International Journal of Hematology (2020) 111:51–56
https://doi.org/10.1007/s12185-019-02767-y

ORIGINAL ARTICLE

Genetic analysis of a compound heterozygous patient with congenital factor X deficiency and regular replacement therapy with a prothrombin complex concentrate

Tomoki Togashi[1] · Satomi Nagaya[2] · Masayuki Nagasawa[3] · Makiko Meguro-Horike[4] · Keiji Nogami[5] · Yuta Imai[1] · Kana Kuzasa[1] · Akiko Sekiya[2] · Shin-ichi Horike[4] · Hidesaku Asakura[6] · Eriko Morishita[2,6]

Received: 8 May 2019 / Revised: 16 October 2019 / Accepted: 17 October 2019 / Published online: 30 October 2019

Abstract

Congenital factor X (FX) deficiency is a rare bleeding disorder with an incidence of one in one million. The proband, a 2-year-old girl, exhibited easy bruising and a history of umbilical cord bleeding at birth. Prothrombin time (> 40 s) and activated partial thromboplastin time (65.0 s) were prolonged. Marked declines in FX activity (< 1%) and FX antigen levels (5%) were also observed. Genetic analysis of the proband identified two types of single-base substitutions, c.353G>A (p.Gly118Asp) and c.1303G>A (p.Gly435Ser), indicating compound heterozygous congenital FX deficiency. Genetic analysis of family members revealed that her father and older sister (5-year-old) were also heterozygous for p.Gly118Asp, and that her mother was heterozygous for p.Gly435Ser. To improve the bleeding tendency, the proband received regular replacement of 500 units of PPSB-HT, a prothrombin complex concentrate (PCC). Following continued regular replacement of 500 units of PPSB-HT once per week, the proband has exhibited no bleeding tendencies and no new bruises have been observed. There are no previous report of the use of PPSB-HT for regular FX replacement. Regular replacement therapy with PPSB-HT may be an effective method for preventative control of bleeding tendencies in FX deficiency.

Keywords Factor X deficiency · Genetic analysis · Regular replacement therapy · Prevent bleeding tendency · Prothrombin complex concentrate

Introduction

Factor X (FX) is a vitamin K-dependent serine protease precursor synthesized in the liver and plays a central role in blood coagulation [1]. FX is a disulfide-bonded two-chain glycoprotein in the blood plasma with a 17-kDa light chain and a 45-kDa heavy chain [2]. The light chain consists of a -carboxyglutamic acid-rich domain (Gla domain), which is involved in Ca^{2+}-dependent conformational change, and two epidermal growth factor-like domains (EGF-like domain), while the heavy chain contains an activation peptide and a serine protease domain. FX is activated to its active form, activated FX (FXa), by an extrinsic pathway involving complex formation with activated factor VII (FVIIa), tissue factor (TF) and Ca^{2+}, or by an intrinsic pathway involving complex formation with activated factor IX (FIXa), activated factor VIII (FVIIIa), Ca^{2+} and a phospholipid. Prothrombin is activated by the interaction between FXa and activated factor V (FVa), leading to the progression of a coagulation cascade.

✉ Eriko Morishita
eriko86@staff.kanazawa-u.ac.jp

1 Department of Laboratory Sciences, School of Health Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan

2 Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, 5-11-80 Kodatsuno, Kanazawa 920-0942, Ishikawa, Japan

