Clinical Case Report

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Factor X heterozygous mutation in a patient with potential risk of bleeding

A case report

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Abstract

Rationale: Factor X (FX) deficiency is a rare autosomal recessive bleeding disorder. The majority of patients carry a missense mutation in *F10*, and patients with bleeding disorders are either homozygous or compound heterozygous for *F10*. Nonsense mutations are exceptionally rare, and a heterozygous nonsense mutation is not considered to cause bleeding disorders.

Patient concerns: A 35-year-old Japanese female with an incidental hemorrhage after gynecologic polypectomy was referred to our hospital.

Diagnoses: Following differential diagnostic workup, including cross-mixing test, congenital FX deficiency was strongly suspected.

Intervention: Coagulation tests and mutation analyses were conducted for the patient and her parents.

Outcomes: Mutation analysis revealed that she carried a heterozygous nonsense mutation in *F10*. Pedigree analysis revealed that the mutation was inherited from her mother although there was no familial history of bleeding or hemostatic disturbance.

Lessons: Hemostatic disturbance may occur even in a patient with heterozygous *F10*. Because heterozygous nonsense mutation in *F10* is expected to be hidden in an apparently healthy population, as observed in our patient, unexpected hemostatic disturbance may occur, particularly during the use of direct oral anticoagulant (DOAC)-targeting factor Xa for thrombotic diseases. FX activity should be evaluated before prescribing DOACs to patients.

Abbreviations: aPTT = activated partial thromboplastin test, CL = cardiolipin, CNS = central nervous system, DOAC = direct oral anticoagulant, FLC = free light chain, FX = Factor X, OMIM = Online Mendelian Inheritance in Man, PT = prothrombin time.

Keywords: anticoagulants, factor X deficiency, heterozygote, nonsense mutation

1. Introduction

Factor X (FX), a serine protease synthesized in hepatocytes in the presence of vitamin K, is a pivotal coagulation factor in the common coagulation pathway. Based on the increasing prevalence of thrombotic disease, direct oral anticoagulants (DOAC) targeting factor Xa is increasingly used for the treatment and prevention of thrombotic diseases.[1] On the contrary, factor X deficiency (OMIM #227600), an autosomal *recessive* bleeding disorder, is uncommon in the general population. The estimated prevalence of the homozygous form is approximately 1:1,000,000.[2] A decrease in FX activity is strongly associated with bleeding complications; patients with <10% FX activities frequently experience severe events, including CNS and gastrointestinal bleeding. By contrast, patients with >40% of FX activity are largely asymptomatic.[3]

F10 gene is located on chromosome 13q34 and consists of 8 exons. Among 130 genomic aberrations reported so far (available at the Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/gene.php?gene=F10), most are missense mutations that results in hypofunction of FX produced by the affected allele.[4] Small insertions, small and gross deletions, and splice variants are also reported.[4,5] However, nonsense mutations with bleeding complication are described only in four reports, and of those, two are homozygous mutations,[4,6] one is a compound heterozygous,[7] and one is unknown.[8] Heterozygous *F10* nonsense mutation with bleeding complication has never been reported previously. Given this, FX deficiency is caused by homozygous or compound heterozygous mutations in *F10*, especially in the case of nonsense mutations.

Here, we first report a female patient with a *heterozygous, neither homozygous nor compound heterozygous, F10* nonsense mutation who unexpectedly experienced bleeding complication after a gynecologic polypectomy. Family study revealed that the mutation is inherited from her mother. Such undiagnosed heterozygous nonsense mutations are hidden in the apparently healthy population; therefore, it potentially increases the risk of bleeding in the DOAC era for thrombotic diseases.

2. Case presentation

A 35-year-old Japanese female consulted us for the possibility of bleeding disorders. She experienced a prolonged hemorrhage after the resection of endocervical polyp, which was considered a

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cause of her infertility. She denied past histories of bleeding in childhood, menorrhagia, and prolonged menstruation. No family members had experienced unexpected bleeding events. Her physical examination showed only anemia (RBC, $418 \times 10^4/mm^3$; Hb, 11.4 g/dL; and MCV, 86.8 fL), and iron supplementation had already been started. She did not take any other medication. Prothrombin time (PT) and activated partial thromboplastin test (aPTT) was 15.6 seconds and 44.4 seconds, respectively. FX activity using the PT method was 33% (normal range: 71%–128%). Factor II and V activities were 83% (74%–146%) and 69% (70%–152%), respectively. Liver function tests were normal, and prothrombin induced by vitamin K absence-II was not elevated. The burden of the free light chain (FLC) and the kappa-lambda ratio of FLC were within normal levels, which are frequently abnormal in AL amyloidosis.[9] The antinuclear antibody titer was low (1:160, homogeneous and speckled patterns), and specific autoantibodies [anti-double stranded DNA, SS-A, SS-B, cardiolipin (CL), and CL-beta 2 glycoprotein 1 complex] were not detected. Lupus anticoagulant test using the phospholipid neutralization method was negative. Cross-mixing test showed a deficiency pattern. As shown in the above differential diagnostic workup, congenital FX deficiency was strongly suggested. Mutation analysis of the gene was approved by the Ethical Committee of Toyama University Hospital, and written informed consent was obtained from the patient. The genomic exam revealed a heterozygous E117X nonsense mutation (c.349G>T) in *F10* (Fig. 1, left). The mutation had not been reported previously in the Human Gene Mutation Database or polymorphism databases, including Japanese genome variations (https://ijgvd.megabank.tohoku.ac.jp/).[10] No other mutations were detected in *F10*.

