

Fibrinogen Yecheon: Congenital Dysfibrinogenemia with Gamma Methionine-310 to Threonine Substitution

This case study reports a rare fibrinogen variant, γ Met310Thr mutation, for the first time in Korea. The case shows a point mutation from T to C in the 1,007th nucleotide of the *FGG* gene. This report describes a variant fibrinogen, hereinafter called "fibrinogen Yecheon", using the name after the town where the patient was living at the time of diagnosis. Fibrinogen Yecheon has a de novo heterozygous point mutation of *FGG* resulting in γ Met310Thr and subsequent extra N-glycosylation at γ Asn308. Extra N-glycosylated fibrinogen is considered a main inhibitor of normal fibrinogen activity.

Key Words : Fibrinogen; Dysfibrinogenemia; *FGG* Mutation; Fibrinogen Yecheon

Eunkyung Park¹, Geumbore Park²,
Rojin Park³, Hee-Jin Kim⁴,
Sang Jae Lee¹, and Young Joo Cha²

Departments of Internal Medicine, Hemato-oncology¹ and Laboratory Medicine², Chung-Ang University Hospital, Seoul; Department of Laboratory Medicine³, Soonchunhyang University Hospital, Seoul; Department of Laboratory Medicine⁴, Samsung Medical Center, Seoul, Korea

Received : 8 August 2009
Accepted : 31 August 2009

Address for correspondence

Young Joo Cha, M.D.
Department of Laboratory Medicine, Chung-Ang University Hospital, 224-1 Heukseok-dong, Dongjak-gu, Seoul 156-755, Korea
Tel : +82.2-6299-2720, Fax : +82.2-6298-8630
E-mail : chayoung@cau.ac.kr

INTRODUCTION

Fibrinogen is a 340-kD glycoprotein, one of the critical proteins of the blood coagulation and platelet aggregation (1). It is composed of six polypeptide chains, (α , β , γ)₂, which are held together by disulfide bonds and organized in a symmetrical dimeric fashion (1).

Until now, a variety of mutations in each of the fibrinogen chain genes have been reported in more than 465 families all over the world, with $A\alpha$ -chain mutation being the most common form, followed by γ -chain mutation (2).

Several cases of congenital dysfibrinogenemia have been reported in Korea. The first report was $A\alpha(517-522)\text{delins}31$, designated as "fibrinogen Seoul" in a 7-yr-old girl associated with amyloidosis (3). The second was $\gamma\text{Ala}341\text{Asp}$, which was designated as "fibrinogen Seoul I", in a 66-yr-old woman with peripheral artery obstructive disease (4). The third was $A\alpha\text{Gln}328\text{Pro}$, which was designated as "fibrinogen Seoul II", in a 51-yr-old man who suffered from recurrent myocardial infarction (5).

Here we describe a γ -chain fibrinogen variant (fibrinogen Yecheon), which is a point mutation from T to C in the 1,007th nucleotide of the *FGG* gene, resulting in a methionine (Met)-to-threonine (Thr) substitution at the 310th residue in the carboxy-terminal region of the fibrinogen γ -chain. This is the

fourth report of γ Met310 to Thr substitution in the world, and the first in Korea. As for genetic dysfibrinogenemia, this is the fourth case report in Korea.

CASE REPORT

A 20-yr-old male patient with past medical history of bleeding tendency in neonate period visited the department of otorhinolaryngology for a preoperative evaluation for operation of chronic maxillary sinusitis. He reported a family history of easy bruisability of his father and conductive hearing difficulty after meningitis in childhood. No laboratory abnormalities indicating coagulation defect had been detected after birth.

