CASE REPORT

Congenital hypofibrinogenemia with a novel mutation BβCys76Phe

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Abstract : Objective : Identified genetic mutation in the patient with unexplained decreased fibrinogen activity. Methods : We conducted a detailed examination of the patient's fibrinogen activity level, antigen level, and genomic sequence. Results : The patient's fibrinogen activity level was 80 mg/dL and the antigen level was 131 mg/dL. Upon sequencing of the fibrinogen gene, a novel heterozygous c.Bβ227G>T mutation was detected in Bβ-chain, which results in the cysteine residue at position 76 in exon 3 being converted to a phenylalanine residue. Conclusions : This mutation reduces fibrinogen activity and antigen levels, but the pathophysiology of this mutation remains unclear. J. Med. Invest. 72 : 182-184, February, 2025

Keywords **:** *congenital hypofibrinogenemia, Bβ chain, cysteine mutation*

BACKGROUND

Fibrinogen is a coagulation factor that plays important roles in hemostasis, fibrinolysis, inflammatory responses, and wound healing. Fibrinogen molecules are elongated 45 nm structures consisting of two outer D domains, each connected by a coiled-coil segment to the central E domain (1). The molecule is comprised of two sets of three polypeptide chains namely, Aα-, Bβ-, and γ-chains, which are joined together in the N-terminal E domain by five symmetrical disulfide bridges (1). Fibrinogen has a molecular weight of approximately 340 kDa ; it is activated by thrombin and converted into fibrin. Numerous congenital genetic mutations in fibrinogen have been reported, and although many patients are asymptomatic, some exhibit bleeding or thrombosis. Herein, we report a case of congenital hypofibrinogenemia with a novel genetic mutation. This research has been approved by the Ethics Committee of Tokushima University Hospital (approval number : 3767-3) and Shinshu University (approval number : 5694).

CASE PRESENTATION

The patient was a woman in her 30s. During pregnancy, it was noted that her plasma fibrinogen activity was low (128-163 mg/dL ; reference range, 200-400 mg/dL), and it did not improve after delivery (69-89 mg/dL). Therefore, the patient was referred to the Department of Hematology. Coagulation tests such as prothrombin time and activated partial thromboplastin time were normal, and the patient showed no bleeding tendency. In addition, there was no abnormal bleeding during childbirth or after tooth extraction, which had been experienced four times in the past. The patient had no history of hepatic disease. Further detailed examination revealed no notable abnormalities other than fibrinogen activity (measured using ThromboCheck Fib (L) [Sysmex Co. Ltd.]) (Table 1). The presence of an inhibitor was ruled out because the activity of the control plasma was not inhibited when mixed with patient plasma. Based on the above observations, a congenital genetic abnormality was suspected.

We requested Shinshu University to conduct a detailed examination of the patient's fibrinogen activity level, antigen level, and genomic sequence. Analysis at Shinshu University showed that the patient's fibrinogen activity level was 80 mg/dL and the antigen level was 131 mg/dL (reference range, 180-350 mg/dL), with a discrepancy between activity and antigen. Shinshu University used a DNA Extraction WB kit (FUJIFILM-Wako Pure Chemical Co. Ltd.) to extract genomic DNA from whole blood cells. To analyze all exons and exon-intron boundaries in the Aα-, Bβ-, and γ-chain genes, long-range PCR for *FGA*, *FGB*, and *FGG* was performed using TaKaRa LA Taq (TaKaRa Bio Inc.) and the three pairs of primers (2). The PCR products were purified from agarose gels using a Gene Clean II Kit (Funakoshi) and sequenced directly using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and a 3500 Genetic Analyzer (Both from Applied Biosystems). Sequencing of DNA obtained from whole blood revealed a heterozygous c.Bβ227G>T mutation in exon 3 of Bβ-chain, which resulted in the cysteine residue at position 76 in exon 3 being converted to a phenylalanine residue (Figure 1).

DISCUSSION

This report is the first to describe a patient with a c.Bβ227G>T genetic mutation, and its corresponding amino acid mutation, BβCys76Phe. We named this mutation Tokushima (BβCys76Phe). Since this mutation involves an amino acid substitution, it may affect the patient's fibrinogen structure and function. Notably, it is known that the cysteine residue located at position 76 of the Bβ-chain forms a disulfide bond with the

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cysteine residue located at position 49 of the Aα-chain (3). As a result of this mutation, one disulfide bond in fibrinogen would become dysfunctional, which may have various effects.

