Congenital Factor X Deficiency, Diagnosis, and Management

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12.1 Introduction

Coagulation factor X (FX; synonyms: autoprothrombin III, Stuart–Prower factor) is a vitamin K-dependent glycoprotein that occupies a pivotal position in the coagulation cascade. After activation, activated FX (FXa) is the first enzyme in the common coagulation pathway and plays a key role in thrombin generation. Congenital FX deficiency is a very rare bleeding disorder that is inherited in an autosomal recessive manner estimated to occur in 1:1,000,000 individuals, although the prevalence is much greater in areas with a high rate of consanguineous marriage. Bleeding manifestations usually occur in homozygous and compound heterozygous cases, while heterozygous cases are generally asymptomatic. Regardless of the severity of FX deficiency, the most common bleeding symptoms are mucocutaneous bleedings including epistaxis, gum bleeding, and easy bruising. Patients with severe FX deficiency have a high incidence of spontaneous major bleeding. The bleeding may occur at any age; however, the cases with severe FX deficiency [FX:C) <1%] may be diagnosed early in life with an abnormal bleeding tendency. The affected women at reproductive age may present with menorrhagia. The diagnosis of FX deficiency is suspected following the finding of a prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT) which corrects (unless an inhibitor is present) in a 50:50 mix with normal plasma and confirmed by measuring plasma FX levels either immunologically or functionally. Management includes on demand

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and prophylactic therapy (if and when indicated). Current available therapies include antifibrinolytic agents; tranexamic acid and aminocaproic acid and blood-derived products; fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), FIX products, and plasma-derived FX concentrate (pdFX).

Factor X is synthesized by the liver and as an inactive zymogen secreted into the plasma where the excision of a tribasic peptide Arg-Lys-Arg ("RKR": residues 180–182) occurs;. It circulates as a two-chain molecule with a concentration of 8–10 μg/mL (~135 nmol/L) and a half-life of 40 h in the plasma. FX belongs to the peptidase S1 family of proteins.

12.2 Factor X Structure and Function

FX, also known as autoprothrombin III and Stuart–Prower factor, is a serine protease mainly synthesized in the liver. Its zymogen form secretes to plasma and circulates as a two-chain molecule with a concentration of 8–10 μg/mL (~135 nmol/L) and a half-life of 40 h. FX belongs to the peptidase S1 family of proteins (Table 12.1) [1]. FX contains a 40-residue pre-propeptide and hydrophobic signal sequence (−37 to −22) in homology with the other vitamin K-dependent coagulation factors. The signal sequence is cleaved in a two-step process. The first cleavage occurs after Leu-29 and then the propeptide (−1 to −18) is removed from the N-terminus of the light chain by the second peptidase (between −1Arg and + 1Ala). The propeptide is essential for intracellular post-translational modifications and the proper function of FX. The light chain of FX contains 11 glutamic acid residues which are modified to γ-carboxyglutamic acid (Gla) domains. Ten residues are encoded by exon II and the last one is encoded by exon III. The Gla domain has a key role in the binding of Ca^{2+} ions and anchoring to negatively charged phospholipid membranes.

Table 12.1 Characteristics of *F10* gene

Exon	Size (bp)	Domain
1	70	Pre-pro-leader sequence (signal peptide) (aa-40 to −17)
2	161	Propeptide region containing Gla domain (aa-17 to +37)
3	25	Linking segment of the aromatic amino acid (aromatic stack) (aa38 to 46)
4	114	EGF-1 (aa46 to 84)
5	132	EGF-2 (aa84 to 128)
6	245	Connecting region and activation peptide (aa128 to 209)
7*	118	Catalytic domain (aa210 to 249)
8*	599	Catalytic domain (aa249 to 448)

bp base pair; *aa* amino acid; *Gla* γ-carboxyglutamic acid-rich; *EGF* epidermal growth factor; *Exons VII and VIII encode two active serine protease domains containing the residues for the catalytic triad (His236, Asp282, and Ser379)

Mature FX consists of two light (17 kDa) and heavy chains (45 kDa), covalently linked by a tripeptide residue (Arg-Lys-Arg) and a disulfide bond between Cys 89 and Cys124. The light chain consists of 139 amino acids organizing the Gla-rich domain (which contains 11 Gla residues from Ala1-Gla39, three α-helices A1–3 and one π-helix P1), a high proportion of aromatic amino acids segment (hydrophobic aromatic stack, residues Phe40-Lys45) and two epidermal growth factor (EGF)-like domains: EGF1 (Asp46-Phe84 forming two β-strands A-B) and EGF2 (thr85-Gly128 forming four β-strands A-D). Each EGF-like domain contains three conserved Gly and six Cys residues forming three unique disulfide bonds; these domains are important to maintain the correct conformation of FXa. The heavy chain consists of 346 residues forming a catalytic serine protease domain (254 residues forming 13 β-strands A-M, three minor α-helices, three 310-helices, and one π-helix) and a 52-amino acid activation peptide. In homology with other vitamin K-dependent factors and trypsin-like enzymes, the catalytic domain contains the highly conserved catalytic site—His236, Asp282, and Ser379. When FX is activated, the activation peptide cleaves (Fig. 12.1) [2–7]. A number of crystal structures for FX exist and have been used to model a number of the naturally occurring FX mutations [8].

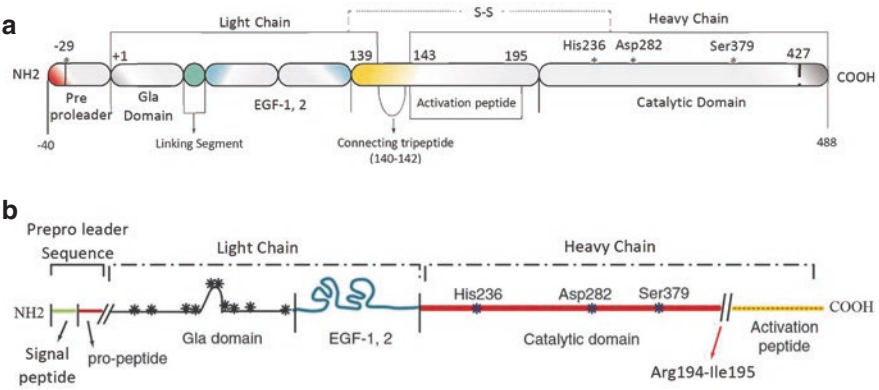

Fig. 12.1 (**a**) Factor (F) X polypeptide structure and its functional domains. The signal peptidase probably cleavages FX protein at residue −29 and the mature protein initiates from residue +1. The light chain consists of 139 amino acids (residues +1 to 139) containing Gla domain and EGF-1, 2 domains, while the heavy chain consists of 364 residues (143–448) containing activation peptide and catalytic domain in which activation peptide is located at residues 143–195. The position of connecting tripeptide (Arg-Lys-Arg) is between residues 140 and 142. Clotting FX is activated by cleavage at Arg194-Ile195. The second cleavage at residue 427 generates FXaβ. The catalytic site within the catalytic domain is formed by His236, Asp282, and Ser379. (**b**) Schematic of FX structure. The separated part indicates cleavage of the pre-pro-leader sequence. The Gla domain is shown with Gla residues and the active sites His, Asp, and Ser are shown in the catalytic domain. Two light and heavy chains are linked by a disulfide bond. The cleavage site of the activation peptide (yellow part) is indicated by the red arrow. *EGF* epidermal growth factor

12.3 Factor X Activation

Normally, there is a mixture of both zymogen and activated forms of FX in plasma with the zymogen form predominating. This balance shifts in favor of FXa concentration once the scissile peptide bond is cleaved between the light and heavy chains. The zymogen form of coagulation FX is cleaved at the Arg194-Ile195 peptide bond in the heavy chain releasing the 52-amino acid activation peptide and activated FXa. When activated, the N-terminus of the serine protease domain is remarkably redirected (a major difference between the zymogen and activated form of FX) and the new N-terminus of Ile195 inserts into the hydrophobic substrate-binding pocket within the catalytic domain by forming a strong salt bridge with Asp378. It is a required orientation to trigger the events promoting the FXa catalytic activity. The second cleavage of FX often occurs at the C-terminus (Lys427-Ser428) to produce FXaβ that has no significant functional hints [2, 4, 9, 10].

FX may be activated either in vivo or in vitro. Physiologically, FX is activated by FIXa (FIXa)/FVIIIa through the "intrinsic pathway" or by TF-FVIIa through the "extrinsic pathway." In-vitro FX can also be activated by Russell viper venom (RVV), a metalloproteinase isolated from the venom of the snake *Vipera russelli*. A complex of tissue factor (TF), FVIIa, Ca^{2+} ion, and appropriate phospholipid membrane is required for in vivo activation of FX and FIX (extrinsic pathway of coagulation cascade) (please refer to Chap. 1). Physiologically activation of FIX by TF/FVIIa complex is more important than direct activation of FX. Generation of FVIIa is amplified via a positive feedback loop by production of FXa. The GLa domain evokes the negative charge essential for Ca^{2+} binding and constructs the required conformation for binding to an anionic phospholipid surface. A variety of cell types such as fibroblast, monocyte-macrophage, and endothelial cells as well as tumor cells provide this anionic phospholipid membrane for activation. Naturally, the membrane of these cells is anticoagulant since phosphatidylserine (PS) and phosphatidylethanolamine (PE) are abundant in the inner leaflet while sphingomyelin and phosphatidylcholine (PC) are sequestered to the outer surface. Disruption and externalization of PS and PE following cellular injuries and activation of platelets provide the suitable binging surface for coagulation factors. Given that mammalian cells' membrane contains about 10% PS, recent studies displayed that in the presence of excess PE, only one molecule of PS is required for binding of FX Gla domain to the negatively charged membrane, indicating the significance of PS/PE synergy to develop membrane binding sites for clotting factors assembly. Conversion of FX to FXa also occurs through coagulation intrinsic pathway by the interaction of FIXa, FVIIIa, Ca^{2+} ion, and phospholipid membrane (platelet and/or endothelial cells) (Fig. 12.2). In vitro activation of FX may occur by Russell viper venom of the snake *V. russelli.* It contains a metalloproteinase which can directly activate FX without Ca^{2+} ion or phospholipid membrane [2, 3, 6, 10, 11]. The presence of all the constituents results in a tenfold decrease in Km for the reaction and a 1000-fold increase in kcat accelerating the generation of FXa by at least 10,000-fold relative to rates observed for FVIIa and Ca2+ alone.

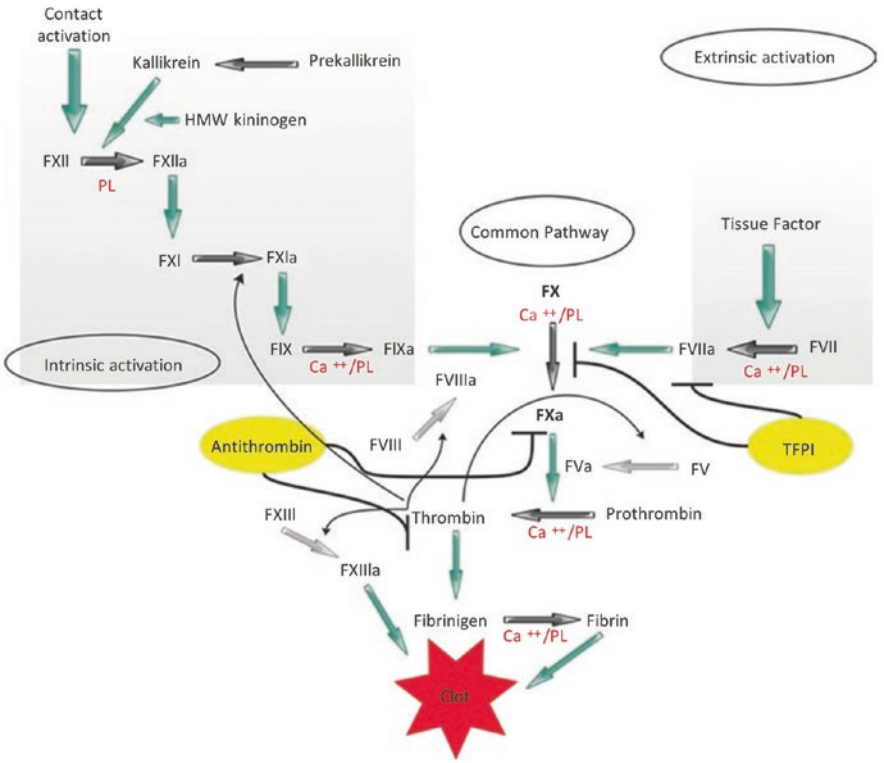

Fig. 12.2 Coagulation cascade. This illustration represents the role and activation of clotting factor (F) X in the coagulation cascade. FX is the first enzyme of the common pathway activated by a complex of TF/FVIIa/Ca^{2+}/PL through the extrinsic pathway and FIXa/FVIIIa/Ca^{2+}/PL via the intrinsic route. Then, a complex of FXa/FVa/Ca^{2+}/PL (prothrombinase complex) cleavages prothrombin to the thrombin subsequently leading to the cleavage of fibrinogen to fibrin. Finally, FXIIIa stabilizes the generated clot. FV, FVIII, FIX, and FXIII also are activated by thrombin. Activation of FX may be inhibited by tissue factor pathway inhibitor (TFPI) and antithrombin. TFPI is a pivotal inhibitor of the extrinsic pathway that in complex with FXa/TF/FVIIa inhibits their catalytic activity. Antithrombin also affects FXa and generates a stable inactive complex (please refer to Chap. 1). *TF* tissue Factor; *FVII* factor VII; *FVIIa* activated factor VII; *FX* factor X; *FXa* activated factor X; *FXII* factor XII; *FXIIa* activated factor XII; *FXI* factor IX; *FIXa* activated factor IX; *FXa* activated factor IX; *FVIII* factor VIII; *FVIIIa* activated factor VIII; *FV* factor V; *FVa* activated factor V; *FXIII* factor XIII; *FXIIIa* activated factor XIII; *TFPI* tissue factor pathway inhibitor; *PL* phospholipid

FXa could be generated independently of either TF/FVIIa or FIXa/FVIIIa, Ca^{2+} ion, and phospholipid membrane. For instance, in some malignant cells, cysteine proteinase may be involved in FX activation [3, 6].

FXa exerts a central role as a key thrombin activator in a way that one molecule of FXa can produce more than 1000 molecules of thrombin. A complex of FXa, FVa, and Ca^{2+} ion at a suitable phospholipid membrane (prothrombinase complex) can 280,000-fold accelerate prothrombin transition. This complex assembles on the

surface of platelets, lymphocytes, monocytes, neutrophils, and endothelial cells. The suggested model for the prothrombinase complex shows that, initially, FVa binds to the negatively charged phospholipid membrane through its light chain and provides a suitable receptor for FXa. In turn, FXa anchors to the membrane surface via Gla domains of the light chain on one side and interacts with FVa by the heavy chain on the other side. FVa then interacts with prothrombin through the heavy chain, independent of Ca^{2+} ions. Subsequently, prothrombin binds to the membrane surface by the formation of Ca^{2+} bridges (Gla residues) and later, following membrane assembly, the prothrombinase complex converts prothrombin to the active form, thrombin which promotes clot formation. FVa is a cofactor of FXa which enhances the catalytic efficacy of FXa, also the negatively charged phospholipid membrane is an appropriate surface that increases the local concentration of FXa, FVa, and prothrombin to accelerate the conversion of prothrombin to the active serine protease, thrombin. In addition to prothrombin, FXa has the potential of converting FV, FVII, and FVIII to their active form. Moreover, activation of FVII and FX is a reciprocal pathway, so that TF/FVIIa complex produces FXa, FXa next activates FVII and increases the level of FVIIa in a positive feedback loop [3], therefore more FXa brings more FVIIa which in turn enhances FXa.