In the preoperative evaluation, the patient had normal blood counts and serum chemistry test results. However, the initial coagulation screening showed prolonged prothrombin time (PT, 24.5 sec; normal 10.4-12.5 sec) and activated partial thromboplastin time (aPTT, 47.3 sec; normal, 26.0-41.0 sec). The bleeding time was normal (2 min and 30 sec; normal, 1-5 min). The PT and aPTT mixing test result indicated factor deficiency. The fibrinogen degradation product (FDP) and d-Dimer were within normal ranges. Other coagulation tests were normal (Table 1). On the other hand, plasma fib-

rirogen activity of the subject examined using the Clauss method was lower than the detection limit (<25 mg/dL; normal, 140–460 mg/dL), while the antigen level measured by fibrinogen ELISA was normal (437 mg/dL). After a written informed consent was obtained, a familial genetic study was started. All exons, exon-intron boundaries, and promoter regions of the fibrinogen $A\alpha$ (*FGA*, GenBank M64982), $B\beta$ (*FGB*, GenBank M64983), and γ (*FGG*, GenBank M10014) chain genes were amplified and sequenced. PCR and direct

sequencing revealed that only the patient in his family was heterozygous for a point mutation in the *FGG* gene. The mutation was a mutation substituting the 310th codon ATG (Met) to ACG (Thr), (c.1007T>C) (Fig. 1A). He had no mutations in the *FGA* or *FGB* gene. We designated the patient's abnormal fibrinogen as "fibrinogen Yecheon", naming after the local city where he and his family are residing. Collectively, congenital dysfibrinogenemia was identified. For further evaluation, fibrinogen mixing test (Table 2) was run and the results showed no significant improvements in the fibrinogen level. This implies the possibility of some inhibitors of fibrinogen activity.

The plasma fibrinogen activities of the patient's parents by Clauss method were within normal range (309 and 254 mg/dL, respectively). His two elder sisters also showed normal coagulation test results. Molecular genetic study was performed only with his parents, including his father who had a history of easy bruising, and they both were homozygous for the wild-type allele (Fig. 1B, C).

Table 1. Coagulation test results

Test	Result	Reference range
FDP ($\mu\text{g/mL}$)	<10	>40 $\mu\text{g/mL}$
D-dimer ($\mu\text{g/mL}$)	0.45	75-150
Plasminogen (%)	130.4	75-125
Antithrombin (%)	93.4	48-151
vWF:Ag (%)	>200	46-164
vWF:RCo (%)	152	
Anti factor IX Ab	Negative	
Anti factor VIII Ab	Negative	
Protein C Ag (%)	113	72-160
Protein C Activity (%)	80	70-130
Protein S Ag (free) (%)	101	50-150
Factor II (%)	79	60-140
Factor V (%)	63	60-140
Factor VII (%)	90	60-140
Factor VIII (%)	87	60-140
Factor IX (%)	97	60-140
Factor X (%)	88	60-140
Factor XI (%)	108	60-140
Factor XII (%)	58	60-140
Factor XIII (%)	Normal	

FDP, fibrinogen degradation product; vWF, von willebrand factor; RCo, ristocetin cofactor; Ab, antibody; Ag, antigen.

Table 2. Fibrinogen mixing test

Sample number	Sample type	Measured (mg/dL)	Expected (mg/dL)
I	Control I	250	
II	Subject	NC	
III	I+II	13	125
IV	I+III	56	187
V	I+IV	100	218
VI	III+VII	88	179
VII	Control II	233	
VIII	VI+VII	99	206

NC, not checkable.

