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## CASE REPORT

# Transient Platelet Dysfunction in Congenital Factor XIII Deficiency with Enhanced Thrombin Generation Potential

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### SUMMARY

***Background:*** **Congenital factor XIII (FXIII) deficiency is an extremely rare bleeding disorder with defects in the *F13A1* or *F13B* genes. Here, we report a case of congenital FXIII deficiency patient who presented with trauma-induced intramuscular hemorrhage accompanied with transient platelet dysfunction with increased endogenous thrombin potential (ETP).**
***Methods:*** **FXIII antigen and activity, *F13A1* gene sequencing, and thrombin generation assay were measured.**
***Results:*** **The diagnosis of FXIII deficiency was confirmed by a double heterozygous mutation of the *F13A1* gene and decreased levels of FXIII antigen and activity. Platelet dysfunction caused by an antiplatelet drug was revealed in both platelet aggregation test and PFA-100. After a bleeding event, the PFA-100 results returned to normal and the thrombin generation assay in patient's plasma showed a higher ETP than normal.**
***Conclusions:*** **This increase in ETP may protect against bleeding and may explain why some patients show only a mild bleeding tendency despite undetectable FXIII activity.**
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### KEY WORDS

factor XIII deficiency, platelet function, thrombin generation potential

### CASE PRESENTATION

A healthy 8-year-old male was admitted with swelling and tenderness in the right thigh. The patient had a history of bumping his right thigh against a chair 8 days ago and taking a cyclooxygenase-1 inhibitor drug because of fever 2 days ago. The patient did not have any obvious perinatal bleeding problems but had one event of multiple bruises on the stomach, legs, and forehead 7 years ago. The family had no history of bleeding disorders.
An X-ray examination of the thigh revealed no fracture, and contrast CT examination confirmed a huge intramuscular hematoma (4.0 cm) in the anterior compartment of the right thigh.
Routine coagulation tests, which included prothrombin time (PT), activated partial thromboplastin time (aPTT),

and fibrinogen, were normal, but PFA-100 (Siemens Healthineers, Munich, Germany) showed prolonged closure times in both cartridges (collagen/epinephrine, 229 seconds; collagen/ADP, 137 seconds; Table 1). Von Willebrand factor (vWF) antigen and ristocetin cofactor activity were normal. Coagulation factor II, V, VIII, IX, X, XI, and XII levels were normal (Table 1). In platelet aggregation tests, platelet aggregation was decreased when induced by ADP, but was normal when induced by other agonists (Figure 1A). FXIII screening using the urea clot solubility test showed absence of FXIII activity. FXIII activity in a chromogenic assay (Technochrom FXIII, Technoclone, Vienna, Austria) was 5% (Table 1). FXIII antigen measured with enzyme-linked immunosorbent assay (Human Factor XIII ELISA Kit, Abcam, Cambridge, UK) was 9.0 ng/mL, well below the normal range.

In a molecular test to screen for the genetic variants of the *F13A1* gene, c.514C>T (p.Arg172Ter) and c.1504 G>A (p.Gly502Arg) were identified (Figure 1B), confirming congenital FXIII deficiency.

The patient was transfused with one unit of fresh frozen plasma on the first day of admission. He was discharged on hospital day 3, when thigh swelling decreased and bleeding stopped. A follow-up PFA-100 test on discharge day 10 was normal in both cartridges. In addition, a thrombin generation assay was performed to investigate thrombin generation potential. Interestingly, the endogenous thrombin potential (ETP) measured with Pool Norm (Stago, Paris, France) after stimulation with either 1 or 5 pM tissue factor was enhanced in the plasma of the patient compared with that of a normal control (Figure 1C).

## DISCUSSION

We reported a case of FXIII deficiency presenting as trauma-induced intramuscular bleeding concomitant with transient platelet dysfunction caused by an antiplatelet drug. Considering that there was no previous tendency of severe bleeding in our patient, the concomitant platelet dysfunction is regarded as one of the causative factors of bleeding [1]. After the bleeding event, the platelet function as measured by PFA-100 returned to normal, and increased thrombin generation was detected in the patient's plasma, which may usually protect the patient against bleeding.

FXIII deficiency is an autosomal recessive disorder, accounting for 6% of rare bleeding disorders [2]. Among clinical manifestations, umbilical cord bleeding is the most common, and intracranial, intramuscular, and subcutaneous soft tissue bleeding is also common [3]. FXIII is a transglutaminase that consists of two A subunits and two B subunits. The defects in the *F13A1* (most often) or *F13B* gene cause FXIII deficiency [4]. In our study, two different heterozygous mutations were found in the *F13A1* gene: one was c.514C>T (p.Arg172Ter) in exon 4, and the other was c.1504G>A (p.Gly502Arg) in exon 12. The former is a nonsense mutation in the β-sandwich domain, which has been reported worldwide including China, England, Spain, and Italy [5]. The latter is a missense mutation in the core domain of the *F13A1* gene that has been reported as a homozygote in only two cases in Israel [6]. Of more than 150 different mutations found in FXIII deficiency, most are missense or nonsense mutations [5]. No mutational hot spot and no apparent correlation of genotype with bleeding tendency has been found [7]. In our study, we could not determine whether the detected mutations where inherited from the parents or were de novo mutations because the parents refused genetic testing as they had no bleeding symptoms.