12.4 Regulation of Factor X Activity

The principal regulators of FX activity (FXa) are antithrombin, TF pathway inhibitor (TFPI), and protein Z-dependent protease inhibitor (ZPI).

Antithrombin (III) FXa is inhibited by antithrombin. The latter forms a stable inactive complex that inhibits FXa. Inactive complexes of FX are then removed from the circulation by the liver. Low molecular weight heparin (LMWH) enhances the anti-Xa activity of antithrombin. Various sulfated glycosaminoglycans may accelerate the antithrombin activity.

Tissue Factor Pathway Inhibitor (TFPI) TFPI is the pivotal inhibitor of the coagulation extrinsic pathway. It binds to FXa in a 1:1 ratio and forms a quaternary complex with TF-FVIIa. The complex of TFPI/FXa/TF/FVIIa lacks TF/FVIIa catalytic activity thereby blocking the extrinsic pathway to thrombin generation (please refer to Chap. 10).

Factor VIII and Activated Factor X FXa is involved in the inactivation of FVIIIa. Therefore FXa is inhibited via a negative feedback loop [6].

Protein Z (PZ) A plasma vitamin K-dependent protein functions as a cofactor to enhance the inhibition of FXa by the serpin ZPI.

Pathophysiological conditions such as inflammation may reduce the levels of FXa inhibitors owing to degradation or devastated synthesis leading to an impaired anticoagulant pathway and unconstrained formation of FXa [12].

12.4.1 Factor X Gene

The human FX gene (*F10*) is 22 kb, mapping on the long arm of chromosome 13 at 13q34, 2.8 kb downstream of *F7*gene (Fig. 12.3) [13]. For the first time, the Davie laboratory isolated the *F10* gene in 1986 [14]. As there is significant homology in the *F10* gene and the other vitamin K-dependent clotting factors (*F9*, *F7,* and protein C), both in structure and organization, an evaluation from a common ancestral gene is suggested. The *F10* gene spread approximately 27 kb of genomic sequence. It contains eight exons and seven introns; each exon encodes a specific part of FX protein. The introns vary in size from 950 bp to 7.4 kb (Fig. 12.6) [5, 15, 16]. Exon I encodes the signal peptide; exon II codes the propeptide and γ-carboxyglutamic acid-rich (Gla) domain. Exon III codes a short linking segment of the aromatic amino acid (aromatic stack), exons IV and V encode two regions homologous to EGF, and exon VI codes activation peptide at the amino terminus of the heavy chain. Exons VII and VIII encode two active serine protease domains containing the residues for the catalytic triad (His236, Asp282, and Ser379) [5] (Fig. 12.4).

The *F10* cDNA consists of 120 bp encoding 40 amino acids pre-pro-leader sequence and 1344 bp coding 488 amino acids of the mature protein. There is a short 3′ untranslated region of 10 bp preceding the poly (A) tail. An obvious TATA box has not been identified at the 5′ end of the *F10* gene, but almost six different transcription initiation sites have been detected at the 5′ region like other TATA-less promoter genes. Analysis of the *F10* gene promoter shows that a 200-bp region upstream of the coding region is vital for its function. *F10* gene has been cloned by different groups. Isolated *F10* colons with a lack of 9-bp sequence encoding a tripeptide (Lys-Val-Arg) have been detected by different groups. At first, it was considered an artifact but later Tuddenham and Copper suggested that it is a result of the formation of a "semi-cryptic" splice site that led to alternative processing. Several polymorphisms also have been detected within the *F10* gene [3, 5].

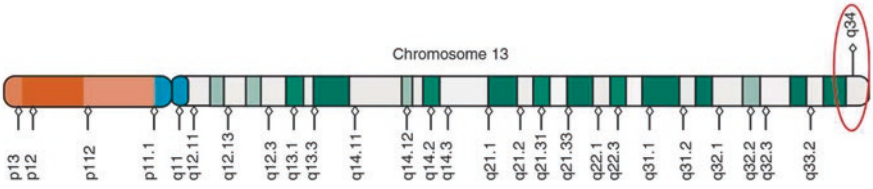

Fig. 12.3 Chromosomal location of *F10* gene. The *F10* gene is located in long arm of chromosome 13 at 13q34

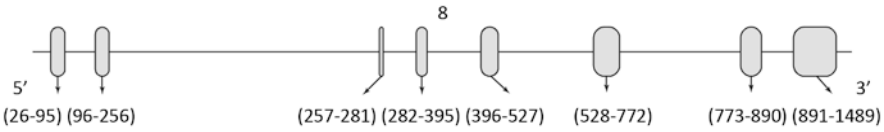

Fig. 12.4 *F10* gene organization. The *F10* gene consists of eight exons. The diagram shows the location of the first and last nucleotides of each exon

12.5 Congenital Factor X Deficiency (FXD)

Historical background of FXD is shown in Fig. 12.5.

The first clues of the FX function date back to **1904** when **Paul Morawitz** brought some evidence of the entity of a physiologic central activator of prothrombin in the coagulation system; it was termed **thrombokinase**. During the following 50 years, the first attempts to isolate and purify thrombokinase were carried out by **Haskell Milstone** in **1947–48**. Later congenital FX deficiency was identified by two independent groups in the 1950s [6, 15, 17]. First**, Telfer et al**. **1956** described menorrhagia and bleeding after dental extraction and tonsillectomy associated with an abnormal thromboplastin generation test and prolonged prothrombin time (PT) in a 22-year-old patient **(Power)**; then 1 year later, **Hougie et al., 1957** reported a 36-year-old man **(Stuart)** with prolongation of the activated partial thromboplastin time (APTT), abnormal thromboplastin generation test (TGT), and a prolonged Russel viper venom time (RVVT). At first, they thought that bleeding tendency in these patients was caused by FVII deficiency, but later they found that direct mixing of the patients' plasma with FVII-deficient plasma led to correction of prolonged prothrombin time (PT) yet aPTT, RVVT, and TGT are prolonged in the patient, while both aPTT and TGT are normal in patients with FVII deficiency highlighting the fact that there was an insufficiency in a clotting factor other than FVII. Hence, **Telfer and Hougie** named it the **Stuart-Prower** factor. Furthermore, **Duckert et al. in 1954** described a type of factor deficiency that was distinct from FVII and FIX shortage in a patient who received coumarin anticoagulants. In 1962 the new characterized factor was officially named **FX** (Fig. 12.5) [3, 6, 13, 17–19].

12.5.1 Prevalence/Inheritance

FX deficiency is inherited in an autosomal recessive manner equally affecting both genders. The estimated incidence of FX deficiency in homozygous form is 1:1,000,000. It is more frequent in areas with a high rate of consanguineous

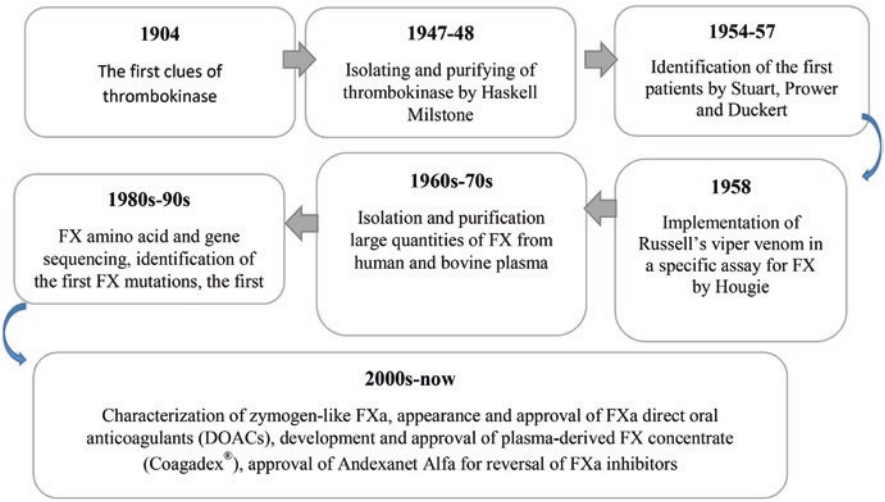

Fig. 12.5 Chronological blocks (timeline) of significant events in the history of FX discovery and development from the beginning until now (adopted from Rodney M.Camire [15] with some modifications)

marriages such as Iran where the frequency is reporetd to be 1:200,000~. The prevalence of heterozygotes is estimated as high as ~ 1:500 [18, 20–23]. FX deficiency accounts for 1.3% of patients with inherited coagulation deficiencies in Iran, 0.4% in Italy, and 0.5% in the UK. Heterozygous FX deficiency (carrier state) is often clinically asymptomatic [24] but can be symptomatic. Although compound heterozygotes have been reported, pedigree analysis frequently identifies consanguineous parents, supporting the presence of a homozygous defect. Indeed in the first case of factor X deficiency reported by Hougie et al., the father and mother of the index case were aunt and nephew. FX deficiency encompassed 8% of all rare bleeding disorders (Fig. 12.6). FX is activated through extrinsic and intrinsic coagulation pathways and has a pivotal role in the common pathway. In view that it is the key part of the prothrombinase complex, FX deficiency causes the impaired formation of the complex which leads to bleeding. Congenital FX deficiency presents with a wide variable bleeding tendency from asymptomatic in heterozygotes to life-threatening manifestation in some homozygous. Bleeding symptoms may occur as a result of insufficient enzymatic activity or interference of mutant gene product with the normal reaction of the coagulation pathway [6, 19].

12.6 Classification

FX deficiency could be simply classified based on the results of both immunological and functional (PT, APTT, and time-based RVV) laboratory assays [19] and highlights the interesting observation that a number of dysfunctional variants of FX show differing FX activities depending upon the method employed in their assay. Type 1 is described by a concomitant reduction in antigenic and functional levels of FX, whereas type 2 is demonstrated with a decrease in FX:C and normal or nearly normal antigenic level of FX (FX:Ag) [3]. The first classification of FX deficiency was established in 1969–1970 including type I or cross-reacting maternal negative (CRM-) (Stuart family), type II or CRM+ (Prower family), and CRM Friuli form. CRM-type is described by simultaneous reduction of both FX:Ag and FX:C levels because of abnormal synthesis or secretion of the protein, while CRM+ form is characterized by normal or nearly normal FX:Ag and reduction of FX:C. For years a wide range of *F10* gene defects has been identified that affect only extrinsic or intrinsic coagulation pathways. So, it became clear that type II or CRM+ is a heterogeneous type with various presentations in different families. Therefore, this classification was insufficient to describe all types of FX deficiency. Establishing molecular biology techniques studying the *F10* gene in the last two decades leads to the identification of several mutations in association with different types of FX

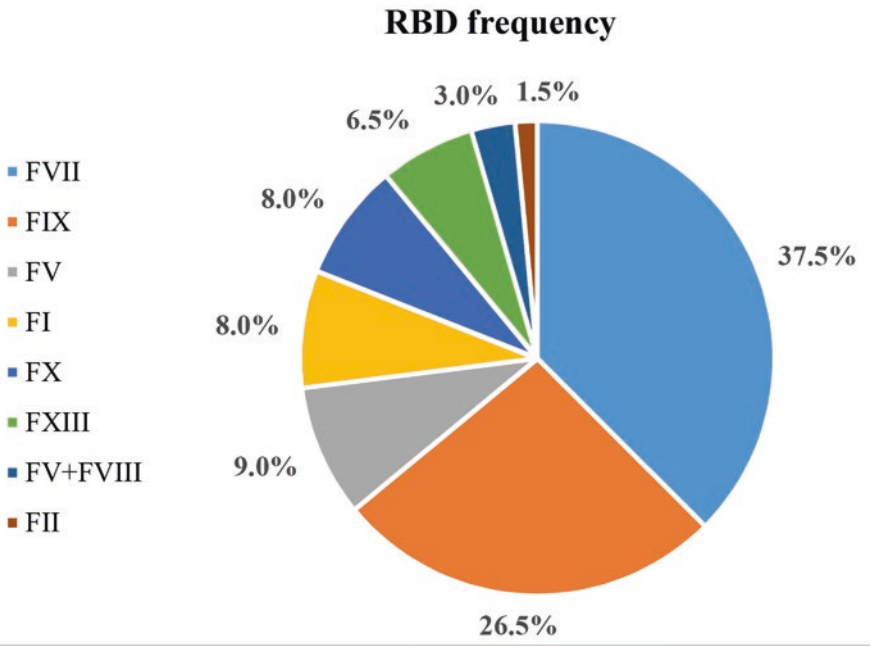

Fig. 12.6 Relative percentage of rare bleeding disorders. (**Worldwide distribution of RBDs derived from the WFH and EN-RBD**)

Table 12.2 Classification of factor X deficiency

Type		Clotting assay			Chromogenic assay	Antigenic assay (FX:Ag)
		PT	aPTT	RVVT		
I	CRM negative	↓	↓	↓	↓	↓
II	CRM positive	↓	↓	↓	↓	N or nearly N
III	Friuli like	↓	↓	N	↓	N or nearly N
	Padua like	↓	N	N	↓	N or nearly N
	Melbourne like	N	↓	N	↓	N or nearly N
	–	↑	↑	↑	H	N or nearly N
IV[a]	–	↓	↓	↓	↓	↓

CRM cross-reacting material; *PT* prothrombin time; *aPTT* activated partial thromboplastin time; *RVVT* Russell viper venom time; *FX* factor X; *FX:Ag* factor X Antigen; *N* normal, *H* high
[a] FX deficiency concomitant deficiency of other clotting factors especially factor VII

deficiency. According to molecular studies, FX deficiency could be classified based on the type of mutations, e.g., nonsense, missense, insertion, deletion, etc. There is also a classification based on of the sites where mutations occur including promoter, Gla domain, EGF domain, activation peptide, and the catalytic domain. Despite these investigations, no obvious correlation has been found between genotype and phenotype in FX deficiency. Mutations in the same area may accompany different phenotypes, whereas the same phenotypes may be due to different mutations. The only exception is variants in the Gla domain of the light chain that present similar phenotypes in different cases.

In conclusion, because of the great heterogeneity in genotype and phenotype of FX deficiency, it seems that a more suitable classification could be achieved by using immunological, functional, and clotting assays as well as molecular biology studies. According to these considerations, the following classification has been proposed by Denson, Fair, Edgington, and then Girolami et al., 2011 [19] that is more acceptable and practical. It is shown in Table 12.2.

- *Type I*: CRM negative or Stuart-like (the classic form of congenital FX deficiency)
- *Type II*: CRM positive or Prower-like, inert protein
- *Type III*: CRM positive with distractive protein that is categorized in four levels:
  - CRM positive *Friuli-like*: Defects in all clotting assays except for RVVT
  - CRM positive *Padua-like*: Defects only or mainly in the extrinsic pathway
  - CRM positive *Melbourne-like*: Defects only or mainly in the intrinsic pathway
  - *Defects with contradiction in the chromogenic assay*
- *Type IV*: concurrent deficiency in FX and other coagulation factors mostly FVII in the effect of abnormality in chromosome 13 (Table 12.2) [19, 25].

It is worth noting that the pattern of clotting assays is variable among different patients based on the involved area of the *F10* gene and protein. For instance, in 2008 Girolami et al. described some cases of mutations in the catalytic domain and light chain C-terminal of FX protein which affect mainly the intrinsic pathway.

They also reported some variants in the Gla domain of the light chain, EGF domain, and catalytic domain that predominantly affect the extrinsic pathway [3, 6, 25].