3 Department of Pediatrics, Musashino RedCross Hospital, Tokyo, Japan

4 Advanced Science Research Center, Kanazawa University, Kanazawa, Japan

5 Department of Pediatrics, Nara Medical University Hospital, Kashihara, Nara, Japan

6 Department of Hematology, Kanazawa University Hospital, Kanazawa, Japan

Congenital FX deficiency is a rare bleeding disorder with an incidence of one in one million [3], which follows an autosomal recessive inheritance pattern [4]. According to the Human Gene Mutation Database (HGMD) professional 2019.2 (https://portal.biobase-international.com), 167 different types of genetic mutations had been reported around the world as of August 11, 2019. As for the types of genetic mutation, missense and nonsense mutations caused by single-base substitutions were the most prevalent, accounting for 78% (130 cases) of all mutations. The FX gene (*F10*), which encodes FX, is a 26.7 kb-long gene located on the long arm of chromosome 13 (13q34), comprising eight exons and seven introns [5]. Congenital FX deficiency is caused by genetic mutations in *F10* and develops due to quantitative and qualitative abnormalities of the resulting FX. This pathology can be classified into a deficiency form characterized by decreases in levels of both antigen and activity, and a molecular abnormality form with normal antigen levels, but decreased activity [6]. FX deficiency is one of the most severe coagulation factor deficiencies [7, 8], and presents primarily with umbilical cord bleeding, mucosal bleeding, gastrointestinal bleeding, central nervous system (CNS) bleeding, excessive bleeding after surgery, and recurrent hematoma. While heterozygotes are often asymptomatic, homozygotes and compound heterozygotes are prone to bleeding, with the degree of bleeding correlating with the degree of reduced FX activity.

Agents such as PCCs, plasma-derived FX/FIX concentrate (pd-FX/FIX) and fresh-frozen plasma (FFP) are used to prevent bleeding in congenital FX-deficient patients [9-11]. Recently, pd-FX is available as replacement therapy in Europe and USA but not in Japan.

We report herein our experience in preventatively controlling bleeding with regular replacement of 500 units of a PCC, PPSB-HT (Nihon Pharmaceutical, Tokyo, Japan), in a compound heterozygous patient with congenital FX deficiency.

Materials and methods

The proband was a 2-year-old girl (body weight, 14.4 kg) who presented to the pediatric department with a chief complaint of easy bruising. Subcutaneous hematomas (3 ~ 5 cm) were sometimes found on the lower extremity, waist and back. The patient had a history of umbilical cord bleeding at birth. Blood coagulation testing showed a prolonged prothrombin time (PT) of > 40 s and an activated partial thromboplastin time (APTT) of 65.0 s. Marked declines were also seen in FX activity (< 1%) and FX antigen levels (5%). Plasma activities of Factor II, VII, and IX are 84%, 82%, and 81%, respectively. The proband was diagnosed with congenital FX deficiency as a cross-mixing test showed a deficiency pattern. The parents were not consanguineous and the grandmother had a history of bleeding tendency.

Measurement of FX activity and antigen levels

FX activity was measured in a one-stage APTT-based coagulation assay using a THROMBOCHECK Factor X (Sysmex, Hyogo, Japan), and FX antigen levels were assessed using a FX Antigen Elisa kit (Affinity Biologicals, Ontario, Canada) at Nara Medical University.

Sequencing

After obtaining informed consent from the proband and her family, genomic DNA was extracted from peripheral blood leukocytes using a GENERATION Capture Column kit (QIAGEN, Tokyo, Japan). All exons and the exon–intron boundaries of *F10* were amplified. KOD-Plus- (TOYOBO, Osaka, Japan) was used for polymerase chain reaction (PCR), and the base sequence of the primer and PCR conditions were adopted from a study conducted by Jayandharan et al. [4]. PCR products were directly sequenced using the dye terminator method with a BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Tokyo, Japan). An ABI PRISM 3100 Genetic Analyzer (Thermo Fisher Scientific) was used for genetic analyses.

This study was approved by the Kanazawa University Human Genome and Gene Analysis Research Ethics Committee (Approval no. 135–7).

Regular replacement therapy with PPSB-HT and measurement of FX activity

Regular replacement with 500 units of PPSB-HT at a frequency of once every 2 weeks was planned. FX activity was measured from the proband's plasma after replacement with 500 units of PPSB-HT. Activity was measured 4 h after replacement, as FX activity peaks approximately 2–4 h after administration. Transfusion of PPSB-HT to the proband was approved through the ethics application process at Musashino Red Cross Hospital (Approval no. 30–9).

Results

Measurement of FX activity and antigen levels

Figure 1 shows FX activity and antigen levels of proband and the family members.

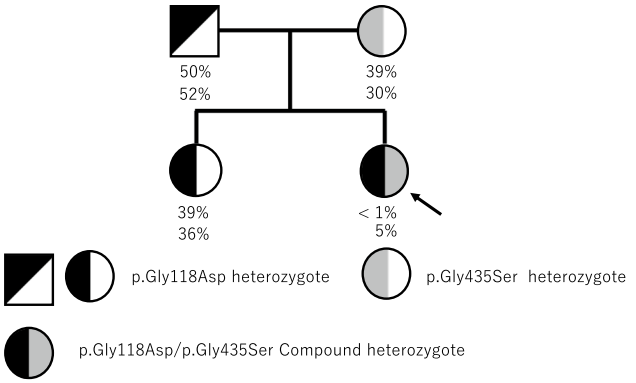

Fig. 1 The pedigree of the proband with FX deficiency. The arrow indicated proband. FX activity levels and antigen levels were shown upper and lower, respectively. The proband was suspected of being a compound heterozygote with p.Gly118Asp and p.Gly435Ser. As a result of genetic analysis of family members, the father and elder sister were heterozygous for p.Gly118Asp and the mother was heterozygous for p.Gly435Ser