Patients with FXIII deficiency have normal values in routine coagulation tests such as PT, aPTT, and platelet function test [8]. In our case, prolonged PFA-100 closure times and normal PT and aPTT results at first suggested platelet-related abnormalities such as von Willebrand disease (vWD) rather than coagulation factor deficiency [9]. However, the subsequent urea clot solubility test showed the absence of FXIII activity, suggesting FXIII deficiency. In the absence of the urea clot solubility test results, the condition could have been misdiagnosed. Therefore, when PT and aPTT results are normal in a bleeding patient, it is important to perform the urea clot solubility test, even if platelet function results are abnormal. On the other hand, the low sensitivity of the urea clot solubility test may result in false positivity in case of hypofibrinogenemia or dysfibrinogenemia [8]. Therefore, when FXIII deficiency is suspected, it is necessary to perform FXIII activity or antigen test even if the urea clot solubility test is negative.

At the time of the bleeding event, our patient showed prolonged PFA-100 closure time and decreased platelet aggregation induced by ADP. This platelet dysfunction was possibly related to taking an antiplatelet drug 2 days before the bleeding event. Since platelets contain cytoplasmic FXIII that interacts with cytoskeletal proteins during platelet activation [10] and platelet contractile force is decreased in FXIII deficiency because of delayed thrombin generation [11], we consider that platelet dysfunction in our patient facilitated bleeding after trauma. The subsequent PFA-100 test showed a normal result, confirming that platelet dysfunction was transient and likely related to an external factor such as the antiplatelet drug.

The lower FXIII activity is, the more severe the bleeding tendency tends to be [1]. Severe bleeding tendency usually occurs in patients with less than 5% FXIII activity, but sometimes only a mild bleeding tendency is observed even in patients with undetectable FXIII activity [1]. Since the spectrum of bleeding tendencies according to FXIII activity is very broad, a protective mechanism against bleeding is thought to exist in case of a severe decrease in FXIII activity [12]. In our patient, FXIII activity was about 5% of normal, but there was no history of umbilical or spontaneous bleeding, suggesting that other protective factors against bleeding

**Table 1. Baseline laboratory results of the patient.**

| | | Patient | Reference range |
|---|---|---|---|
| Coagulation assays | PT (sec) | 11.4 | 10.6 - 12.9 |
| | aPTT (sec) | 29.1 | 26.7 - 36.6 |
| | Fibrinogen activity (mg/dL) | 260 | 157 - 400 |
| | vWF antigen (%) | 154.4 | 72.7 - 234.2 |
| | vWF:RCo activity (%) | 111.9 | 79.5 - 151.9 |
| | Factor II (%) | 70.5 | 67 - 107 |
| | Factor V (%) | 58.1 | 50 - 116 |
| | Factor VIII (%) | 140 | 44 - 144 |
| | Factor IX (%) | 71.9 | 63 - 89 |
| | Factor X (%) | 72.5 | 55 - 101 |
| | Factor XI (%) | 82.7 | 52 - 120 |
| | Factor XII (%) | 67.0 | 60 - 140 |
| | PFA-100, C/EPI (sec) | 229 | 80 - 180 |
| | PFA-100, C/ADP (sec) | 137 | 63 - 109 |
| FXIII assays | FXIII screening | absent | present |
| | FXIII antigen (ng/mL) | 9.0 | 14.0 - 30.4 |
| | FXIII activity (%) | 5 | 69 - 140 |

**Abbreviations: PT - prothrombin time, aPTT - activated partial thromboplastin time, vWF - von Willebrand factor, vWF:RCo - von Willebrand factor ristocetin cofactor assay, C/EPI - collagen/epinephrine, C/ADP - collagen/ADP, FXIII - Factor XIII.**

may exist.

Thrombin generation assay was suggested to predict bleeding tendency and thrombotic risk [13]. In coagulation factor deficiencies other than FXIII deficiency, the ETP and peak thrombin are reduced on the thrombin generation curve and the lag time is prolonged, and the decrease in ETP is correlated with factor level and bleeding tendency [14]. However, in our case of FXIII deficiency, the ETP and peak thrombin were rather increased which is consistent with a previous report of increased ETP and peak thrombin in FXIII deficiency and their normalization by replacement of FXIII [12]. The increased ETP is considered to result from altered fibrin polymerization caused by FXIII deficiency [12]. The altered fibrin structure can provide the surface for hyperactive prothrombin conversion, facilitating active thrombin generation. Enhanced thrombin generation in FXIII deficiency is expected to protect against bleeding. Moreover, the generated thrombin itself can activate platelets, which play a major role in hemostasis. Therefore, enhanced thrombin generation can explain why the bleeding tendency is minimal even in patients with undetectable FXIII activity [4]. Future prospective study is necessary to investigate whether the ETP level can predict protection against bleeding in FXIII deficiency.