12.7 Clinical Manifestations

Although FX deficiency produces a variable bleeding tendency, patients affected by severe FX defects tend to be the most seriously affected among those with rare bleeding disorders (RBDs). Clinical features of the patients with FX deficiency are associated with the plasma level of FX protein, but the correlation between the plasma level of FX and bleeding tendency is not as strong as in hemophilia A and B [13, 26]. On the basis of a one-stage prothrombin-based FX assay using rabbit thromboplastin, patients with FX deficiency have been classified into three grades of severity and correlated with the bleeding phenotype (Table 12.3) [13, 14, 18, 27].

Generally, regardless of the severity of the disease, mucosal bleeding, particularly epistaxis, and easy bruising are the most common symptoms of congenital FX deficiency. Common symptoms reported at all severity levels include epistaxis and menorrhagia. Symptomatic patients with clotting factor deficiencies are usually homozygous or compound heterozygous; however, there are some reports of significant post-dental extraction and postpartum bleeding in about one third of heterozygotes of FX deficiency who did not receive prophylactic replacement therapy [26].

Less severely affected patient may bleed only after some challenge to the hemostatic system, e.g., trauma, surgery. Some cases are identified incidentally during routine screening or family studies (Table 12.3).

Herrmann et al. analyzed clinical manifestations of 102 patients with FX deficiency from Europe and Latin America with *F10* gene mutations in 2006. The most severe bleeding symptoms among 42 symptomatic patients (homozygous, heterozygous, and compound heterozygous) were ICH, GI bleeding, and hemarthrosis which were observed in cases with FX:C <2%. However, there were differences among the bleeding patterns of the patients due to corresponding genotypes and the type of mutation. Among clinically symptomatic subjects, 13% were heterozygous, mainly

Table 12.3 Classification and clinical presentations of patients with congenital factor X deficiency

Severity	Factor X level	Clinical features (predominantly)
Mild	6–10 U/dL	Usually asymptomatic and identified based on family studies or occasionally on routine laboratory evaluations but can present with easy bruising, epistaxis, and menorrhagia [3, 13, 14]
Moderate	1–5 U/dL	Mucosal bleeding and post traumatic or post-surgical bleeding [3, 13, 14]
Severe	<1 U/dL	Mucosal bleeding, CNS, umbilical cord bleeding during the neonatal period, hemarthrosis, hematoma, hematuria, and GI bleeding [1, 5, 13, 14, 26]

CNS central nervous system; *GI* gastrointestinal

Table 12.4 Prevalence of bleeding symptoms in patients with congenital factor X deficiency

Symptom	Herrmann et al. (n = 42) (%)	Peyvandi et al. (n = 32) (%)	Acharya et al.		Anwar et al. (n = 20) (%)
			Homo (n = 19) (%)	S Hetero (n = 15) (%)	
Epistaxis	36	72	45[a]	75[a]	35
Gum bleeding	31	–	–	–	35
Easy bruising	55	–	–	–	45
Hematoma	43	66	27	–	–
Hematuria	7	25	–	–	5
Hemarthrosis	33	69	–	–	5
GI bleeding	12	38	4–9	12	10
CNS bleeding	21	9	15	–	–
Umbilical cord bleeding	–	28	–	–	15
Post-circumcision bleeding	–	–	–	–	30
Menorrhagia [b]	71	505	–	–	10
Post-operative hemorrhage	–	–	4	13	–

Homo homozygous; *S Hetero* symptomatic heterozygous; *GI* gastrointestinal; *CNS* central nerves system

[a] Skin and mucus membrane bleeding

[b] Women in reproductive age

with mucocutaneous hemorrhage. It could occur either due to insufficient enzymatic activity of the FX wild type or inhibition of one reaction in the clotting cascade by FX mutant protein [28].

The most common bleeding symptom among 32 Iranian patients, regardless of the disorder severity, was epistaxis (72%). Other clinical manifestations including GI bleeding and hematuria were mainly observed in the cases with an undetectable level of FX. In this study, umbilical stump bleeding was found unexpectedly in nine patients occurring at the time of stump detachment (7–10 days after birth). A direct correlation between FX level and bleeding diathesis was observed in this study (Table 12.4) [18, 28–30].

The biggest four studies with a total of 128 patients show the wide bleeding phenotype of FXD as shown in Table 12.4 with epistaxis being the commonest noted bleeding symptom.

The thrombogram has been used to investigate the relation between FX levels in an attempt to establish at what level of FX normal thrombin generation is achieved. The thrombogram in rare inherited coagulation disorders: its relation to clinical bleeding. In FX deficiency, half-normal endogenous thrombin potential (ETP) is seen at an FX:C of 5 U/dL. Ten individuals with FX deficiency (FX levels 1–50% of normal) were studied and when functional FX activity was <10 the parameters of the thrombogram (lag time and peak height) were markedly abnormal. The ETP was

similar irrespective of the method of activation of FX (intrinsic or extrinsic pathway). In patients with FX activity between 10 and 50 U/dL only the lag time and peak height were abnormal and the ETP remained within normal limits. These patients had no bleeding even after trauma and this suggests the threshold range of FX required to obtain normal thrombin generation time is approximately10% of normal.

12.8 Diagnosis

Similar to other rare bleeding disorders the diagnosis of FX deficiency includes taking detailed history containing family history, assessment of the patient's bleeding phenotype and clinical evaluation as well as first-line screening tests, and specific coagulation tests.

12.8.1 History Taking and Clinical Evaluation

A detailed history taking including family history and pedigree is mandatory. Bleeding phenotype and severity are assessed using the ISTH-BAT and clinical bleeding severity score. Clinical assessment should include full clinical examination including determination of site, type, and duration of bleeding, treatment received as well as other co-morbidities [31] (Table 12.5).

12.8.2 First-Line Screening Tests (Primary Assays)

Following clinical assessment, laboratory screening should be performed. Complete blood count (CBC) and peripheral blood smear examination are necessary to determine any abnormality in the number and morphology of platelets in a bleeding episode in an undiagnosed patient. PT, aPTT, mixing study, and thrombin time (TT), as well as RVVT, should be established in a case suspected of having FX deficiency. FX has a pivotal role in both intrinsic and extrinsic coagulation pathways so prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT)

Table 12.5 Categories of clinical bleeding severity

Clinical bleeding severity	Definition
Asymptomatic	No documented bleeding episodes
Grade I bleeding	Bleeding that occurred after trauma or drug ingestion
Grade II bleeding	Spontaneous minor bleeding*:* bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis, and menorrhagia
Grade III bleeding	Spontaneous major bleeding: hematomas, hemarthrosis, CNS, GI, and umbilical cord bleeding

which corrects (unless an inhibitor is present) in a 50:50 mix with normal plasma [3, 18, 31–33].

It is noteworthy to mention that, a number of patients with FX deficiency harbor mutants that affect just extrinsic coagulation pathway exhibiting prolonged PT and normal aPTT (type III, Padua-like).On the contrary, other patients harbor mutations in which only intrinsic pathway is affected and they have a prolonged aPTT and normal PT (type III, Melbourne-like). There are also reports of some variants in the *F10* gene that represent a normal RVVT (type III, Friuli-like).

12.8.2.1 Russell Viper Venom FX Assay

The Russell viper venom (RVV) FX assay is designed based on a special component of some snakes' venoms. The family of *Viperidae* snakes produces a specific venom with the ability to influence the hemostatic system by boosting or impeding platelet aggregation, blood coagulation, and fibrinolysis. The venom's protein content includes two types of proteases, snake venom serine protease (SVSPs) and snake venom metalloprotease (SVMPs) that can promote coagulation cascade by converting some of the clotting factors to their active form. SVSPs exclusively activate FV, while either SVSPs or SVMPs can activate FX and prothrombin. The SVMPs in *Daboia russelii* venom cleaves a specific peptide in the N-terminal domain of the FX heavy chain and activate it directly. Therefore, RVV starts up the common pathway of the coagulation cascade. Prolonged RVVT may be associated with a deficiency in FX, FV, prothrombin, and fibrinogen [18, 32], although, by using FX-deficient plasma as substrate, this assay would be specific for FX deficiency [13, 18, 32, 34].

12.8.2.2 The Principle of the Russell Viper Venom FX Assay

After preparing serial dilutions of standard and patients plasma, an equal volume of each dilution is mixed with FX-deficient plasma and incubated at 37 °C. The RVV-platelet substitute is next added and incubated at 37 °C again. Then the clotting time is recorded following the addition of the pre-warmed Cacl2 (Fig. 12.9). At last, the results of clotting time are plotted on logarithmic paper [2, 3, 35–38].

Interpretation RVVT results are interpreted similar to the PT-based FX assay. Note that RVVT is normal in patients with type III (Friuli-like) FX deficiency (Fig. 12.7).

12.8.3 Specific Coagulation Assays

If a patient is suspected of having FX deficiency based on first-line screening tests, more specific analyses should be considered in the next step. There are various methods for the measurement of both antigenic and functional levels of FX. Plasma levels of FX:Ag may be determined by several immunological assays such as

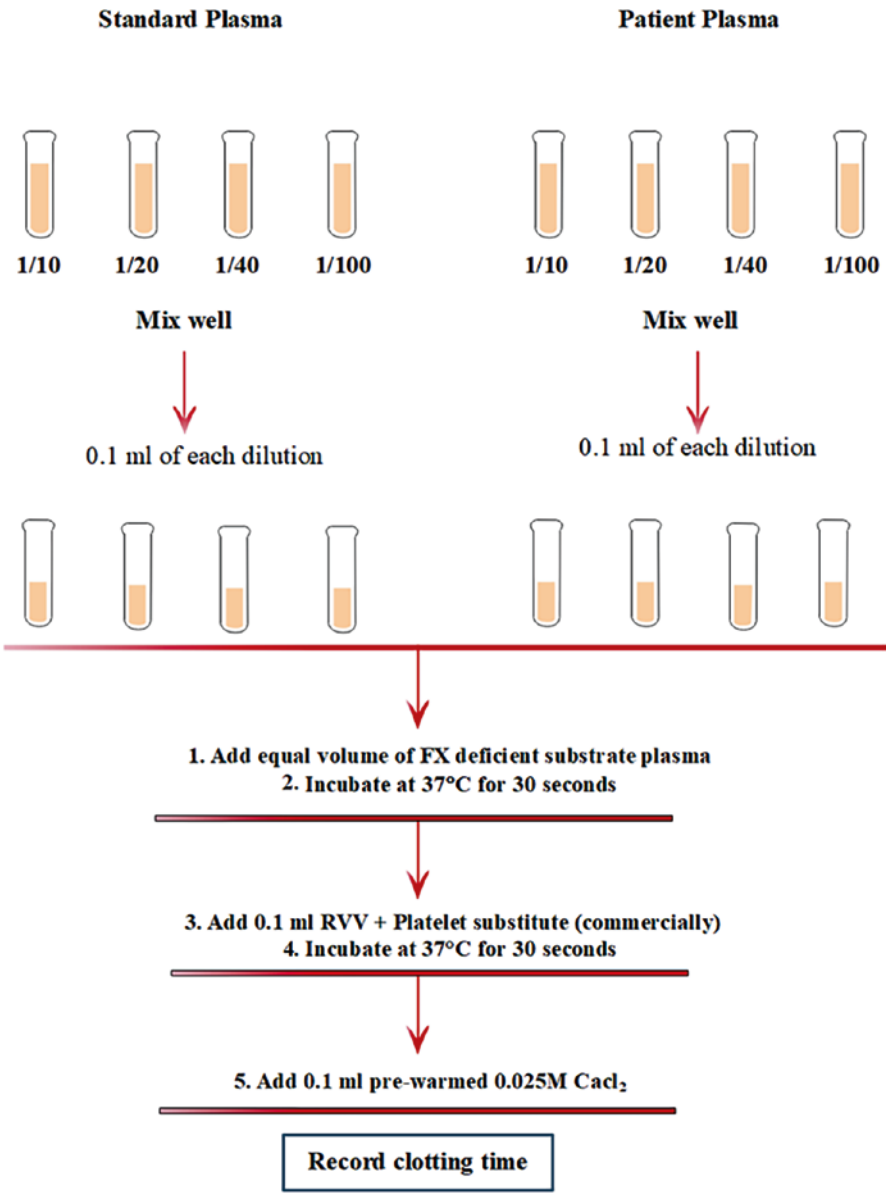

Fig. 12.7 The principle of Russell viper venom (RVV) FX assay

electro-immunoassay, immunodiffusion, radioimmunoassay, antibody neutralization, and laser nephelometry as well as enzyme-linked immunosorbent assay (ELISA) (Figs. 12.8, 12.9, and 12.10).

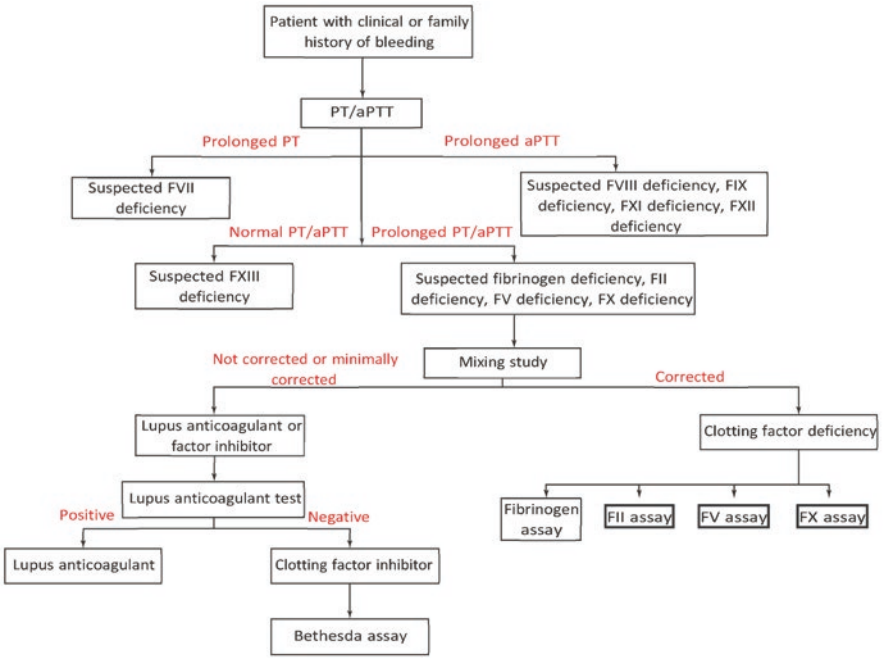

Fig. 12.8 An algorithm approach to diagnose congenital factor (FX) deficiency. To evaluate a patient presenting clinical or family history of bleeding, screening tests such as PT and aPTT are the first step. Prolonged PT with normal aPTT is suspected to FVII deficiency (extrinsic pathway), while normal PT with prolonged aPTT may be due to FVIII deficiency, FIX deficiency, FXI deficiency, or FXII deficiency (intrinsic pathway). Normal PT and aPTT along with bleeding symptom may be suspected to FXIII deficiency or other bleeding disorders that further specific assays are required. In presence of prolonged PT and aPTT, deficiency in fibrinogen, FII, FV, or FX is suspected (common pathway). Then the mixing study by using equal volume of normal and patient plasma could be performed to determine presence of an inhibitor or deficiency in the coagulation factors. Uncorrected or minimally corrected mixing test should be checked for the presence of lupus anticoagulant or coagulation factor inhibitors, while corrected mixing study is an evidence for one or more than one factor deficiency. In the next step, specific assays for the coagulation factors in common pathway are required. *PT* prothrombin time; *aPTT* activated partial thromboplastin time; *FII* factor II; *FV* factor V; *FVII* factor VII; *FVIII* factor VIII; *FIX* factor IX; *FX* factor X; *FXI* factor XI; *FXII* factor XII; *FXIII* factor XIII

12.8.3.1 Functional Assays

- One-stage FX assay (PT-based).
- One-stage FX assay is one of the most common methods to determine precise FX:C level in plasma. It is based on using FX-deficient plasma as substrate in mixing with normal or patient plasma. PT is performed before and after diluting of FX-deficient plasma with patient plasma and the correlated ratio of the clotting time is calculated [13, 18].