Sequencing

Figure 2 shows the results of genetic analysis of the proband and family members. Genetic analysis identified two types of missense mutation, in exon 4 (c.353G>A; p.Gly118Asp) and in exon 8 (c.1303G>A; p.Gly435Ser). The analysis of family members identified heterozygosity, with p.Gly118Asp in the father and elder sister, and p.Gly435Ser in the mother.

Regular replacement therapy with PPSB-HT and measurement of FX activity

To normalize FX activity and control the bleeding tendencies of the proband, regular replacement therapy was initiated with 500 units of PPSB-HT. As shown in Table 1, PT and APTT were normalized, and FX activity improved to 60% by 4 h after replacement with 500 units of PPSB-HT. The initial plan was one administration every 2 weeks, but additional emergency replacement had to be administered as intra-articular bleeding was detected in the right foot (3–4 cm of swelling) on day 9 after starting replacement therapy. This was the first clear evidence of intra-articular bleeding, and replacements were administered every week thereafter. After shifting to replacement therapy once a week, no bleeding tendencies or new bruises have been observed. Moreover, PT-INR and APTT, which were 9.1 and 65.0 s at diagnosis, have been improved to 4.7 and 50.6 s after 8 months of the regular replacement therapy. FX inhibitor assay results 8 months after starting replacement therapy were negative.