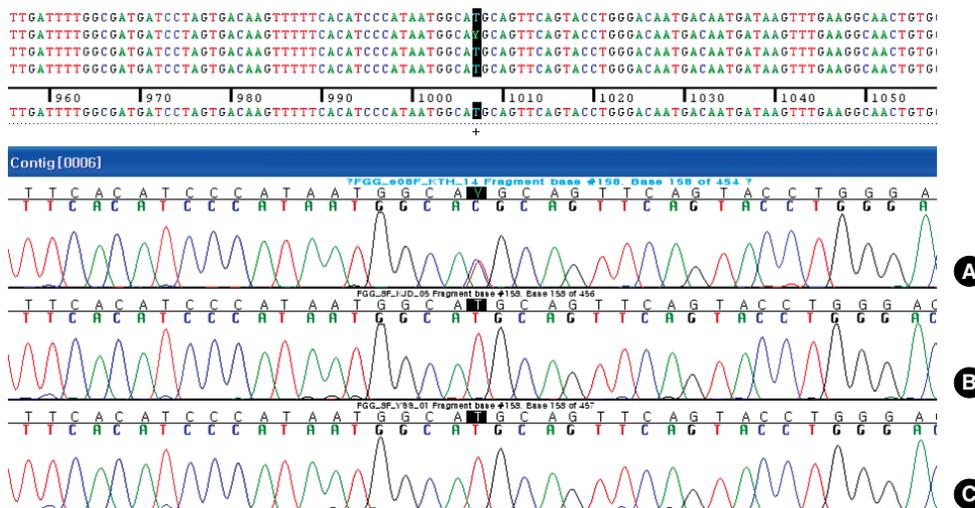


Fig. 1. The chromatogram shows DNA sequencing results of fibrinogen γ -chain gene (*FGG*). The A indicates the sample of the patient. A heterozygous *FGG* mutation (c.1007T>C [p.Met336Thr]) was identified. The B indicates the sample of the father and the C indicates the sample of the mother.

DISCUSSION

Dysfibrinogenemia was suggested in the subject according to the initial plasma fibrinogen activity and antigen levels. DNA sequence analysis confirmed congenital dysfibrinogenemia. The patient himself decided not to take operation after the diagnosis of dysfibrinogenemia, although cryoprecipitate treatment was possible. Cryoprecipitate contains factor VIII, fibrinogen, von Willebrand factor and factor XIII, so it is generally used for dysfibrinogenemia. Although he was born with bleeding tendency, the patient had no symptoms of bleeding or thrombosis after childhood. According to Cote et al.(1), an examination of the clinical symptoms associated with γ -dysfibrinogenemia shows that <5% of the individuals experienced significant bleeding, and <30% showed thrombotic tendencies. Approximately 60% of patients with γ -dysfibrinogenemias were asymptomatic at the time of diagnosis. Unfortunately, data on long-term follow-up of dysfibrinogenemic patients are rarely available. Close observation of clinical symptoms might be the best option for treatment.

γ Met310Thr was already reported by the names of Asahi (6), Frankfurt VII (7), and Hannover XXIV (2). Details of biochemical mechanisms involved in fibrin polymerization, release of fibrinopeptides A/B, factor XIIIa-mediated cross-linking profiles, and the position of glycosylation were elucidated in the study of fibrinogen Asahi (6). Asahi fibrinogen showed normal release of fibrinopeptides A/B, but fibrin polymerization and factor XIIIa-mediated γ -chain cross-linking were severely impaired regardless of calcium ions, which was caused by N-glycosylation consensus sequence Asn (308)-Gly (309)-Thr (310) conferred by the mutation (1, 6, 8). Specifically, severely impaired polymerization of Asahi fibrin monomers may account for extremely retarded cross-linking between the two Asahi γ -chains, although the amine receptor γ Gln (398) was found to function normally (1, 6, 8). Those functional abnormalities could explain fibrinogen Asahi patient's bleeding tendency as well as that of the fibrinogen Yecheon subject, which was supported by the ultrastructure of fibrinogen Caracas II (A α Ser434 to N-glycosylated Asn) detected with the use of scanning electron microscopy. In the microscopic image, large pores bounded by local fiber networks made up of thin fibers were observed (9). Normally, fibrinogen contains approximately 3% carbohydrate consisting of NeuAc, Gal, Man, and GlcNAc, which is linked to Asn 52 on the γ chain and Asn 364 on the B β chain (10). The role of glycosylation in fibrinogen function has been studied and its involvement in the clotting process was proposed (10), although contradictory results have been reported (11). Steric hindrance by the extra carbohydrate itself, the disruption of D:D interactions by the new glycosylation, and strong negative charges of sialic acids in the extra carbohydrate could be raised to account for disturbed fibrin polymerization or impaired cross-linking in Niigata (B β Asn160Ser), Asahi, and Lima (A α Arg141Ser) fibrins, respectively (1, 12-14).