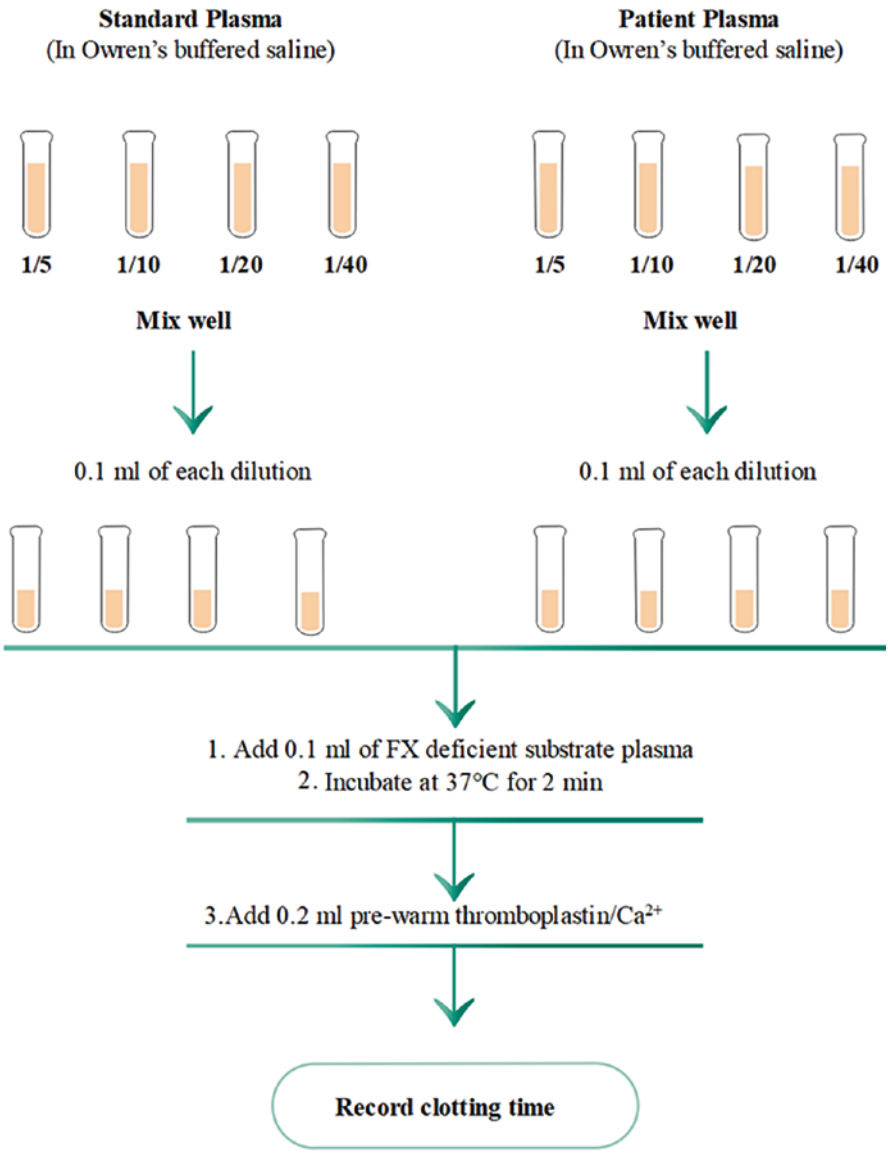

Fig. 12.9 The principle of one-stage FX assay (PT based). Serial dilutions of the standard and test plasma are prepared. The equal volume of each dilution is mixed with substrate plasma. Following the addition of the activating agent, the clotting time is recorded. The FX activity level is determined based on the prepared standard curve

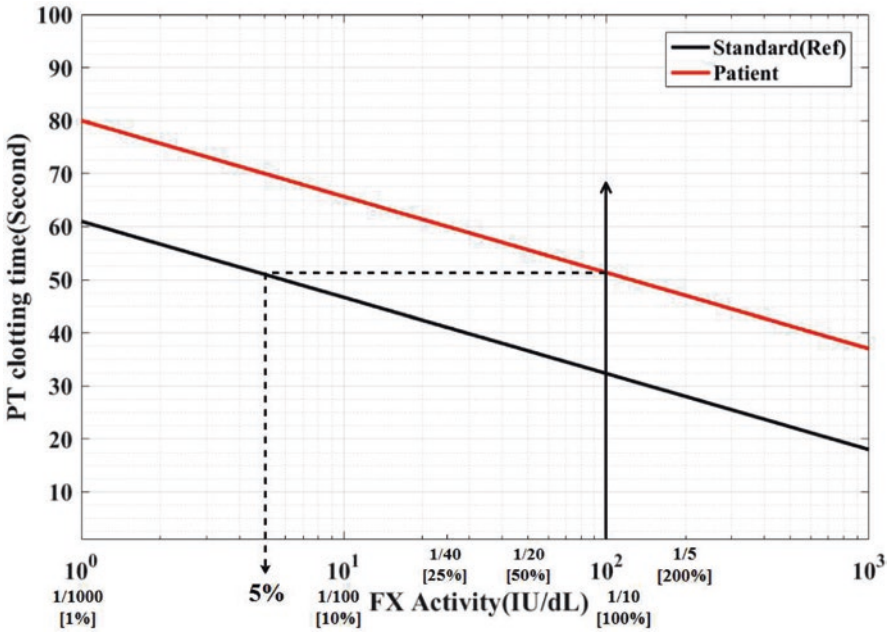

Fig. 12.10 The standard and patient curve of one-stage FX assay (PT based). For determining FX activity level in a patient's specimen, from 1/10 standard point (FX:C 100%), a vertical line is drawn to discontinue the patient's curve. Then, the horizontal line is drawn to intersect the standard curve. This new point shows the FX activity level of the patient specimen (FX:C 5%)

12.8.3.2 The Principle of the One Stage PT-Based FX Activity Assay

First, four dilutions of the patient and standard plasma in Owren's buffered saline at 25 °C (1/5, 1/10, 1/20, and 1/40) are prepared and an equal volume of each dilution is mixed with FX-deficient substrate plasma and incubated at 37 °C. Then, the pre-warmed thromboplastin containing calcium is added to each dilution and immediately the clotting time is recorded. Each standard and patient dilutions should be performed in duplicate and included the blank (Fig. 12.11). Finally, the clotting times of the standard and patient are plotted on logarithmic paper. The Y-axis represents the clotting time and the X-axis represents the FX activity level (U/dL) on the curve. All points of standard and patient are connected by drawing a line with a downward slope. The 1/10 standard dilution is arbitrary considered as the value of 100%. Based on 1/10 standard point, FX activity level in the patient's specimen is determined (Fig. 12.12) [14, 39, 40].

Interfering variables Disproportionate amount of anticoagulant to blood, not reaching the temperature of the reagents to 37 °C, and presence of inhibitors may interfere with the test.

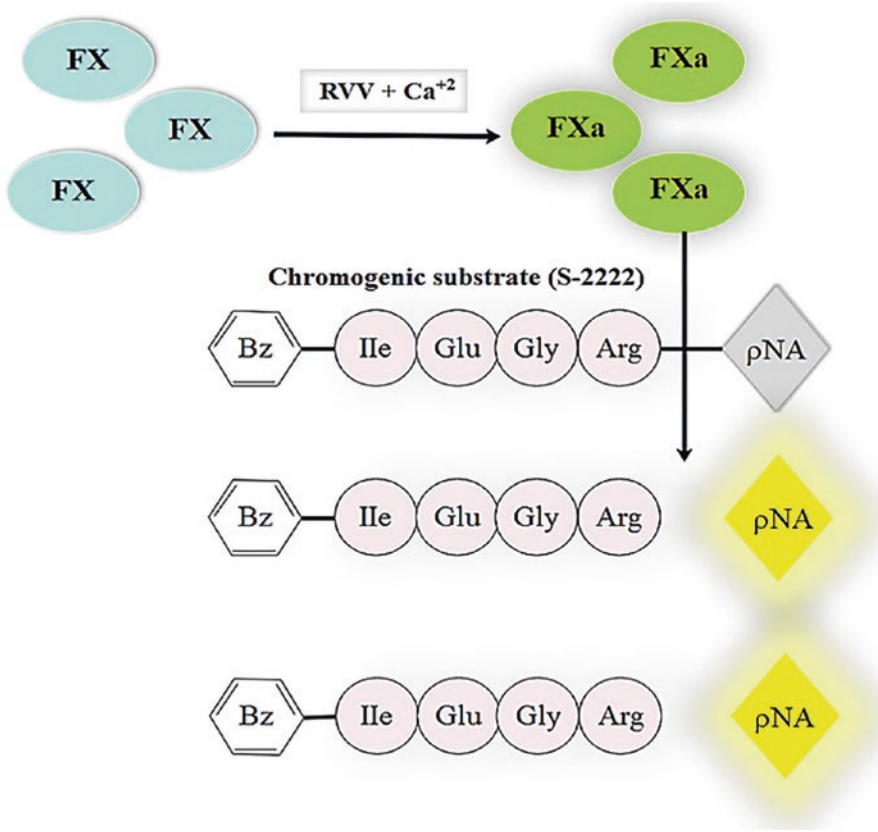

Fig. 12.11 The two-stage mechanism of the chromogenic assays of FX. First, activation of plasma FX to FXa by Russell viper venom and calcium. Second, the generated FXa proteolysis the chromogenic substrate (S-2222) and releases a yellow para-nitroaniline (pNA) chromophore

12.8.3.3 Chromogenic Assays

Chromogenic assay of FX (CFX) is another trustable quantitative method to assess plasma FX:C level in citrated plasma samples using a specific chromogenic substrate. The method is performed based on the two-stage assay. First, CFX is initiated by activation of FX by RVV and calcium, and then aFX proteolyzes a FXa-specific chromogenic substrate (S-2222 or spectrozyme FXa) resulting in the release of a yellow para-nitroaniline (pNA) chromophore (Fig. 12.13). The maximum absorbance of the generated color is next detected by spectrophotometer at 405 nm wavelength, the color intensity is directly proportional to the plasma FXa enzymatic activity. More activity levels of FX result in more FXa, so the color will be more intense [2, 3, 35]. It is important to know that because of nonspecific nature of the chromogenic substrate, the FX level may be estimated higher in some cases [13].

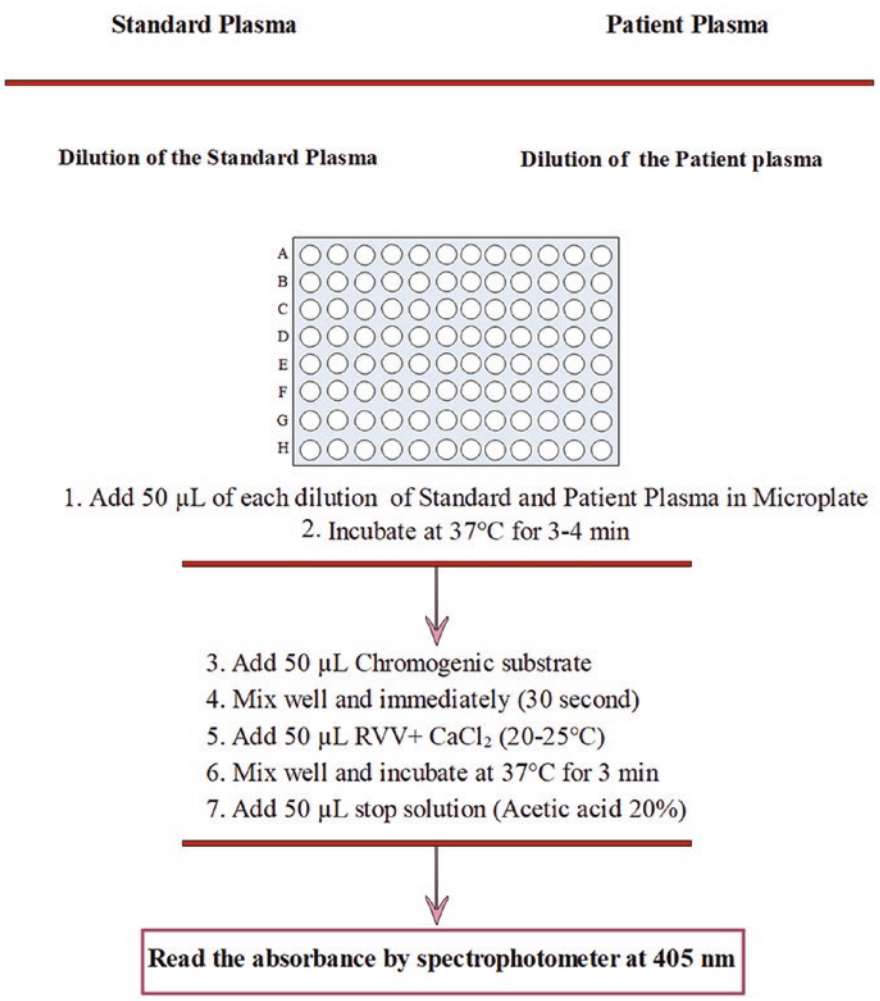

Fig. 12.12 The principle of the chromogenic assay of FX. Each standard and patient dilutions should be performed in duplicate and averaged

12.8.3.4 The Principle of the Chromogenic FX Assay

At the onset, the dilutions of the standard plasma are prepared. Afterward, each volume of prepared dilutions of the standard plasma or patient plasma is added to the microplate wells to start the assay. After incubation at 37 °C, the chromogenic substrate is added and mixed well. The RVT+ Cacl2 is then added and incubated at 37 °C. Subsequently, the reaction is stopped by using the acidic solution and the maximum absorbance of the standard and patient plasma is measured by spectrophotometer at 405 nm (Fig. 12.14). Finally, the standard curve is drawn by using the standards absorbance values against the FX concentrations. Based on the absorbance of the patient plasma, the FX concentration of the patient is determined on the

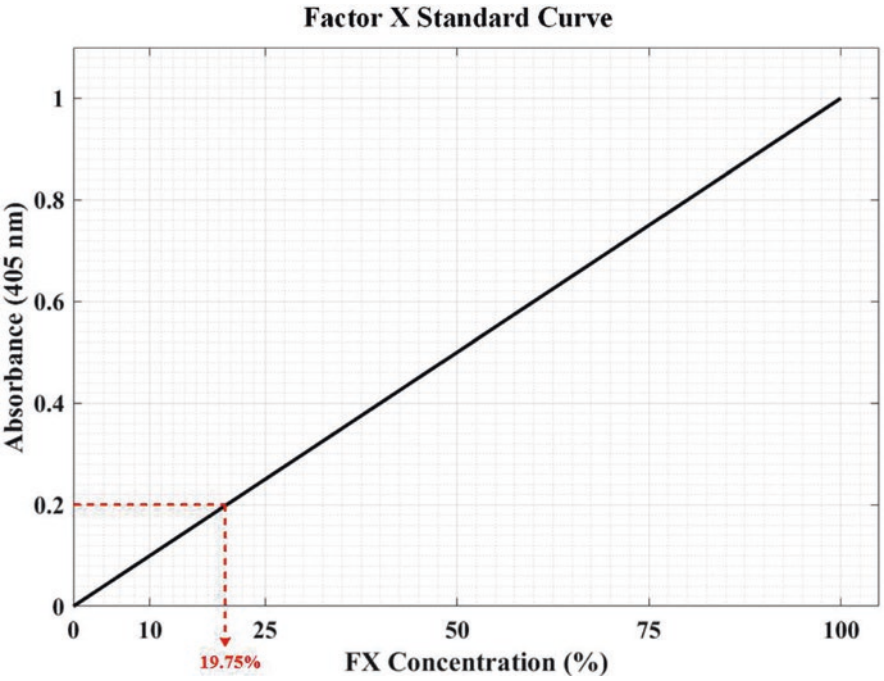

Fig. 12.13 The FX standard curve of the chromogenic assay. The standard curve is drawn using the standard absorbance values against the FX concentrations and based on the absorbance of the patient (OD = 0.2), the FX concentration of the patient is determined (FX:C 19.75%). Each standard and patient dilutions should be performed in duplicate and averaged

standard curve (Fig. 12.15). Each standard and patient dilutions should be performed in duplicate.

