hexamer secretion [10]. This could explain the milder symptoms in both siblings. Considering this case study, we should keep in mind that patients presenting with mucocutaneous bleeding may need to have further investigations for abnormal fibrinogens. Our findings emphasize the importance of coagulation tests and fibrinogen assays in mild bleeders and demonstrate the clinical implication of genetic testing in providing precise diagnosis of patients with afibrinogenemia or hypo/dysfibrinogenemia which could lead to early diagnosis and prevention of severe bleeding in the future.

In conclusion, we report for the first time a Thai family with congenital hypofibrinogenemia caused by a novel 15-bp deletion (c.745_759delGGTGAAACATCTGAA) in the *FGB* gene. To make a definite diagnosis and proper genetic counseling, measuring fibrinogen activity and antigen levels along with genetic testing are required.

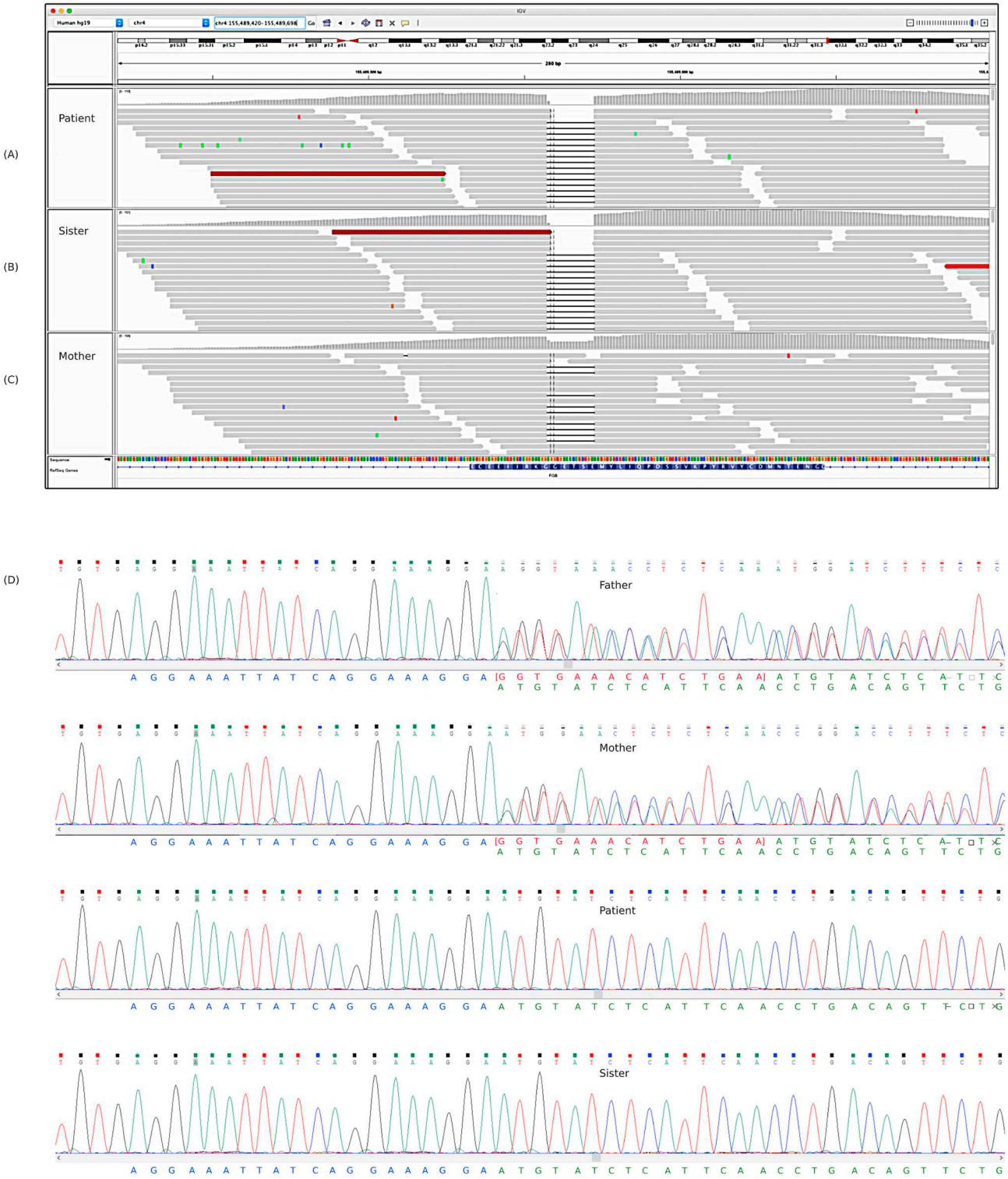

Fig. 1. Nucleotide sequences from BAM files of the patients and their mother. Aligned read coverage of the DNA regions harboring mutations identified in the patients and their parent. The proband (A) and her sibling (B) are homozygous for the15-bp deletion, c.745_759delGGTGAAACATCTGAA in the *FGB* gene while their parents are heterozygous for the deletion (C). Electropherograms showing heterozygous and homozygous deletions in both parents and siblings, respectively (D).

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