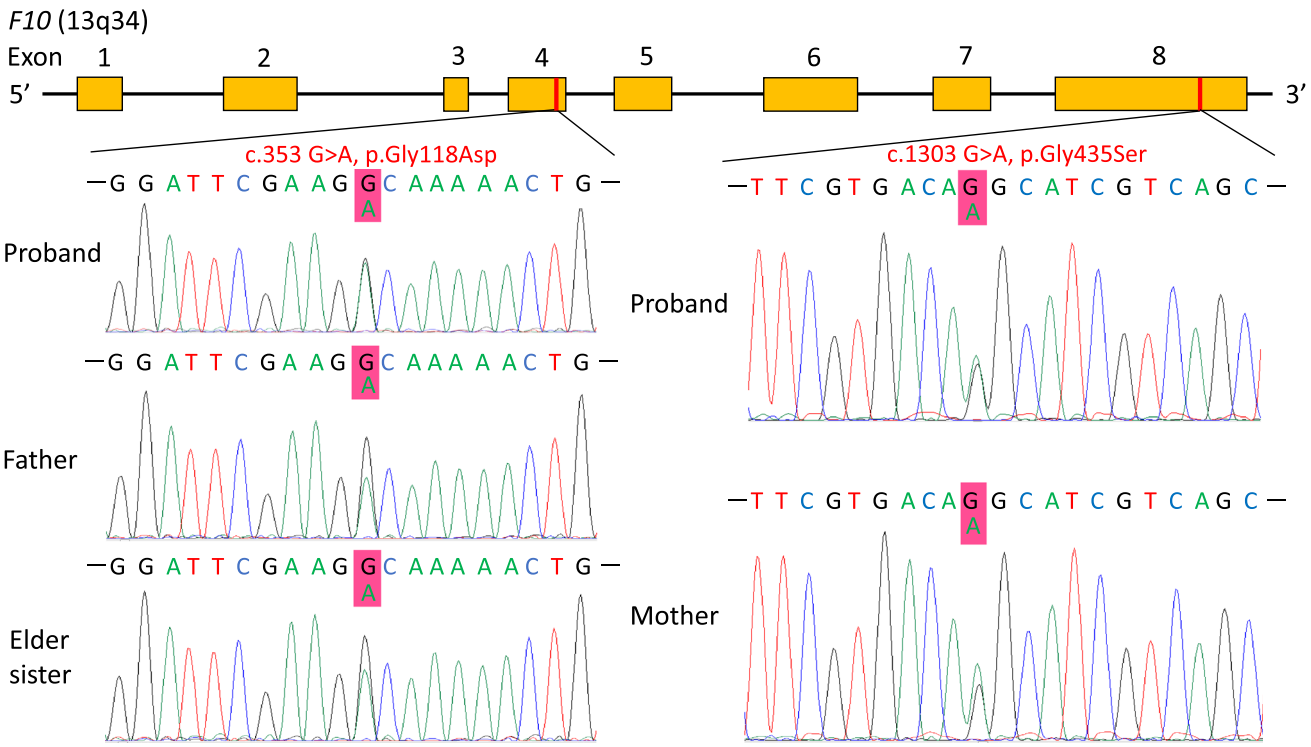

Fig. 2 Sequencing analysis of the proband and family members. This Fig. shows the result of the genetic analysis of the proband and family members. The sequencing analysis revealed her to have two types of missense mutation, in exon 4 (c.353G>A; p.Gly118Asp) and in exon 8 (c.1303G>A; p.Gly435Ser). The analysis of family members identified heterozygous missense mutations, with p.Gly118Asp in the father and elder sister, and p.Gly435Ser in the mother

Table 1 Blood coagulation tests of the proband at diagnosis and after administration with PPSB-HT

	Before administration	After administration			
	At diagnosis	4 h later	1 week later	3 months later	8 months later
PT (sec)	> 40	13.3	> 40	> 40	> 40
PT-INR	9.1	1.0	5.3	5.2	4.7
APTT (sec)	65.0	35.6	59.2	62.7	50.6
FX activity level (%)	< 1	60	< 1	< 1	< 1
FX antigen level (%)	5	ND	ND	ND	ND
Other coagulation factors					
FII activity level (%)	84	129	ND	ND	ND
FVII activity level (%)	82	102	ND	ND	ND
FIX activity level (%)	81	100	ND	ND	ND

ND indicates not done

Discussion

Genetic analysis of the proband identified two types of missense mutations (c.353G>A, p.Gly118Asp and c.1303G>A, p.Gly435Ser), demonstrating compound heterozygosity of the proband for congenital FX deficiency. The p.Gly118Asp mutation was reported by Flora et al. in 2001, and Matsuo et al. reported the p.Gly435Ser mutation in 2016.

Based on previous reports, the following is the suspected mechanism of amino acid substitution at the site of each mutation that triggers FX deficiency. Gly118 is located within the EGF-1 domain. The EGF-1 domain is in a region involved in Ca^{2+} binding and in the structural maintenance of FXa. Gly118 is a highly conserved amino acid among vitamin K-dependent coagulation factors (VKDCF) and among mammals (Fig. 3), and plays a crucial role in protein folding [5]. Gly118Asp mutation may have caused the decline in FX activity by causing protein misfolding leading to impaired secretion. However, more detailed experiments such as functional analysis of a mutant FX protein should be conducted to confirm this hypothesis. On the other hand, p.Gly435Ser is a missense mutation within the serine protease domain. Gly435 is highly conserved among VKDCF and among mammals (Fig. 3) and completely conserved in the serine protease family, and thus appears to represent a particularly important amino acid in the catalytic region [12]. Gly435Ser mutation may have caused the decline in FX activity by destabilizing the three-dimensional structure of the protein in the catalytic region, leading to impaired secretion.

As rare coagulation disorders (RCDs) such as congenital FX deficiency are not encountered very often, specialist physicians select the course of treatment based on experience, due to the lack of epidemiological findings and clinical results [13]. The half-life of FX in the blood is approximately 40–60 h, relatively longer than other coagulation