Interfering variables All samples should be mixed well to form a homogeneous reaction mixture. Extreme shaking of the specimens causes the deposition of protein in the foam. Observing the time and temperature of incubation is very important. The icterus, hemolysis, and lipemic plasma may interfere with absorbance readings. In these cases, the same patient's plasma blank should be used.

12.8.3.5 Immunological Assays

Various immunological assays including radioimmunoassay, laser nephelometer, immunodiffusion, antibody neutralization, and electro immunoassay as well as enzyme-linked immunosorbent assay (ELISA) have been used for the detection of congenital FX Deficiency. *Radioimmunoassay* is a more objective method that measures very low plasma levels of FX antigen. It is noticeable that other plasma proteins do not interfere with this technique. *The laser nephelometer* is a rapid method in comparison to other immunological assays and may be used for the rapid evaluation of FX antigen. The possibility of several plasma testing in a batch is another

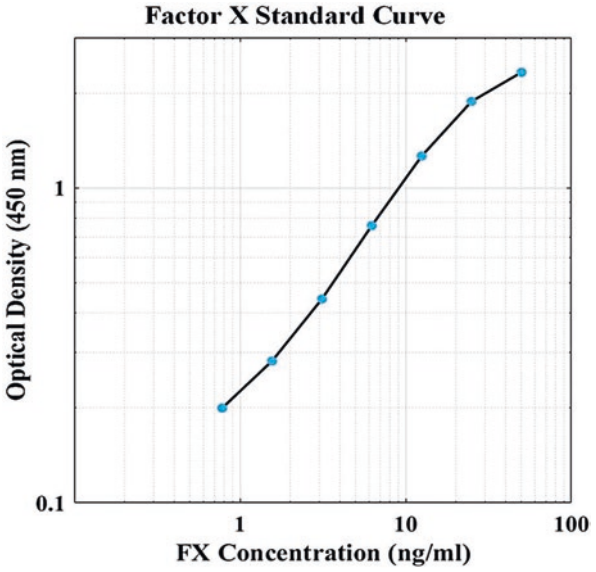

Fig. 12.14 The standard curve of FX antigen assay. The standard curve is plotted based on the optical density (OD) of the standards against FX antigen. The patient concentration of FX antigen is determined by the OD of patient plasma on the standard curve

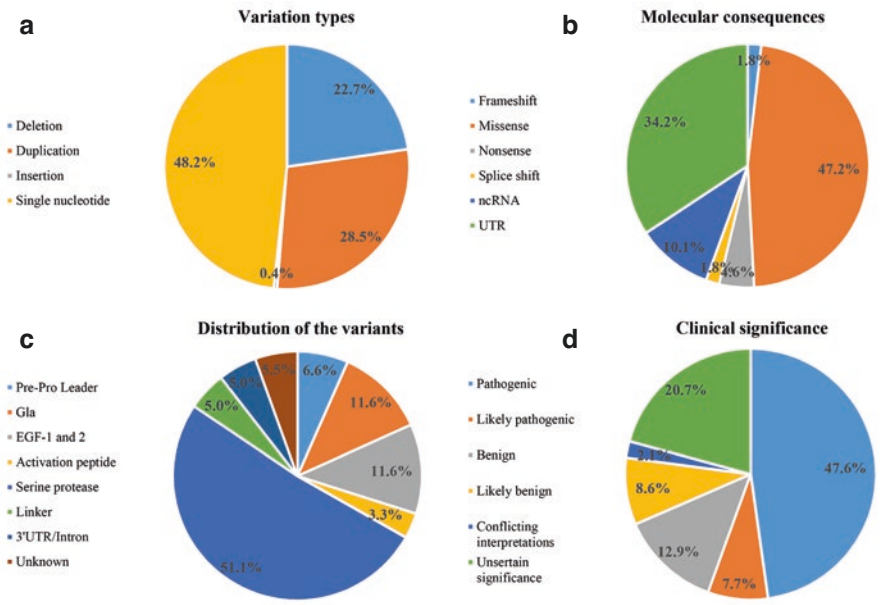

Fig. 12.15 Distribution and clinical significance of the recognized 180 mutations within the *F10* gene. The (**a**–**d**) charts represent relative frequency of the variations types, molecular consequences, and relative distribution of the variants across the protein domains as well as the clinical significance of the variants identified within the *F10* gene, respectively

advantage of this assay, though it needs a relatively large amount of antiserum. *ELISA* is one of the most commonly used immunological assays.

12.8.3.6 The Principle of the Enzyme-Linked Immunosorbent Assay (ELISA)

In ELISA assay, a polyclonal antibody against FX is coated at the bottom of the microplate wells. The standard and patient's plasma are applied and FX binds to the coated antibodies. After washing the microplate, unbound antibodies and materials are removed, a peroxidase conjugated antibody is added to bind to the captured antigen. After washing, a TMB substrate is added and the generated color is measured at 450 nm. Finally, the standard curve is plotted based on optical density (OD) of standards against standards concentrations of FX antigen (commercial standards). The patient concentration of FX antigen is determined by OD of the patient plasma on the standard curve (Fig. 12.14).

Interpretation The FX:Ag is reduced in *Type I* and *IV* of FX deficiency but type *II* and *III* with dysfunctional FX protein (CRM positive) have normal or nearly normal FX: Ag levels [3, 37, 38, 41].

FX antigen levels are reduced to approximately 50% of normal in patients being treated with vitamin K antagonists, e.g. warfarin although FX activity is lower [42]. The reduction in FX antigenic levels in such patients may reflect an increased catabolism of the acarboxylated form or to a reduced secretion by the hepatocyte. In addition to routine coagulation assessments, using other processes including *thrombin generation* and *fibrin polymerization* as well as *thromboelastography* to evaluate the total blood coagulation pathway dynamically is useful in the diagnosis of FX deficiency.

It is noteworthy that the reliability of results of FX assays depends on both preanalytical and analytical variables for example amount and type of anticoagulant, sampling, centrifuging of the sample, preservation of reagents and instruments as well as using suitable quality controls in analytical phase [14].

Anti-FXa assay is a chromogenic method monitoring the level of low molecular weight heparin (LMWH). LMWH binds to antithrombin specifically and more effectively inactivates FXa than thrombin. The remaining FXa in the patient's plasma cleaves a chromogenic substrate and produces color. The intensity of the produced color is measured by a spectrophotometer. The intensity of color is inversely associated with the amount of LMWH. It should be noticed that in the case of renal failure or due to contamination of the sample with heparin, the level of anti-Xa may be overestimated. This method is also appropriate to monitor direct oral FXa inhibitors if a suitable standard curve is available [43].

12.9 Important Points

1. Immunological assays measure only antigenic levels of FX, so cases with dysfunctional FX protein without any reduction of antigenic level may be missed. During warfarin therapy, both antigenic and activity levels of FX are decreased to about 50%, although the functional levels may be lower [13].
2. It is noticeable that the FX level in newborns and infants under 6 months is lower than in adults. Therefore, FX assay results should be calculated according to age- and gestational age-matched reference ranges. The FX level in healthy term newborns ranges from 0.12 to 0.68 IU/mL and increases gradually during the first 6 months.
3. FX level can be affected by liver disease and vitamin K deficiency so it is necessary to rule out acquired causes of FX deficiency. On the other hand, vitamin K deficiency in preterm or term neonates may complicate the diagnosis of FX deficiency, especially in cases with mild deficiency. Therefore, a re-assessment should be done after replacement therapy with vitamin K and also at 6 months of age [13, 18].
4. Human (15/102) is a new reference reagent for FXa which has established by WHO Expert Committee on Biological Standardization in October 2017. Each vial has a potency of 6.7 units. Currently, several laboratories use an existing non-WHO Reference Material (75/597) for the measurement of FXa and calibration of local standards. It is a bovine FXa reference material provided by the National Institute for Biological Standards and Control (NIBSC. Since it was not formulated by NIBSC and there was no information about the uniformity or stability of the content of the vials using it can be inaccurate. On the other hand, due to noticeable differences between the coagulation and chromogenic activity of bovine and human FXa, using a bovine standard to assess human FXa can cause discrepancies in the assay [44].

12.9.1 Molecular Diagnosis

12.9.1.1 F10 Gene Mutations and Its Clinical Significance/ Genotype Phenotype Correlation

During the last two decades, several *F10* gene mutations have been identified and the majority were unique and restricted to special families. DNA amplification and direct sequencing mostly are used to find underlying mutations. Therefore, establishing a genetic diagnosis method is challenging and costly, identification of recurrent variants of each population is more practical and also is helpful in reducing the use of direct sequencing, but it is not applicable because in most families the mutations are unique.

FX has a leading role in the coagulation cascade common pathway as absolute lack of FX seems to be incompatible with life. Some surveys on ***F10* gene** knockout mice have proven that the complete absence of FX is lethal for mice, but it is different in humans. Patients with the undetectable activity of FX as a result of

null mutations can survive, although they are dependent on constant replacement therapy. However, mice with FX activity levels of 1–3% of normal show a complete rescue of lethality. In **2019**, **Ferrarese et al.** investigated the structure and function of FX in a neonate who had almost complete FX deficiency presenting with life-threatening ICH due to underlying molecular defects of *Trp421Ter (missense, c.1382G>A) and Leu211Pro (nonsense, c.752 T>C)*. Interestingly, the trace level of FX:C was sufficient to arrest a lethal phenotype, though the patients needed regular prophylaxis because of life-threatening breakthrough bleeds. Moreover, maternal transferring of a trace amount of FX might be effective for embryonic survival. The variants of *Glu29Lys and Phe31Ser in the Gla domain* are other examples. They are not potentially severe defects, but some situations such as the deficiency of vitamin K and required cofactors during pregnancy causing imperfect carboxylation of the Gla domain and FX binding defect can compromise the patient's condition. This concomitant presence of congenital and acquired deficiency of FX can be prenatally lethal [3, 5, 45]. There is no hot spot region in the *F10* gene, and the number of mutations occurring in each exon relatively is proportional to its length; however, most mutations happen in exon 8 [3, 14, 16]. Almost all types of mutations such as deletion, nonsense, missense, splice site, and frameshift have been reported in the ***F10* gene** in which *missense single-point mutations, mostly localized in Gla and catalytic domains (exons 2, 7, 8)*, are the most reported *F10* gene lesions (Fig. 12.15).

According to the reports, more than half of the identified variants within the F10 gene are pathogenic or likely pathogenic that can be along with clinical manifestations (Fig. 12.8d) [46]. Generally, *missense mutations* are the most frequent variants that account for most congenital bleeding disorders. Based on a published report from **Harris et al. in 2021**, approximately 46% and 16% of pathogenic mutations cause phenotypically type I and type II of FX deficiency, respectively whilst 38% of the variants have unknown phenotypes. Most **type II-causing mutations are distributed** in the catalytic (~64%) and Gla (~14%) domains, while the **type I-causing mutations** are more frequently distributed in the EGF-1 and EGF-2 domains. The way the variants are distributed within the FX protein domains highlights the point that catalytic and Gla domains are involved in the functional activity, while EGF-1 and EGF-2 domains have a central role in the term of correctly-FX protein folding. Accordingly both FX:C and FX:Ag levels are reduced in type I FXD, but the level of FX:C is predominantly lower than FX:Ag in type II FXD.

There are also some reports of FX deficiency as a part of vitamin K-dependent clotting factors deficiency due to some defects in either γ-glutamyl carboxylase (*GGCX*) or subunit 1 of vitamin K epoxide reductase complex (*VKORC1*) (please refer to Chap. 9). Among different reported *F10* gene mutations, some mutations are in association with severe and life-endangering manifestations. *Gly380Arg, IVS7-1G>A,* and *Tyr163delAT* mutations are associated with a *high incidence of ICH*, and *Gly-20Arg mutation* is associated with *severe hemarthrosis*. Also, some mutations such as *Leu32pro, Glu102Lys, and Gly114Arg* are more common in patients with *mild deficiency* of FX [3, 4, 6, 13, 14, 18, 26, 28, 47].

1. **Chromosomal abnormalities/gene deletions**

   The first molecular abnormality affecting the *F10* gene was 13q34 monosomy reported by **Scambler and Williamson**, **1985** who described a patient having a concomitant deficiency of FX and FVII, while her brother was trisomic for 13q34 and had increased levels of FX and FVII. Interestingly *FX* chromosomal deletions affecting both the F10 and the F7 genes which are positioned close to each other form part of the 13qsyndrome. *San Antonio* arises from an 838-T single nucleotide deletion that leads to frameshift mutation and production of a stop codon at residue 232. *San Giovanni Rotondo FX* is also due to a single nucleotide deletion (556-C) that leads to the creation of a stop codon in residue 226.

2. **Point mutations**

   The following ten mutations are the most frequent variants occurring in the *F10* gene, the first four mutations are associated with *type I* FXD whilst the rest are associated with *type II* FXD:

   Factor X Voralberg (type I): two variants Glu14Lys (c.160G>A) and Glu102Lys (c.424G>A), Known as *FX Voralberg* were reported by Watzke in 1990 for the first time. Glu14Lys occurs in the Gla domain (Exon 2) and interferes with FX activation by FVIIa/TF and FIXa/FVIIIa complexes, while Glu102Lys involves the EGF-2 domain (exon 5). In homozygous cases of *FX Voralberg*, *FX activity is <10% (PT based) and the antigen level is about 20%.*

   FX Santo Domingo (type I): It was reported by Watzke in 1991. The variant results from c.61G>A substitution leading to the replacement of glycine at position −20 to arginine in the Pre-Pro leader domain (Gly-20Arg, exon 1), and interferes with FX cleavage signal peptidase impairing FX secretion into the endoplasmic reticulum. *FX activity is <1% and the antigenic level is <5% in the homozygous stat*e [3].

   Gly222Asp (type I): This mutation, reported by **Peyvandi et al. in 2002**, occurs in c.785G>A substitution and involves the serine protease domain (exon 7). It disrupts the protein folding and catalytic activity due to its vicinity to the catalytic triad.