We conducted coagulation tests and mutation analysis for her parents because they gave consent to the pedigree analysis. Although the parents' PT and aPTT were within normal levels, FX activity and FX antigen concentrations using an enzyme-linked immunosorbent assay of the mother were decreased by half of the normal level (Table 1). Mutation analysis revealed that the same mutation was found in *F10* of the mother (Fig. 1, right). The sequence of the promoter region of *F10* was completely matched. In contrast, her father had no mutation in *F10*. After explaining the findings, informed consent for publication was obtained from the patient and her parents.

3. Discussion

Because E117 resides in exon 4 of *F10*, of which the stop codon is normally in exon 8, the affected gene transcripts are eliminated by a nonsense-mediated mRNA decay pathway.[11] FX activity is comparable with the FX concentration in our patient and her mother, which means that the gene product of the affected allele is virtually absent. Interestingly, the mother, who has the same mutation as the patient, never experienced bleeding, and her FX activity was approximately half of the normal level. Although mutations of the 5′ flunking region have been known to decrease FX activity,[12] the promoter regions of F10 of the patient and of the mother were matched; therefore, the burden of gene product from the unaffected allele is theoretically equivalent. Several hemostatic factors are known to be affected by age,[13] which is important when considering the mechanism of thrombotic disease, whereas FX activity is not affected by age, sex, or ABO blood type.[14] Why such an individual difference between the patient and her mother occurs is not revealed in our study, a part of which may be explained by physiological changes (the normal range of FX activity by one allele is thought to be 35.5% to 64% according to the reference level).

Table 1

Coagulation tests of the patient and her parents.

	Normal range	Patient	Father	Mother
PT	>80%	69	>100	90
APTT	26.5–41.8 seconds	43.8	33.9	35.6
FX activity	71%–128%	33	97	54
FX antigen	15.0 μg/mL	4.9	14.7	7.8

APTT = activated partial prothrombin test, FX = factor X, PT = prothrombin time.
Note: All parameters of the patient were re-evaluated at the same time as when her parents were tested.

DOAC has been increasingly used for thrombotic diseases. DOAC reduces the risk of bleeding compared with vitamin K-dependent anticoagulant warfarin.[15] Moreover, DOAC could readily manage patients without the usual clotting exam or fine tuning of dosage, which is one of the reasons why DOAC is used worldwide. Such a trend raises a concern about the possibility that patients with an undiagnosed *F10* mutation receive DOAC treatment, leading to unexpected hemostatic disturbance. As demonstrated in our study, several patients with a heterozygous aberration of *F10* are asymptomatic. PT and aPTT are not prolonged even when the FX activity decreases to half of the normal level, as was the case with the patient's mother. Rivaroxaban, one of the DOACs interacting with factor Xa, decreases the FX activity in PT method by approximately 20% even under the therapeutic dose, and its inhibitory effect is dose dependent,[16] which means that the FX activity easily decreases above the safety range if patients with heterozygous *F10* mutation take a DOAC. Because thrombotic diseases become increasingly common with age, the patient's mother can possibly be administered with DOAC without knowing of her bleeding tendency. Several guidelines recommend that DOAC withdrawal is not required when patients receive minimal bleeding risk procedures,[17] and a few studies discussed that DOAC could continue even when patients undergo low to moderate risk procedures.[18,19] Given that the prevalence of a heterozygous *F10* mutation is approximately 1:2,000, unexpected adverse events of hemostatic disturbance may occur in patients with hidden heterozygous mutations in *F10*. At present, an appropriate assay to measure DOAC effects is still debated, and no specific reversal agents are widely available,[20] which requires prompt resolution to reduce undesired bleeding in such patients.

In summary, we experienced a patient with incidental hemorrhage who had a novel, hereditary heterozygous nonsense mutation in *F10*. Although evidence should be validated whether

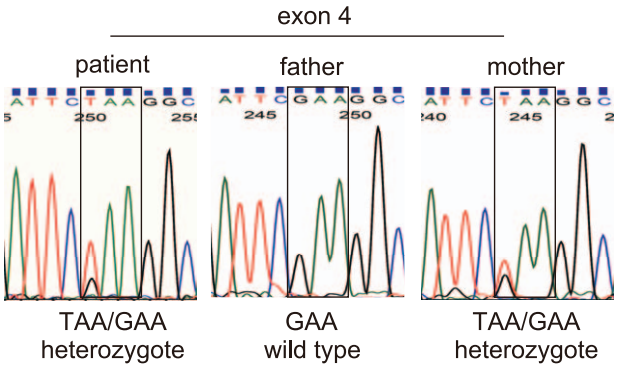

Figure 1. Sanger sequence of *F10* in the patient and her parents. c.349G>T heterozygous single nucleotide transversion found in the patient and her mother causes E117X nonsense mutation.

heterozygous nonsense mutation per se causes bleeding, we emphasize that several patients have a potential to experience unexpected bleeding from DOAC use due to heterozygous *F10* mutations. FX activity should be evaluated before we prescribe DOACs for patients.

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Author Contribution: KA planned the outpatient managements and analyzed the data; HN, NY, TU, and IK performed genetic exams and analyzed the data; TS organized the therapeutic team; KA drafted the paper; and all authors critically revised the manuscript and agree with its contents.

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