Amino acid		113					118					123		430					435					440	
Homo sapiens	…	L	E	G	F	E	G	K	N	C	E	L	……	T	Y	F	V	T	G	I	V	S	W	G	…
Mus musculus	…	S	E	G	F	E	G	K	N	C	E	L	……	T	Y	Y	V	T	G	I	V	S	W	G	…
Rattus norvegicus	…	T	E	G	F	E	G	K	N	C	E	L	……	T	Y	F	V	T	G	I	V	S	W	G	…
Bos taurus	…	A	E	G	F	E	G	K	N	C	E	F	……	T	Y	F	V	T	G	I	V	S	W	G	…
Equus caballus	…	L	E	G	F	E	G	K	N	C	E	L	……	T	Y	F	V	T	G	I	V	S	W	G	…
Physeter catodon	…	L	E	G	F	E	G	K	N	C	E	L	……	T	Y	F	V	T	G	I	V	S	W	G	…
Orcinus orca	…	L	E	G	F	E	G	G	N	C	E	L	……	T	Y	F	I	T	G	I	V	S	W	G	…
Amino acid		113					118					123		430					435					440	
FX	…	L	E	G	F	E	G	K	N	C	E	L	……	T	Y	F	V	T	G	I	V	S	W	G	…
FIX	…	P	F	G	F	E	G	K	N	C	E	L	……	T	S	F	L	T	G	I	I	S	W	G	…
FVII	…	L	P	A	F	E	G	R	N	C	E	T	……	T	W	Y	L	T	G	I	V	S	W	G	…
FII	…	T	P	R	S	E	G	S	S	V	N	L	……	R	W	Y	G	M	G	I	V	S	W	G	…

Fig. 3 Comparison of amino acid alignments around Gly118 and Gly435. Gly118 and Gly435 are highly conserved among vitamin K-dependent coagulation factors and among mammals

factors, and a FX level of 10–20% is considered to be needed for sufficient hemostasis [14, 15]. Agents such as PCCs, pd-FX/FIX, and FFP are used in the treatment of congenital FX-deficient patients. However, FFP is known to have side-effects such as circulatory overload, anaphylactic shock and transfusion-related acute lung injury (TRALI) [16, 17]. When administering pd-FX/FIX concentrate and PCCs, there is a risk of thrombosis from the accumulation of non-specific coagulation factors other than FX [15, 18]. Currently available PCCs contain various anti-coagulants (such as heparin, antithrombin, protein C, protein S, and protein Z) to minimize this risk. However, for FX deficiency, coagulation factor concentrate containing only FX should ideally be used [19]. In 2016, a high-purity pd-FX (Bio Products Laboratory, Elstree, UK) was developed as a therapeutic agent for congenital FX deficiency [3] and has since been approved for use in the United States and Europe. While FX replacement with pd-FX is recommended for congenital FX deficiency patients, the use of pd-FX has not yet been approved in Japan. FX replacement was thus performed using PCCs as a substitute for pd-FX. Cases have been reported of PCCs and pd-FX being used in FX replacement therapy during bleeding, but no reports have described PCCs usage in regular replacement therapy.

In the present study, PPSB-HT was used as a substitute for pd-FX in regular replacement therapy, and proved effective in preventative control of bleeding tendency in congenital FX deficiency. As shown in Table 1, PT and APTT were normalized, and FX activity improved by 4 h after administration. Plasma activities of factor II, VII, and IX increased slightly by contrast with before administration. Although PT and APTT were prolonged and FX activity reduced below 1% at 1 week after the first administration, her bleeding tendency disappeared clinically. Even more surprising, PT-INR and APTT gradually were shortened as the regular replacement therapy continued. In general, the amount of FX replacement increases as the child grows. It seems that the improvement in the bleeding tendency by the regular replacement therapy has reduced the consumption of FX. Therefore, it is considered that the administration every week was effective in improving the bleeding tendency in this patient.

In the future, we need to examine the replacement dosage and frequency to account for increased body weight during the growth period, and we hope to elucidate the optimal replacement dosage and frequency through the accumulation of more cases. As FX replacement consequently results in excessive administration of factor II, VII, and IX, possibly increasing the risk of thrombosis due to hypercoagulability, monitoring by means such as measurement of coagulation factor activities is important. It is also helpful to try to exclude thrombosis by measurement of coagulation activity markers such as thrombin-antithrombin complex, prothrombin fragment 1 + 2 and soluble fibrin in plasma. Recently, comprehensive coagulation assays, such as clot waveform analysis (CWA), Thrombelastography and thrombin generation assay (TGA), have been developed to assess physiological hemostasis. Therefore, these assays could provide valuable information on coagulation potential in the patient with the regular replacement therapy of a PCC. Monitoring for the emergence of inhibitors is also essential.