   FX Friuli (type II): FX Friuli was the first dysfunctional mutant FX and the best well-characterized mutation in the *F10* gene reported by **Girolami et al. in 1970**. It came from a northeastern region, named Friuli in Italy and all the recognized patients originated from this area, never reported in other areas of Italy or other countries. Pro343Ser (c.1147C>T) causes new hydrogen bridge formation between Ser343 and Thr318 in the catalytic domain. So the tertiary structure of the domain and its catalytic potential are affected. FX Friuli is characterized by FX normal antigen level and near-normal Stypven time, but prolonged PT and aPTT-based clotting assays. It occurs in both heterozygous and homozygous states. The *FX:C level is about 3–6%* in Friuli cases which seems adequate to arrest hemarthrosis and brain hemorrhage, usual symptoms in the classical form of FX deficiency. Another difference between these two conditions is the heterozygous cases where heterozygote cases diagnosed with Friuli type and have

FX:C level of 40–60% sometimes experience mild bleeding tendency while the heterozygote cases of the classic disease are commonly asymptomatic.

FX Riyadh (type II): Glu51Lys substitution, was reported by **Al. Hilali et al. 2007**, involves the EGF-1 domain (exon 4) disturbing the structure folding and activity of FX.

FX Stockton (type II): Asp282Asn (c.964G>A) was reported by **Messier et al. in 1996**. The substitution effects residue 282 in the catalytic triad and FX catalytic activity.

FX Marseille (type II): Ser334Pro (c.1120C>T) was reported by **Bernardi et al. 1994** affects the serine protease domain (exon 8) causing the reduction of FX activation by FIX and RVV. It hinders the cognition of FX by its activators.

FX Nagoya II (type II): Gly366Ser (c.1216G>A) that was identified by **Miyata et al. in 1998** is due to the substitution of Gly366 in the serine protease domain (exon 8) by a larger hydrophilic residue, serine, possibly affecting the substrate-binding and losing FX activity.

Gly381Asp (type II): The substitution of Gly381 residue by Asp in the serine protease (exon 8) disturbs key active site interactions causing the loss of FX activity and function. Because the side chain of Asp has a bulky charged nature. The variants were recognized by **Camire et al. in 2001** [3, 4, 6, 48–51].

Leu211Pro (type I): It is reported by **Ferrarese et al. in 2019**. The residue of Lue 251 is located in a highly conserved hydrophobic region in the serine protease domain (exon 7). Its substitution by proline would change the residue hydrophobicity of the region and affect the native structure of FX protein. FX folding, secretion, and activity properties are disrupted by this alteration, though they are not fully suppressed. The variants of Gly204Arg, Gly380Arg, and Cys364Arg also involve the catalytic domain in a manner similar to Leu211Pro, resulting in a very low FX level in plasma. Gly204Arg or FX Debrecen (c.730G>A, exon 6) was identified first by **Bereczky et al. in 2008**. The change of Gly204 which is located in a small disulfide-bridge loop induces structural changes in terms of default secretion. Gly380 is a conserved residue in the catalytic domain among the serine protease family including FVII, FIX, FX, and protein C, even among different species. In the alteration of Gly380Arg or FX Padua III (**reported by Vianello et al. in 2001**), the side chain of Arg may interact with the catalytic triad and change its function. Cys364Arg **(reported in 2000 by Millar et al.)** interferes with a highly conserved intra-chain disulfide bond in the serine protease domain (exon 8) disturbing the native FX folding and stability [4, 45, 52].

FX Nice I (type I): It arises from Met-40Val (c.1A>G) substitution in the pre-pro leader (exon 1). FX Nice I usually occurs in concomitant with *FX Nice II* (Pro304Ser, c.1030C>T), a substitution in the serine protease domain (exon 8).

The reported levels *The FX:C and FX:Ag were about 4% (based on PT assay) and 7%,* respectively, in the patients. The replacement of methionine with valine results in the elimination of the ATG start codon and the complete loss of translation of FX, while the Pro304Ser mutation destabilizes the FX hydrophobic core interfering with the interactions between EGF-2 and protease domain. It results in a defective FX secretion. Both mutations were recognized by **Miyata et al. in 1998** [3, 4, 6, 52].

FX St. Louis II: The variant of Glu7Gly (c.140A>G) in the Gla domain (exon 2) was reported by **Rudolph et al. in 1996**. The replacement of a Gla by glycine causes the loss of one Gla residue affecting the Ca^{2+} binding interactions in the molecule. It does not affect the synthesis or secretion of FX protein. FX St. Louis II presents with a *normal level of FX:Ag and FX:C less than 1%.*

FX Frankfurt I (type I): Glu25Lys (c.193G>A), reported by **Huhmann et al. in 1998**, occurs in the Gla domain (exon 2). It was identified in a patient with *FX:C and FX:Ag levels of 56% and 55%,* respectively [3, 6, 48].

Ser322Asn (type II): it is a novel homozygous missense mutation in the serine protease domain (exon 8), reported by **Lu et al.** in a Chinese family, **in 2020**. The substitution of highly conserved Ser322 by Asn (c.1085G>A) probably destabilizes the catalytic structure leading to the *reduction of FX:C level and secretion* [4, 52, 53].

Leu487Phe (type II): It is a novel homozygous variant in the catalytic domain (exon 8), reported by **Fallah et al. in 2022** in a male Iranian patient presenting with *FX:C level of 13%*. The mutation probably reduces the flexibility and also increases the number of H-bond in the catalytic domain [54].

Cys17Arg and Gln135:* These two new variants were reported by **Borhany et al.** in two Pakistani patients in **2018**. Cys17Arg (c.169T>C), identified at a compound heterozygous state with Gly380Arg, occurs in the Gla domain (exon 2) and apparently disrupts the disulfide bond formation (Cys57-Cys62). The homozygous nonsense variant of Gln135* (c.523T>C) in the linker segment (exon 6) was recognized in an infant with *delayed ICH, at 10 days of birth*. This mutation results in premature chain termination and a *lack of functional FX in plasma* [52, 55].

Ser322Asn and Tyr344Ter (type II): It is a compound heterozygote of two missense and termination mutations, reported **by Lu et al.** in a Chinese pedigree in **2020**. The proband presented with moderate bleeding, prolonged PT and aPTT, and *FX:C level of 1.7%*. They occur in the serine protease domain (exon 8) causing destabilization of the catalytic site structure that leads to reduced activity and secretion of FX protein [52, 56].

Characterizations of some of the FX gene mutations are listed in Table 12.6.

Table 12.6 Characterizations of some FX gene mutations

Name	Mutation	Involved exon and domain	Type of variant	Type of FXD/no of patients	Clotting assay			FX:C	FX:Ag	Comments
					PT	aPTT	RVVT			
Nice I	Met-40Val (c.1A>G)	Exon 1/ preproregion	Missense	I/3	↓	ND	ND	ND	↓	Start codon is destroyed by loss of Met, first report by Miyata et al. 1998
Nice II	Pro304Ser (c.1030C>T)	Exon 8/ catalytic domain	Missense	I/2	↓	ND	ND	ND	↓	Hydrophobic core is destabilized, problem in secretion, first report by Miyata et al. 1998
Stuart	Val246Met (c.856G>A)	Exon 7/ catalytic domain	Missense	I/1	↓	↓	↓	↓	↓	First report by Perry 1997
Santo Domingo	Gly-20Arg (c.61G>A)	Exon 1/ pre-propeptide	Missense	I/21	↓	↓	↓	↓	↓	Disturbing the FX cleavage, preventing of the secretion, first report by Watzke et al. 1991
St Louis II	Glu7Gly (c.140A>G)	Exon 2/Gla domain	Missense	II/2	↓	↓	ND	ND	N	Disturbing Gla domain interactions, Affects Ca^{2+} binding, first report by Rudolph et al. 1996
Ketchikan	Glu14Gly (c.161A>G)	Exon 2/Gla domain	Missense	I/1	↓	↓	↓	ND	↓	Changing of FX affinity for Ca^{2+} binding, first reported by Kim, Thompson and James 1995
Vienna	Gly204Glu (c.731G>A)	Exon 6/ activation domain	Missense	I/1	↓	↓	ND	↓	↓	First reported by Watzke et al. 1993
Voralberg	Glu14Lys (c.160G>A)	Exon 2/Gla domain	Missense	I/16	↓	↓	↓	↓	↓	Interfering with FX activation by FIXa/FVIIIa and FVIIa/TF complexes, first reported by Watzke et al. 1990
Voralberg	Glu102Lys (c.424G>A)	Exon 5/ EGF-2	Missense	I/12	↓	↓	↓	↓	↓	First reported by Watzke et al. 1990
Malmo 4	Glu26Asp (c.198G>C)	Exon 2/Gla domain	Missense	U/U	↓	↓	ND	↓	ND	First reported by Wallmark et al. 1991

Tokyo	Glu32Gln (c.214G>C)	Exon 2/Gla domain	Missense	II/U	↓	NN	↓	ND	N	Affects Ca^{2+} binding Preventing of standard carboxylation of FX, interfering with processing, first report by Zama et al. 1999
Wenatchee I	Arg139Cys (c.535C>T)	Exon 6/ activation domain	Missense	II/U	↓	↓	↓ND	↓	↓	Preventing FX proteolytic cleavage and standard processing, first reported by Kim et al. 1995
Wenatchee II	Asn57Thr (c.290A>C)	Exon 4/EGF	Missense	II/U	↓	↓	↓ND	↓	↓	First reported by Kim et al. 1995
Kurayoshi	Arg139Ser (c.535C>A)	Exon 6/ activation domain	Missense	II/2	↓	↓	↓	N	N	Preventing FX proteolytic cleavage and standard processing, disturbing FX interactions with FVa and FVIIIa, first reported by Iijima et al. 2001
Nagoya I	Arg306Cys (c.1036C>T)	Exon 8/ catalytic domain	Missense	I/U	↓	↓	ND	ND	↓	Alpha-helix is destroyed, first reported by Miyata et al. 1998
Nottingham	Ala404Thr (c.1332G>A)	Exon 8/ catalytic domain	Missense	II/3	↓	↓	↓	ND	N	Inducing conformational alteration, interfering with the nearby disulfide bond, probably causing complete unfolding of the protein, first reported by Deam et al. 2001
Taunton	Arg405Gly (c.1335C>G)	Exon 8/ catalytic domain	Missense	II/5	BL	BL	BL	ND	N	Disturbing catalytic activity and local conformation, first reported by Deam et al. 2001
Marseille	Ser334Pro (c.1120C>T)	Exon 8/ catalytic domain	Missense	II/14	↓	↓	↓	↓	N	Reduction of FX activation by FIXa and RVV, first reported by Bernardi et al. 1994
Stockton	Asp282Asn (c.964G>A)	Exon 8/ catalytic domain	Missense	II/15	↓	↓	ND	↓	N	Affecting catalytic activity of FX, first reported by Messier et al. 1996

(continued)

Table 12.6 (continued)

Name	Mutation	Involved exon and domain	Type of variant	Type of FXD/no of patients	Clotting assay			FX:C	FX:Ag	Comments
					PT	aPTT	RVVT			
Friuli	Pro343Ser (c.1147C>T)	Exon 8/ catalytic domain	Missense	II/25	↓	↓	NN	↓	N	Changing in FX catalytic domain structure, hindering the activation, first reported by Girolami et al. 1970
Roma	Thr318Met (c.1073C>T)	Exon 8/ catalytic domain	Missense	II/7	↓	↓	N	↓	N	Interfering with catalytic activity without changing in the structure, first reported by De Stefano et al. 1988
Frankfurt1	Gla25Lys (c.193G>A)	Exon 2/Gla domain	Missense	I/U	↓	N	↓	ND	↓	First reported by Nöbauer-Huhmann et al. 1998
Unnamed	Asp63His (c.307G>C)	Exon 4/ EGF-1	Missense	U/3	ND	ND	ND	↓	ND	Disruption with β-hydroxylation at residue 63, loss FX function, first reported by Karimi et al. 2008
Unnamed	Leu-34Ile (c.19C>A)	Exon 1/ preproregion	Missense	U/U	↓	↓	ND	ND	↓	First reported by Camire et al. 2001
Unnamed	Gly323ser (c.1087G>A)	Exon8/serine protease	Missense	I/3	↓	↓	ND	ND	↓	Conformational changes and misfolding of the protein, first reported by Millar et al. 2000
Unnamed	Asp368Asn (c.1222G>A)	Exon8/serine protease	Missense	U/U	↓	↓	ND	ND	↓	First reported by Camire et al. 2001
Unnamed	Gly381Asp (c.1262G>A)	Exon8/serine protease	Missense	II/13	↓	↓	ND	ND	↓	Interfering with active site interactions, FX activity, and function, First reported by Camire et al. 2001
Unnamed	Arg-1Thr (c.119G>C)	Exon 2/ preproregion	Missense	II/6	↓	↓	↓	N	N	Disrupting FX cleavage by signal peptidase, production of dysfunctional FX, first reported by Peyvandi et al. 2002

Unnamed	Cys81Tyr (c.362G>A)	Exon 4/ EGF-1	Missense	I/2	↓	↓	↓	↓	↓	Disulfide bond formation, EGF-1 domain folding, and overall structure of FX are disrupted, first reported by Peyvandi et al. 2002
Unnamed	Gly94Arg (c.400G>A)	Exon 5/ EGF-2	Missense	I/5	↓	↓	↓	↓	↓	Interfering with FX structure and function, first reported by Peyvandi et al. 2002
	Asp95Glu (c.405C>A)	Exon 5/ EGF-2	Missense	I/2	↓	↓	↓	↓	↓	First reported by Peyvandi et al. 2002
Unnamed	Cys109Tyr (c.446G>A)	Exon 5/ EGF-2	Missense	I/2	↓	ND	ND	ND	↓	Disulfide bond is disrupted, interfering with folding and overall structure of FX, first reported by Millar et al. 2000
Unnamed	Thr-2Met (c.116C>T)	Exon 2/ pre-proleader	Missense	I/1	↓	ND	ND	ND	↓	First reported by Millar et al. 2000
Unnamed	Cys111Tyr (c.452G>A)	Exon5/EGF-2	Missense	I/1	↓	ND	ND	ND	↓	Disulfide bond is disrupted, interfering with folding and overall structure of FX, first reported by Millar et al. 2000
FX Debrecen	Gly204Arg (c.730G>A)	Exon 6/serine protease	Missense	I/10	↓	↓	ND	ND	↓	Changing the structure and interfering with the secretion of FX, first reported by Bereczky et al. 2008
Unnamed	Glu264Lys (c.910G>A)	Exon 8/serine protease	Missense	I/3	NN	ND	ND	ND	NN	First reported by Millar et al. 2000
Unnamed	Arg287Trp (c.979C>T)	Exon 8/ catalytic domain	Missense	U/1	↓	ND	ND	ND	ND	Changing overall FX electrostatic potential, affects FVa binding, first reported by Cooper et al. 1997

(continued)

Table 12.6 (continued)

Name	Mutation	Involved exon and domain	Type of variant	Type of FXD/no of patients	Clotting assay			FX:C	FX:Ag	Comments
					PT	aPTT	RVVT			
Unnamed	Glu310Lys (c.1048G>A)	Exon 8/ catalytic domain	Missense	I/3	↓	ND	ND	ND	↓	First reported by Millar et al. 2000
Unnamed	Gly323Ser (c.1087G>A)	Exon 8/ catalytic domain	Missense	I/3	↓	ND	ND	ND	N	Conformational changing and the protein mis-folding, first reported by Millar et al. 2000
Unnamed	Cys364Arg (c.1210T>C)	Exon 8/ catalytic domain	Missense	I/5	↓	ND	ND	ND	↓	Disulfide bond formation is disrupted, disturbing folding and stability of FX, first reported by Millar et al. 2000
Unnamed	His383Gln (c.1269C>G)	Exon 8/ catalytic domain	Missense	U/1	ND	ND	ND	↓	ND	Substitution of His is near to catalytic domain, affecting catalytic triad interactions, disrupting FX catalytic activity, first reported by Odom et al. 1994
Unnamed	Trp421Arg (c.1381T>C)	Exon 8/ catalytic domain	Missense	U/1	ND	ND	ND	↓	ND	First reported by Odom et al. 1994
Unnamed	Gly380Arg (c.1258G>A)	Exon 8/serine protease	Missense	I/10	ND	ND	ND	↓	ND	Immediately after the active site, causes formation of a new hydrogen bond with Ala234, causes ICH, first reported by Vianello et al. 2001
Unnamed	Tyr163delAT	Exon 6/ activation peptide	Frameshift	I/U	↓	↓	↓	↓	↓	Interruption with reading frame leading to a stop codon 163 in exon 6, premature chain termination, degradation of the truncated protein, causes ICH, first reported by Herrmann et al. 2005