Being a heterozygote, in fibrinogen Yecheon, as in fibrinogen Asahi, fibrin monomer containing at least one gamma chain mutated is not likely to function on making polymerization properly. Fibrinogen mixing test results (Table 2) demonstrated the presence of inhibitor. FDP level was within normal range. Those facts, therefore, suggested extra glycosylated gamma chain not only dysfunction on polymerization but also inhibit normal fibrin and fibrinogen resulting in hardly clotting with thrombin in fibrinogen activity assay by Clauss method. In addition, the subject's coagulation abnormality was thought to be caused by extra glycosylation of γ -chain and subsequent disruption of D:D interaction because other coagulation factors were within normal ranges (Table 1). The subject with fibrinogen Yecheon showed bleeding tendency. Usually, dysfibrinogenemias having extra glycosylations concerned hemorrhagic tendency unlike Tokyo V (γ Ala327-Thr) fibrinogen which was somewhat unique in that its propositus was thrombophilic partly owing to fibrinolysis-resistant fibrin clots despite the very low clottability of the fibrin. It was suggested that tissue plasminogen activator binding to Tokyo V fibrinogen might have been affected directly. The subject of fibrinogen Yecheon presented de novo mutation in *FGG*, while his parents did not show any coagulation abnormalities including DNA sequencing. De novo mutations are associated with advancing paternal age (15) and pollutants (16, 17).

One thing different between Yecheon and Asahi subjects was the level of FDP, which was normal (2.3 μ g/mL) for the Yecheon subject while that was markedly increased to 160 μ g/mL for the Asahi patient. Because d-dimer level was within normal range, the Yecheon subject was not considered in the state of hyperfibrinolysis, which was partially supported by the normal levels of plasminogen and antithrombin. As a follow-up, a further study can be conducted to understand what brought about marked increase in FDP level for Asahi subject.

Fibrinogen Yecheon is the only congenital dysfibrinogenemia in Korea associated with bleeding tendency, and not thrombosis or amyloidosis. Our subject was born with bleeding tendency, although he had no history of serious bleeding symptoms. In general, mutations within the γ chain of fibrinogen are not associated with serious bleeding disorders. Two patients, Baltimore I (Gly292Val) and Giessen IV (Asp318Gly), who experienced mild bleeding symptoms, also suffered from thrombotic tendencies. The only γ -dysfibrinogenemia associated with a serious bleeding diathesis is Asahi I (Met310Thr). In this instance, the bleeding symptoms were probably related to the extra glycosylation resulting from the substitution (1).

In conclusion, this report describes a variant fibrinogen, hereinafter called "fibrinogen Yecheon", using the name after the town where the patient was living at the time of diagnosis (2). Fibrinogen Yecheon has a de novo heterozygous point mutation of *FGG* resulting in γ Met310Thr and subsequent

extra N-glycosylation at γ Asn308. Extra N-glycosylated fibrinogen is considered a main inhibitor of normal fibrinogen activity.