Acknowledgements This work was supported by grant from the Ministry of Health, Labor and Welfare of Japan (E.M.) (Grant number 6046619-01), the Ministry of Education, Culture, Sports, Science and Technology of Japan (E.M.) (Grant number 18K07442) and Japan Agency for Medical Research and Development (E. M.) (Grant number 19ek0109210h0003).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Nagaya S, Akiyama M, Murakami M, Sekiya A, Asakura H, Morishita E. Congenital coagulation factor X deficiency: Genetic analysis of five patients and functional characterization of mutant factor X proteins. Haemophilia. 2018;24:774–85.
2. Takabe K, Holman PR, Herbst KD, Glass CA, Bouvet M. Successful perioperative management of factor X deficiency associated with primary amyloidosis. J Gastrointest Surg. 2004;8:358–62.
3. Menegatti M, Peyvandi F. Treatment of rare factor deficiencies other than hemophilia. Blood. 2019;133:415–24.
4. Jayandharan G, Viswabandya A, Baidya S, Nair SC, Shaji RV, George B, et al. Six novel mutations including triple heterozygosity for Phe31Ser, 514delT and 516TG factor X gene mutations are responsible for congenital factor X deficiency in patients of Nepali and Indian origin. J Thromb Haemost. 2005;3:1482–7.
5. Peyvandi F, Menegatti M, Santagostino E, Akhavan S, Uprichard J, Perry DJ, et al. Gene mutations and three-dimensional structural analysis in 13 families with severe factor X deficiency. Br J Haematol. 2002;117:685–92.
6. Shapiro A. Plasma-derived human factor X concentrate for on-demand and perioperative treatment in factor X-deficient patients: pharmacology, pharmacokinetics, efficacy, and safety. Expert Opin Drug Metab Toxicol. 2017;13:97–104.
7. Menegatti M, Peyvandi F. Factor X deficiency. Semin Thromb Hemost. 2009;35:407–15.
8. Dorgalaleh A, Zaker F, Tabibian S, Alizadeh S, Dorgalele S, Hosseini S, et al. Spectrum of factor X gene mutations in Iranian patients with congenital factor X deficiency. Blood Coagul Fibrinolysis. 2016;27:324–7.
9. Peyvandi F, Menegatti M. Treatment of rare factor deficiencies in 2016. Am Soc Hematol Educ Program. 2016;2016:663–9.
10. Lim MY, McCarthy T, Chen SL, Rollins-Raval MA, Ma AD. Importance of pharmacokinetic studies in the management of acquired factor X deficiency. Eur J Haematol. 2016;96:60–4.
11. Franchini M, Lippi G. Prothrombin complex concentrates: an update. Blood Transfus. 2010;8:149–54.
12. Matsuo Y, Mizuochi T, Miho M, Nakagawa S, Ozono S, Ueda K, et al. Factor X deficiency with heterozygous mutations of novel

p. G435S and known p. G244R in a patient presenting with severe umbilical hemorrhage. Kurume Med J. 2017;63:23–8.
13. Peyvandi F, Garagiola I, Biguzzi E. Advances in the treatment of bleeding disorders. J Thromb Haemost. 2016;14:2095–106.
14. Kulkarni R, James AH, Norton M, Shapiro A. Efficacy, safety and pharmacokinetics of a new high-purity factor X concentrate in women and girls with hereditary factor X deficiency. J Thromb Haemost. 2018;16:849–57.
15. Austin SK, Kavakli K, Norton M, Peyvandi F, Shapiro A, FX Investigators Group. Efficacy, safety and pharmacokinetics of a new high-purity factor X concentrate in subjects with hereditary factor X deficiency. Haemophilia. 2016;22:419–25.
16. Kouides PA, Kulzer L. Prophylactic treatment of severe factor X deficiency with prothrombin complex concentrate. Haemophilia. 2001;7:220–3.
17. Seki M, Koh K, Inoue T, Tomita Y, Kato M, Shimizu M, et al. Prophylactic administration of prothrombin complex concentrates for congenital prothrombin deficiency with a novel frameshift mutation, prothrombin Saitama. Pediatr Blood Cancer. 2013;60:503–5.
18. Apak H, Celkan T, Ozkan A, Yüksel L, Bilgi Z, Yildiz I. Severe factor X deficiency treated with heparin-added prothrombin complex concentrate. Ann Hematol. 2003;82:710–1.
19. Liesner R, Akanezi C, Norton M, Payne J. Prophylactic treatment of bleeding episodes in children %3c12 years with moderate to severe hereditary factor X deficiency (FXD): efficacy and safety of a high-purity plasma-derived factor X (pdFX) concentrate. Haemophilia. 2018;24:941–9.