Unnamed	Glu32Asp	Exon 2/Gla domain	Missense	U/U	ND	ND	ND	ND	ND	First reported by Girolami et al. 2019
Unnamed	Cys17Arg (c.169T>C)	Exon 2/Gla domain	Missense	U/1	ND	ND	ND	ND	ND	Disrupting the formation of the disulfide bond and FX structure, first reported by Borhany et al. 2018
Unnamed	Cys17Phe (c.170G>T)	Exon 2/Gla domain	Missense	I/1	ND	ND	ND	ND	ND	Disrupting the formation of the disulfide bond and FX structure, first reported by Mitchell et al. 2019
Unnamed	p.Glu77* (c.349G>T)	Exon 4/ EGF-1	Nonsense	I/2	ND	ND	ND	ND	ND	Premature chain termination, first reported by Arita et al. 2018
Unnamed	Cys81Arg (c.361T>C)	Exon 4/ EGF-1	Missense	I/3	ND	ND	ND	ND	ND	Interfering with disulfide bond formation, EGF-1 folding, and FX structure, first reported by Jin et al. 2018
Unnamed	Cys206Arg (c.736T>C)	Exon 6/serine protease	Missense	I/6	ND	ND	ND	ND	ND	Interfering with disulfide bond formation and FX structure, first reported by Mitchell et al. 2019
Unnamed	Leu211Pro (c.752T>C)	Exon 7/serine protease	Missense	I/1	ND	ND	ND	ND	ND	The native FX structure is disrupted, but the secretion or activity of FX is not completely suppressed, first reported by Ferrarese et al. 2019
Unnamed	p.Gly298= (c.894C>T)	Exon 8/serine protease	Silent	None/U	N	N	N	N	N	Polymorphism, first reported by Borhany et al. 2018

(continued)

Table 12.6 (continued)

Name	Mutation	Involved exon and domain	Type of variant	Type of FXD/no of patients	Clotting assay			FX:C	FX:Ag	Comments
					PT	aPTT	RVVT			
Unnamed	Tyr279His (c.955T>C)	Exon 8/serine protease	Missense	II/1	ND	ND	ND	ND	ND	Localization of the residue in the domain interferes with catalytic activity of FX, first reported by Mitchell et al. 2019
Unnamed	Ser322Asn (c.1085G>A)	Exon 8/serine protease	Missense	II/5	ND	ND	ND	ND	ND	Destabilization structure of FX, reduction in activity and secretion, first reported by Lu et al. 2020
Unnamed	P.Tyr344* (c.1152C>A)	Exon 8/serine protease	Nonsense	II/4	ND	ND	ND	ND	ND	Destabilization structure of FX, reduction in activity and secretion, first reported by Lu et al. 2020
Unnamed	p.Gln371* (c.1231C>T)	Exon 8/serine protease	Nonsense	I/1	ND	ND	ND	ND	ND	Premature chain termination, first reported by Mitchell et al. 2019
Unnamed	Gly410Arg (c.1348G>A)	Exon 8/serine protease	Missense	II/1	ND	ND	ND	ND	ND	Catalytic activity is affected without interfering with the native structure, first reported by Mitchell et al. 2019
Unnamed	p.Trp421* (c.1382G>A)	Exon 8/serine protease	Nonsense	I/1	ND	ND	ND	ND	ND	Premature chain termination, first reported by Mitchell et al. 2019
Unnamed	Cys50Arg (c.268T>C)	Exon 4/ EGF-1	Missense	I/1	ND	ND	ND	ND	ND	Disturbing FX structure, first reported by Mitchell et al. 2019
Unnamed	c.70+4A>G	Splice site	Splice site	U/1	ND	ND	ND	ND	ND	Formation of a new splice site, first reported by Ferrarese et al. 2019
Unnamed	c.71-1G>C	Splice site	Splice site	U/1	ND	ND	ND	ND	ND	First reported by Mitchell et al. 2019
Unnamed	c.257-1G>C	Splice site	Splice site	U/1	ND	ND	ND	ND	ND	Abolishing of donor/acceptor splice site, first reported by Mitchell et al. 2019

Unnamed	Lue487Phe	Exon 8/ catalytic domain	Missense	U/1	ND	ND	ND	↓	ND	Reduction in flexibility and increasing H-bond numbers, negative effects of the function of FX, first reported by Fallah et al. 2022
Deletion	Del (exon 2)	Exon 2/Gla domain	Deletion	U/1	ND	ND	ND	ND	ND	First reported by Mitchell et al. 2019
Deletion	Complete deletion of gene and partial deletion including exon 7–8	–	–	–	↓	↓	↓	ND	↓	Probable germline mosaicism in the father
San Antonio	c.813delC	Exon 7/serine protease	Frameshift	I/1	↓	N	ND	↓	↓	leading to stop codon, truncated protein is not functional, first reported by Reddy et al. 1989
San Giovanni Rotondo	c.556delC	Exon 6/ activation peptide	Frameshift	I/7	↓	↓	↓	↓	N	leading to stop codon at residue 226, limited synthesis or secretion of truncated protein, first reported by Simioni et al. 2001
Deletion	17 bp deletion in exon 8	Exon 8/ catalytic domain	–	–	↓	ND	ND	ND	↓	Additional mutation at GTG > ATG Val298Met

PT prothrombin time; *aPTT* activated partial thromboplastin time; *RVVT* Russell viper venom rime; *FVa* activated factor V; *FVIIIa* activated factor VIII; *FX:Ag* factor X antigenic assay; *FX:C* factor X activity by chromogenic assay; *FX* factor X; *N* normal; *ND* no data; *BL* borderline; *ICH* intracranial hemorrhage; *NN* near normal; *U* unknown

12.10 Prenatal Diagnosis (PND)

Prenatal diagnosis (PND) can be used for families at risk of having a severely affected infant in cases when both parents are carriers or have at least one affected child. To diagnose an affected fetus, chorionic villous sampling (CVS) is performed at weeks 10–12 of gestation, and then extracted DNA is analyzed to determine the parents' underlying mutations. In special geographic areas with recurrent mutations, molecular characterization, carrier detection, and prenatal diagnosis remain key steps for genetic counseling and education. So establishing a mutation-screening method may be helpful to reduce the rate of affected offspring [14].

12.11 Diagnosis of Acquired Factor X Deficiency

In the case of dealing with a patient suffering from plasma cell dyscrasias and/or Al amyloidosis concomitant with bleeding, acquired FX deficiency should be considered, and investigating the patient's coagulation profile is essential. On the other side, when a patient refers with known or new-onset coagulopathy without any personal or family history, the related underlying diseases to acquired FX deficiency should be concerned as a differential diagnosis. Prolonged PT and aPTT with normal bleeding time are suspicious and more study is required; the level of FX:C and FX:Ag should also be measured along with hematological assessment. Besides, correction or not of PT and aPTT with normal plasma, in the mixing study, can differentiate the acquired FX deficiency with inhibitor presence (e.g. lupus anticoagulant or AiFXD with neutralizing specific FX antibody) from the other acquired types.

It is worth noting in this regard, that the levels of FDPs and D-dimer are increased moderately in Al-amyloidosis, while they are normal or slightly elevated in AiFXD; therefore these parameters are valuable in the differential diagnosis. Plasmin-α_2-plasmin inhibitor complex (PIC) in a hyperfibrinolytic state and thrombin-anti-thrombin complex (TAT) in a hypercoagulation state can be mentioned as other valuable parameters in the differential diagnosis between Al amyloidosis and AiFXD [57, 58].

12.12 Treatment

The proportion of patients with FX deficiency that require treatment is higher than most other rare bleeding disorders according to UK Hemophilia Centre Doctors' Organization (UKHCDO) registry data. Management of FXD can be difficult as evidence-based guidelines are lacking. The UK Haemophilia Centre Doctors' Organisation (UKHCDO) has published guidelines on the management of FX deficiency (and other rare inherited bleeding disorders) based upon a literature review and personal experience. The main mode of therapy in patients with congenital FX deficiency is on-demand therapy (stopping hemorrhage as soon as possible) whilst regular prophylaxis is used in patients with the risk of severe hemorrhagic manifestations. Replacement therapy in congenital FX deficiency is guided by the particular hemorrhagic episode. Current therapeutic options for the management of FXD

patients include antifibrinolytic agents; tranexamic acid and ε-aminocaproic acid (EACA) and blood-derived products; solvent detergent treated fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), FIX products, and plasma-derived FX concentrate (pdFX) [13, 18, 21, 59, 60]. The biological half-life of FX is 20–40 h so an adequate level can be achieved with repeated infusions. Factor levels of 10–20 U/dL are generally sufficient for hemostasis, even in the immediate postoperative period although some data suggest that levels of 5 IU/dL may be sufficient for adequate hemostasis and the thrombogram would suggest that levels of >10 U/dL are sufficient to restore thrombin generation to normal.

12.12.1 Antifibrinolytic Agents

They are indicated in mucous membrane bleeding including epistaxis, oral bleeding, and menorrhagia and can be quite effective either alone or as adjunctive therapy to blood products or FX concentrates.

Aminocaproic acid is usually administrated either as mouthwash (15 mL every 6 h) or orally taken (50–100 mg/kg every 6 h) to control nosebleeds and bleeding from the oral cavity.

Tranexamic acid can be quite effective in managing menorrhagia. It is prescribed orally in dosage of 20–25 mg/kg/6–8 h or IV in a dose of 10–15 mg/kg/6–8 h or as local TAMW (tranexamic acid mouthwash).

Nosebleeds Quick Release™ powder (Biolife; LLC, Sarasota, FL, USA) is a hydrophilic polymer that is administrated for the management of epistaxis [14, 18].

12.12.2 Blood-Derived Products

12.12.2.1 Nonspecific FX Replacement Therapy

Fresh Frozen Plasma (FFP) Although virus-inactivated FFP is an option to control both traumatic and spontaneous bleeding, it is not universally available. A level of 10–20% of FX:C is sufficient for normal hemostasis, so given that the half-life of FX is 20–40 h in plasma, doses of 3–6 mL/kg twice a day increase FX:C level to 10–20%. In cases of surgery and active or severe bleeding, a dosage of 15–20 mL/kg of FFP is required [3, 14, 18, 20]. Due to the low or actually unknown concentration of other clotting factors in FFP, a large volume is required to achieve the hemostatic level of FX; therefore fluid overload is a challenging issue, especially in children and elderly patients suffering from cardiovascular disease. Allergic reaction, elevated risk of transfusion-associated lung injury (TRALI), and delayed efficacy are other concerns. Antihistamines can be given in patients with a history of allergy and using plasma only from men and/or non-multiparous women, as well as using diuretics and slower infusion rate can help in managing these complications [13, 14, 18, 61, 62].

Prothrombin Complex Concentrates (PCC) Virally inactivated PCC is plasma-derived concentrates in which there are three (FII, FIX, and FX) or four (FII, FIX, FVII, and FX) clotting factors and also some anticoagulants such as heparin, anti-

thrombin and protein C, S, and/or Z. It is noticeable that different products contain different amounts of each clotting factor, or even more, product batches may be variable in the contents. This variability can be challenging for severe deficient patients who need frequent infusions.

PCC contains a 1:1 ratio of FX/FIX that is administrated to manage bleeding diathesis in patients with FX deficiency. By a dose of 1 IU/kg, PCC increases the level of FX:C to 1.5%, and due to FX's long half-life (about 30 h), a daily infusion is not necessary. In case of severe deficiency, a daily dosage of 20–30 IU/kg PCC is expected to elevate the FX activity level by 40–60 IU/dL; however, the optimal dose should be determined with regard to the type of bleeding and residual level of FX:C [3, 14, 18]. The efficacy of PCC has been shown in various bleeding scenarios including control of minor bleeding diathesis, hemostatic maintenance in operations, and prophylactic treatment in severe FX deficient patients. It is important to note that PCC also contains an unknown amount of other activated clotting factors (FVIIa, FIIa, and FIXa), so it may be associated with a high risk of thrombotic complications. As FII and FX (60 and 30 h respectively) have long half-lives recurrent infusion of PCC may result in a cumulative effect Therefore, during long-term treatment or in case of regular prophylaxis, monitoring of FIX and FX and D-dimer should be monitored. Several factors can predispose to thrombosis related to PCC including the quality of products, patients' risk profile, and infusion dose and rate. Thrombotic complications have been reported more in acquired coagulation factor deficiencies rather than congenital ones. PCC also should be administrated with caution in cases of liver disease, major trauma, large hematoma, and antithrombin deficiency as well as in neonates [62, 63]. It is noticeable that the concomitant use of antifibrinolytic as tranexamic acid and PCC is contraindicated due to the high risk of thromboembolism (Table 12.7) [13, 14, 18].

Table 12.7 PCC concentrates available in the United State, Canada, or Europe and their FX content

Name of the product	The content of FX (IU)
Profilnine®, three-factor	100
Prothrombinex® VF, three-factor	100
Prothromplex TIM3, three factor	100
Prothromplex total, four-factor	100
Proplex-T, four-factor	100
Confidex, four-factor	100–200
Beriplex® P/N, four-factor	110–190
Octaplex®/Ocplex®, four-factor	72–120
Cofact, four-factor	56–140
Kcentra®, four-factor	125–165
Kanokad, four-factor	56–140
Kaskadil, four-factor	160
Uman complex DI, three-factor	80
FEIBA NF or VH	Not available

12.12.3 Factor IX/X Products

In view of several complications related to PCCs and FFP administration, freeze-dried concentrates containing specific quantities of human FIX and FX were developed to retrieve these factors' deficiencies. Several products have been introduced to the market including:

- **Factor X P Behring (CSL Behring AG, Bern, Switzerland):** a dual-factor powder containing 800–1200 IU and 600 IU human FX and FIX respectively, with heparin and antithrombin III. The efficacy of the product has been approved by a recent trial involving 10 FX-deficient patients who were on prophylactic treatment for 1 year (a dosage of 20 IU/kg once a week). *It is the only approved dual-factor product for FX deficiency treatment and is licensed just in Switzerland for now.*
- **Factor IX HS® (ZLB Behring [now CSL Behring])** another two-factor product, pasteurized and derived from plasma containing 800 IU and 1200 IU human FX and FIX respectively. According to Greifswald Registry, the efficacy of this product on seven FX-deficient patients taking regular prophylactic treatment to control the bleeding episodes was satisfactory.
- **Immune VH (Baxter AG, Vienna, Austria):** a lyophilized human single-FIX powder which is highly purified and virus-inactivated. It also contains a smidgen amount of other clotting factors including FII, FVII, and FX (<0.02 IU per IU of FIX).
- **AlphaNine® SD (Grifols Biologicals, Inc, Los Angeles, CA, USA):** another purified freeze-dried human single-FIX concentrate, virus-inactivated and derived from plasma, also containing trace amounts of FII, FVII, and FX (<0.05, <0.04, and <0.05 U/IU FIX respectively).