REFERENCES

1. Cote HC, Lord ST, Pratt KP. *Gamma-chain dysfibrinogenemias: molecular structure-function relationships of naturally occurring mutations in the gamma chain of human fibrinogen*. *Blood* 1998; 92: 2195-212.
2. Hanss M, Biot F. *A database for human fibrinogen variants*. *Ann N Y Acad Sci* 2001; 936: 89-90.
3. Kang HG, Bybee A, Ha IS, Park MS, Gilbertson JA, Cheong HI, Choi Y, Hawkins PN. *Hereditary amyloidosis in early childhood associated with a novel insertion-deletion (indel) in the fibrinogen Aalpha chain gene*. *Kidney Int* 2005; 68: 1994-8.
4. Song KS, Park NJ, Choi JR, Doh HJ, Chung KH. *Fibrinogen Seoul (FGG Ala341Asp): a novel mutation associated with hypodysfibrinogenemia*. *Clin Appl Thromb Hemost* 2006; 12: 338-43.
5. Park R, Doh HJ, An SS, Choi JR, Chung KH, Song KS. *A novel fibrinogen variant (fibrinogen Seoul II; AalphaGln328Pro) characterized by impaired fibrin alpha-chain cross-linking*. *Blood* 2006; 108: 1919-24.
6. Yamazumi K, Shimura K, Terukina S, Takahashi N, Matsuda M. *A gamma methionine-310 to threonine substitution and consequent N-glycosylation at gamma asparagine-308 identified in a congenital dysfibrinogenemia associated with posttraumatic bleeding, fibrinogen Asahi*. *J Clin Invest* 1989; 83: 1590-7.
7. Galanakis D, Spitzer S, Scharrer I, Peerschke E. *Impaired platelet aggregation support by two dysfibrinogene: a gamma319-320 deletion and a gamma310Met->Thr substitution*. *Thromb Haemost* 1993; 69: 1261.
8. Yamazumi K, Shimura K, Maekawa H, Muramatsu S, Terukina S, Matsuda M. *Delayed intermolecular gamma-chain cross-linking by factor XIIIa in fibrinogen Asahi characterized by a gamma-Met-310 to Thr substitution with an N-glycosylated gamma-Asn-308*. *Blood Coagul Fibrinolysis* 1990; 1: 557-9.
9. Woodhead JL, Nagaswami C, Matsuda M, Arocha-Pinango CL, Weisel JW. *The ultrastructure of fibrinogen Caracas II molecules, fibers, and clots*. *J Biol Chem* 1996; 271: 4946-53.
10. Townsend RR, Hilliker E, Li YT, Laine RA, Bell WR, Lee YC. *Carbohydrate structure of human fibrinogen. Use of 300-MHz 1H-NMR to characterize glycosidase-treated glycopeptides*. *J Biol Chem* 1982; 257: 9704-10.
11. Nishibe H, Takahashi N. *The release of carbohydrate moieties from human fibrinogen by almond glycopeptidase without alteration in fibrinogen clottability*. *Biochimica et biophysica acta* 1981; 661: 274-9.
12. Sugo T, Nakamikawa C, Takano H, Mimuro J, Yamaguchi S, Moseson M, Meh D, DiOrio JP, Takahashi N, Takahashi H, Nagai K, Matsuda M. *Fibrinogen Niigata with impaired fibrin assembly: an inherited dysfibrinogen with a Bbeta Asn-160 to Ser substitution associated with extra glycosylation at Bbeta Asn-158*. *Blood* 1999; 94: 3806-13.
13. Sugo T, Sekine O, Nakamikawa C, Endo H, Arocha-Pinango CL, Matsuda M. *Mode of perturbation of Asahi fibrin assembly by the extra oligosaccharides*. *Ann N Y Acad Sci* 2001; 936: 223-5.
14. Maekawa H, Yamazumi K, Muramatsu S, Kaneko M, Hirata H, Takahashi N, Arocha-Pinango CL, Rodriguez S, Nagy H, Perez-Requejo JL. *Fibrinogen Lima: a homozygous dysfibrinogen with an A alpha-arginine-141 to serine substitution associated with extra N-glycosylation at A alpha-asparagine-139. Impaired fibrin gel formation but normal fibrin-facilitated plasminogen activation catalyzed by tissue-type plasminogen activator*. *J Clin Invest* 1992; 90: 67-76.
15. Reichenberg A, Gross R, Weiser M, Bresnahan M, Silverman J, Harlap S, Rabinowitz J, Shulman C, Malaspina D, Lubin G, Knobler HY, Davidson M, Susser E. *Advancing paternal age and autism*. *Arch Gen Psychiatry* 2006; 63: 1026-32.
16. Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ, Berndt ML, Pogribny IP, Koturbash I, Williams A, Douglas GR, Kovalchuk O. *Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location*. *Proc Natl Acad Sci USA* 2008; 105: 605-10.
17. Hu C, Jiang L, Geng C, Zhang X, Cao J, Zhong L. *Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells*. *Mutat Res* 2008; 652: 88-94.