There are many reports of fibrinogen genetic mutations and their associated functional abnormalities. Cysteine residues form disulfide bonds and are involved in protein structure ; when a cysteine residue is lost or gained, it may form new disulfide bonds at unusual locations or bind to external proteins, thereby affecting protein function. Fibrinogen Marburg is one such mutation (4). In this mutation, the codon encoding lysine 461 (AAA) of the Aα-chain is changed to a stop codon (TAA). Consequently, 150 amino acids are deleted from position 461 onwards. Fibrinogen Marburg is known to form an extremely thin and tight fibrin network ; as a result, strong resistance to plasmin and decreased permeability of liquid components cause thrombosis. Furthermore, due to the deletion, cysteine 442 of the Aα-chain becomes free and binds to albumin, and approximately one-third of fibrinogen becomes bound to albumin. Mutations in the Bβ-chain that similarly cause thrombosis have been reported, including fibrinogen Ijmuiden (BβArg14Cys), fibrinogen New York 1 (Bβ del 9-72), and fibrinogen Naples (BβAla68Thr) (5-7). Although the ability of these mutations to bind albumin is unknown, it has

Table 1. Laboratory data

【CBC】			【coagulation】			【bio chemistry】		
WBC	5.2	$\times10^3/\mu L$	PT	13.2	sec	AST	30	U/L
RBC	4.42	$\times10^6/\mu L$	PT-INR	1.09		ALT	43	U/L
Hb	14.2	g/dL	APTT	36.3	sec	LDH	179	U/L
HCT	42	%	Fib activity	84	mg/dL	ALP	87	U/L
MCV	95	fL	FDP	<2.5	μg/mL	γ-GT	13	U/L
PLT	176	$\times10^3/\mu L$	D-dimer	<0.5	μg/mL	T-bil	1.2	mg/dL
seg	71	%	TAT	<1.0	ng/mL	TP	7.2	g/dL
lymph	21	%	PIC	<0.5	μg/mL	BUN	13	mg/dL
mono	3	%				Cre	0.61	mg/dL
eosino	5	%				IgG	1036	mg/dL
baso	0	%				IgM	140	mg/dL
						IgA	327	mg/dL

Abbreviation
PT : prothrombin time, APTT : activated partial thromboplastin time, FDP : fibrin-fibrinogen degradation product, TAT : thrombin anti-thrombin complex, PIC : plasmin plasmin-inhibitor complex

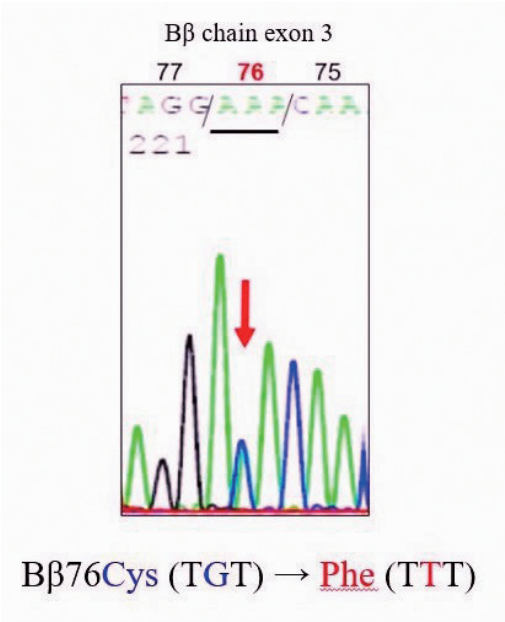

Figure 1. Sequence of the fibrinogen gene
Waveform of the sequence data obtained when using the reverse primer. The change in DNA sequence from TGT to TTT resulted in a substitution of the cysteine residue with phenylalanine. That is a heterozygous mutation because the waveforms of guanine and thymine overlap.

been reported that fibrinogen New York 1 and fibrinogen Naples have a low affinity for thrombin. Although the Tokushima (BβCys76Phe) mutation occurred at a location different to that of other reported fibrinogen mutations, it may cause a similar result because it also generates a free cysteine.

There are three types of congenital fibrinogen deficiencies : afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia. These diseases can be differentiated by measuring fibrinogen activity and antigen levels. Our patient was diagnosed with hypofibrinogenemia with decreased activity and antigen levels ; however, there was a discrepancy between the activity and antigen levels, suggesting that the patient also had functional abnormalities. Clinically, the patient exhibited almost no bleeding or thrombosis. This may be a characteristic of the Tokushima (BβCys76Phe) mutation.

In conclusion, we identified a new fibrinogen gene mutation accompanied by an amino acid mutation. This mutation reduces fibrinogen activity and antigen levels. The pathophysiology of fibrinogen with the Tokushima (BβCys76Phe) mutation remains unclear, and further functional analysis is required.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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