## CONCLUSION

We report a case of congenital FXIII deficiency confirmed by a double heterozygous mutation. The initial detection of platelet dysfunction on the basis of prolonged PFA-100 closure time and decreased ADP-induced platelet aggregation could lead to an erroneous assumption of vWD, but subsequent normal levels of vWF antigen and activity excluded vWD, and finally the decreased levels of FXIII antigen and activity and a double heterozygous mutation of *F13A1* confirmed congenital FXIII deficiency. The bleeding event after bumping injury could be facilitated by transient platelet dysfunction caused by an antiplatelet drug. Of note, the thrombin generation assay revealed enhanced plasma thrombin generation, which may play a role in protection against bleeding in FXIII deficiency. It may explain why some patients show only a mild bleeding tendency despite undetectable FXIII activity. Further study of the relationship between bleeding tendency and enhanced thrombin generation in FXIII deficiency is needed.

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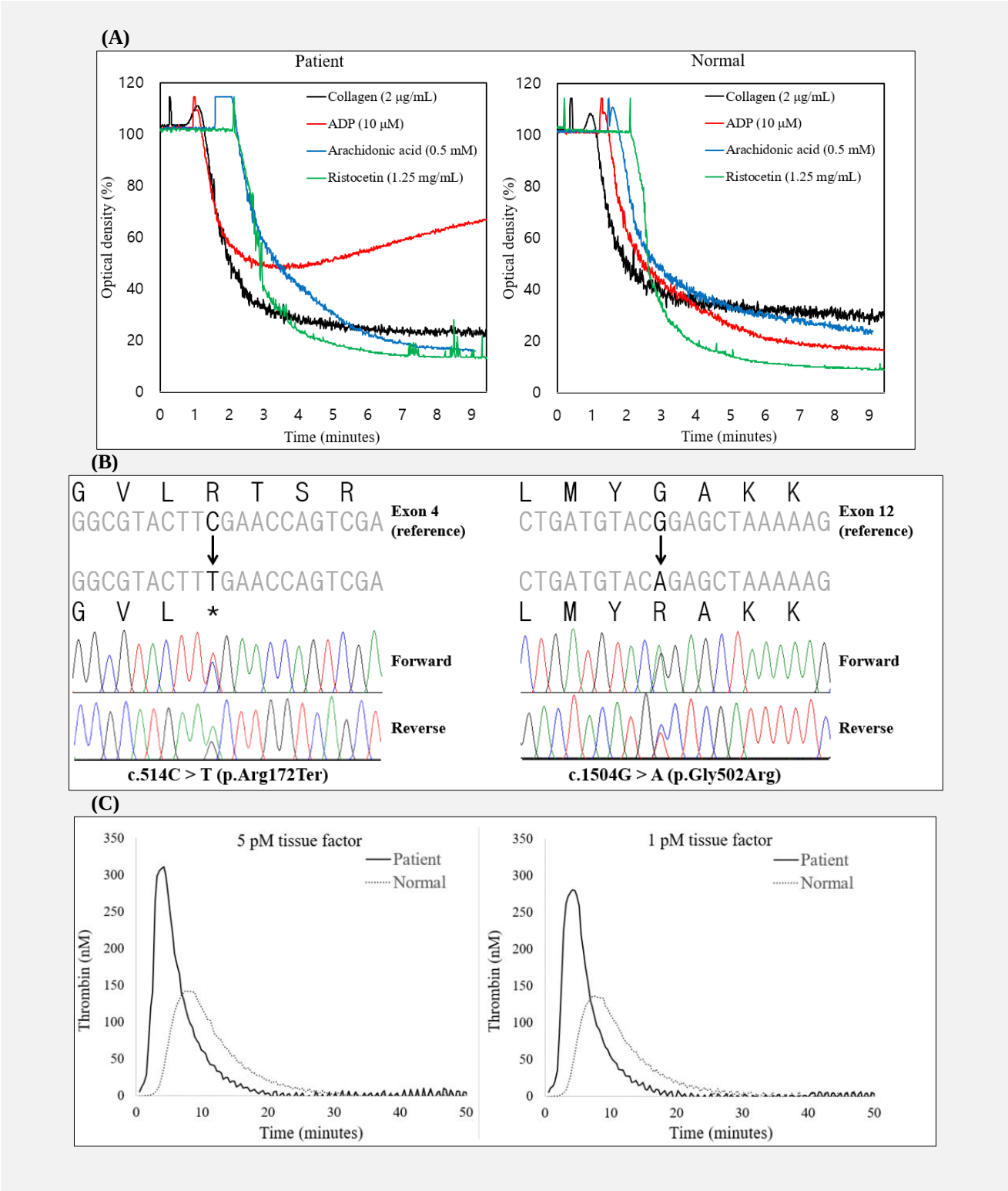


**Figure 1. (A) Platelet aggregation tests in our patient (left) and a normal control (right). (B) Sanger analyses of the *F13A1* gene in the patient showing mutations in exon 4 (left) and exon 12 (right). (C) Plasma thrombin generation curves in calibrated thrombograms after stimulation of platelet poor plasma with 1 pM (right) or 5 pM tissue factor (left). The curves represent duplicate tests.**

**Declaration of Interest:**
The authors declare that they have no potential conflicts of interest.

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