When using these products, associated complications such as thrombosis, nephrotic syndrome, and hypersensitivity reactions as well as the development of inhibitors should be considered [14, 60, 62].

Fibrin Glue Fibrin glue is generally used to facilitate local hemostasis, particularly in sites of surgery [18, 20].

12.12.3.1 Specific Factor X Products

High-Purity Human Plasma-Derived Factor X Concentrate (pdFX) The first high-purity and high-potency single-factor FX concentrate (pdFX) with the commercial name of Coagadex (Bioproducts Laboratory, Elstree, UK) was approved by the Food and Drug Administration (FDA) in 2015. Coagadex is manufactured from plasma of healthy and virally negative (hepatitis A, B, and C and HIV-1 and HIV-2 viruses) donors. Virus-inactivated pdFX is produced as a lyophilized powder that is injected after reconstitution with sterile water. It contains 100 IU/mL FX, less than 1 IU/mL of FII and FIX, and no added proteins. pdFX activity level is more than 100 IU/mg protein by using the chromogenic FX activity assay.

Indication of Coagadex It is indicated in the United States (in adults and children) and Europe (in all age groups) for on-demand and prophylactic treatments of bleeding episodes, perioperative management of bleeding and in order to achieve sufficient hemostasis in subjects with congenital FX deficiency. Coagadex could be administrated as pre-operative management in patients with mild FX deficiency although its efficacy for major surgery in patients with moderate and severe deficiency has not been investigated. It should be avoided in patients with allergies to any components of the product.

The safety, clinical efficacy, and pharmacokinetics of pdFX were assessed in two open-label, multicenter, and nonrandomized phase III trials, Ten01 and Ten03. Moreover, the efficacy of pdFX as prophylaxis was evaluated in the phase III TEN02 study and the TEN05 retrospective study (Table 12.8) [60, 62, 64].

12.12.3.2 TEN01

The first study (Ten01) was conducted on a population of 16 patients (208 bleeding episodes) aged >12 years with moderate to severe FX deficiency (<5 IU/dL) that experienced ≥1 bleed in the last year. pdFX was administrated at a dose of 25 IU/kg for the baseline pharmacokinetics (PK1) measurement including recovery rate and half-life. Then the patients are treated on-demand for spontaneous, traumatic, and heavy menorrhagia bleeding diathesis for more than 6 months and till >1 bleeding episode had been treated. After which, the secondary endpoints including PK2, and the number of required infusions, were evaluated. The mean recovery rate was 2.0 IU/dL per IU/kg, and the terminal half-life ($t_{½}$) of pdFX was approximately 30 h suggesting that a prophylactic regime once or twice infused weekly is adequate (depending on the required activity level of FX). *The results of pdFX efficacy were good or excellent in treating 98.4% of the 187 bleeding episodes.* The score of 'excellent' was described as stopping obvious bleeds or menorrhagia with one dose within 12 h or with ≤2 doses within 48 h, while the 'good' score meant overt bleeding and menorrhagia stopping with ≤2 doses within 24 h with 3 or 2 doses within 48 h. Clearance (CL) of pdFX through the hepatic route is slow so it remains in plasma for a long time. It is noteworthy that the PK of pdFX depends on the plasma volume as well as the body weight of patients. Therefore, it varies from younger children to older children and adults.

12.12.3.3 TEN02

This study was designed with the aim of assessing the potency of pdFX in reducing/preventing and/or treating the bleeds in nine children aged <12 years with FX:C <5 IU/dL and a history of severe bleeding. All patients received beginning, routine prophylaxis and end doses of 50, 40–50 and 50 IU/kg respectively, for ≥26 weeks. Totally, 537 prophylactic infusions with the mean dose of 38.8 IU/k every 3.1 days were taken by all patients. The mean IR was 1.74 IU/dL per IU/kg and all patients maintained levels >5 IU/dL after the fourth visit. *The pdFX preventing efficacy was 'excellent' meaning that the patients indicated no minor or major bleeding or that it was lower than expected based on the patient's history and condition.*

Table 12.8 Summary of data of clinical studies of Coagadex

Name of the study	Ten01 (NCT00930176)	Ten03 (NCT01086852)	Ten02 (NCT01721681)	Ten05
Design	Multicenter, nonrandomized, open-label, and prospective phase III trial	Multicenter, nonrandomized, open-label, and prospective phase III trial	Multicenter, nonrandomized, open-label, and prospective phase III trial	Retrospective, multicenter, open-label, international
Patients	– 16 patients aged ≥12 years with severe or moderate congenital FXD (FX:C <5 IU/dL) – Required replacement therapy for ≥1 spontaneous bleed	Two patients aged ≥12 years with plasma FX:C <20 IU/dL	– Nine patients aged <12 years with moderate or severe congenital FXD (FX:C <5 IU/dL) – History of severe bleeding – Or an *F10* mutation causing a severe bleeding phenotype	15 patients (13 aged ≥12 years and two patients aged <12 years) with moderate or severe congenital FXD
Aim of the study	– Overall efficacy assessment and safety in the management of bleeding diathesis up to 2 years – On-demand treatment for ≥6 months – Short-term preventing therapy – PK after a single dose of 25 IU/kg – Perioperative management for the surgical patients	Perioperative management for the surgical patients	Assessment of pdFX in preventing/treating bleeding episodes over ≥26 weeks	Assessment of pdFX based on compassionate use between March 30, 2011, and December 31, 2015, for: – Routine prophylaxis – On-demand treatment – Short-term preventing – perioperative hemostatic coverage

(continued)

Table 12.8 (continued)

Name of the study	Ten01 (NCT00930176)	Ten03 (NCT01086852)	Ten02 (NCT01721681)	Ten05
Dosing	– The mean dose per injection: 25.3 IU/kg – The mean total dose: 30.4 IU/kg – Supporting dose of 25 IU/kg twice a week for prophylaxis in adolescents and children	–	– A beginning and last dose of 50 IU/kg on day one and end of the study – Routine prophylaxis with 40–50 IU/kg twice weekly with adjustment of dose and frequency through week 6 to maintain a trough level FX:C >5 IU/dL	– The specific dosing was tailored to each patient and the dosing regime was decided by the investigator – Mean dose per infusion 32.5 IU/kg for routine prophylaxis
Findings	– Mean half-life: 29.4 h – Mean IR: 2.00 IU/dL per IU/kg – Excellent or good efficacy for on-demand management of episodes: 98% of cases	– Excellent efficacy for the management of blood loss before surgery: 100% – No bleeding after surgery – No need for blood transfusion – Expected or less than expected blood loss	– Overall mean IR: 1.74 IU/dL per IU/kg – Excellent efficacy in all the patients – No patient experienced minor or major bleeding or the frequency of bleeding was lower than expected based on the patient's medical history	– The investigators rated the efficacy of pdFX as 'excellent' in all patients – The overall median bleeding rate per patient per month was 0.04 in the prophylaxis group compared with 0.8 in the on-demand group
Complications	– Mild headache – One subject with pain in the injection site – One subject with erythema in the injection site, fatigue and back pain	No possibly complications	No serious adverse reaction was reported	Not mentioned

FX factor X; *FX:C* factor X functional activity; *PK* pharmacokinetic; *IR* incremental recovery

12.12.3.4 TEN03

The second study investigated the pre-operatively treatment of Coagadex in two surgical patients aged >12 years with mild to severe FX deficiency (FX:C <20 IU/dL).

12.12.3.5 TEN05

TEN05 was a multicenter data-collection study on 15 patients with moderate or severe congenital FX deficiency to assess the efficacy of pdFX for on-demand and routine prophylactic treatment, short-term prevention and perioperative management for 5 years. Thirteen patients aged ≥12 and two patients were <12 years from whom seven patients were on prophylaxis, seven patients took on-demand treatment and one patient was between these two conditions. The dosing regime was decided by the researchers and designed for each patient. A total of 1239 infusions were taken as prophylactic treatment with a mean dose of 32.5 IU/kg per infusion. Four out of eight patients who were on prophylaxis experienced 17 bleeds totally. *The effectiveness of pdFX was rated as 'excellent' in all patients meaning that pdFX regularly met or overstepped expectations.*

- The most commonly reported adverse event was a mild headache in the TEN01 study, while erythema and pain in the site of fusion, back pain, and fatigue were observed in both TEN01 and TEN03 studies. No associated cases of thrombosis, inhibitor development, safety concerns, and serious side reaction were reported in clinical studies.
- In brief, according to the evidence, the effectiveness and tolerability of pdFX were promising for children<12 years, adults, and adolescents. *The recommended doses for prophylaxis are 25 IU/kg twice per week for adolescents and adults and 40 IU twice a week in children <12 years.*
- As Coagadex is manufactured from human plasma, there may be contamination with infectious agents such as viruses or the agent of Creutzfeldt-Jakob disease (theoretically). Also, because possibly the formation of FX inhibitors and other complications by using Coagadex, closely monitoring and dosages adjustment of clinical responses and FX levels, via suitable laboratory, assays are necessary [60, 62, 64–27].

12.13 Management of Factor X Deficiency in Surgery

The target level of FX in surgery is variable in different patients according to the residual FX level in the plasma and based on the type and duration of surgery. A satisfactory surgery could be achieved in severely affected patients with FX deficiency (FX <1%) by carefully using FFP, PCC, or other products. A level of 20 IU/dL FX in plasma seems to be adequate for hemostasis and controlling of bleeding, so a loading dose of 15–20 IU/kg PCC followed by 10–15 IU/kg daily or every other day for minor surgery is recommended (Table 12.9) [13, 14, 63].

Table 12.9 Recommended dose of different products for the management of bleeding episodes or prophylaxis patients with factor X deficiency

	Products	Recommended dose
Mucosal bleeding	TA	10 mL of a 5% solution as mouthwash every 8 h
	FFP	A loading dose of 10–20 mL/kg followed by 3–6 mL/kg twice a day to keep FX:C level above 10–20 IU/dL[a]
	PCC	1 IU/kg, PCC raises FX:C level by 1.5%[b]
Acute bleeding in severe factor X deficiency	FFP	A loading dose of 10–20 mL/kg followed by 3–6 mL/kg twice a day to keep FX:C level above 10–20 IU/dL
	PCC	20–30 IU/kg once a day
	pdFX	25 IU/kg (patients aged ≥12 years)
Surgery in severe factor X deficiency[c]	FFP	15–25 mL/kg, an alternative if PCC is unavailable
	PCC	A loading dose of 15–20 IU/kg followed by 10–15 IU/kg after surgery
Prophylaxis	PCC (FIX)	15–40 IU/mL two or three times per week 20–70 IU/mL weekly
	FX	15–20 IU/kg weekly

TA tranexamic acid; *FFP* fresh frozen plasma; *PCC* prothrombin complex concentrate; *pdFX* plasma-derived factor X; *FX* factor X

[a] Level of 10–20 IU/dL FX seems to be sufficient for hemostasis; however, some reports suggest that 5 IU/dL FX may also be adequate

[b] As biological half-life of FX is 20–40 h daily infusion is not usually required

[c] Replacement therapy is not required in patients with factor IX level >10% without significant bleeding history

[d] Generally a daily dose is sufficient; however, in minor surgery, a dose every other day may be adequate

12.14 Treatment in Women: Pregnancy, Delivery, and Menorrhagia

Menorrhagia occurs in more than 50% of women with FX deficiency. Therapeutic options for the control of menorrhagia include medical treatments (such as antifibrinolytics, oral contraceptives, levonorgestrel intrauterine device, and clotting factor replacement), and surgical treatments (such as endometrial ablation and hysterectomy). In particular, tranexamic acid was reported to be useful (tranexamic acid 15 mg/kg every 8 h, in practice 1 g every 6–8 h, may be effective when taken for the duration of the menstrual period).

Affected women are also at risk of gynecologic problems including hemoperitoneum and corpus luteum bleeding in relation to ovulation.

12.14.1 Factor X Deficiency in Pregnancy

Pregnancy is accompanied by increased concentrations of FX. However, there are some concerns regarding FX-deficient women during their childbearing period. Recurrent abortion and placental abruption appear only in cases with severe

deficiency. Their opportunity for fertilization is lower than the normal population because of either higher frequency of bleeding in some organs such as the ovary or prolonged menstruation, especially in women with FX levels less than 1%. Also, they are at high risk of severe hemorrhagic complications following some necessary invasive procedures, e.g., chorionic villus sampling (CVS), amniocentesis, or axillary reproductive techniques. A sampling of CVS and amniocentesis are required for prenatal diagnosis (PND). Another concern is the mode of inheritance of FX deficiency so in countries with a high rate of consanguinity, either affected or mandatory carrier offspring would be expected more than in other areas [67].

Therefore, for FX-deficient pregnant women or who want to try pregnancy, a team of experts and an adequately equipped centre preferably a hemophilia treating center or a tertiary hospital are required for appropriate management of patients during pregnancy and labor. In such conditions, more affected women can experience low-risk pregnancy and delivery of healthy infants [3, 67].

Women with severe FX deficiency and a history of adverse outcome such as abortions, placental abruption, or premature births may benefit from replacement therapy throughout pregnancy. However, the associated risk of thrombosis must be carefully evaluated, particularly with the use of PCCs that contain appreciable quantities of coagulation factors other than FX. It is of relevant importance to note also that heterozygote subjects were reported to have bleeding after delivery without prophylactic replacement therapy, which required treatment with FFP. However, management of women with FX deficiency requires additional monitoring of the hemostatic parameters and awareness of the increased risk of bleeding with any surgical intervention.

During pregnancy, sufficient replacement therapy is required to prevent adverse outcomes such as miscarriage, preterm birth, and placental abruption. In severely affected patients (FX:C <2 IU/dL), who are in the third trimester of pregnancy and require a cesarean section, a loading dose of 20–40 IU/kg, PCC is required to achieve FX:C >4 IU/dL and then followed by 10–20 IU/kg once a day to keep FX:C >3 IU/dL for at least 3 days [68].

Among patients participating in the TEN01 study, ten women and girls were registered that received a total of 267 pdFX infusions, 178 for on-demand (a mean dose of 30.5 IU/kg per bleed) and 89 for prophylactic treatment. A total of 149 bleeding episodes were treated in which the pdFX efficacy score was “excellent,” “good,” “poor,” and “un-assessable” in 116, 13, 2, and 1 episodes, respectively. The overall efficacy score was 97.7% for the women and girls. Generally, in this study, women and girls took more infusions than men and boys (average 2.48 vs. 1.62 per month).

FX evaluation is required either before delivery or at birth (cord blood sample) to reduce the risk of hemorrhage in neonates. Further, severely affected neonates should carefully be evaluated by cranial ultrasound due to the high risk of ICH and then re-evaluated and screened at 6 months of age. Prophylactic care also must be considered a necessity during the neonatal period.

No trauma during expected vaginal delivery or using ventouse, or forceps during delivery.

Circumcision and vaccination should be postponed till the baby is screened.

Replacement therapy is not usually required to manage moderately factor X deficiency (FX:C >2 IU/dL) without significant bleeding history (despite hemostatic challenges). However, any prior hemostatic challenges related to bleeding history and the kind of surgery must be considered.

12.14.2 Recommendation for Management of Women with FX Deficiency

- Women suffering from severe deficiency and a history of unsuccessful delivery or unfortunate outcome should be treated with aggressive FX replacement.
- Pregnant women with heterozygous mutations should be evaluated for the risk of bleeding before delivery.
- Women with severe deficiency who experienced recurrent bleeding or unfavorable outcomes during pregnancy should receive prophylactic therapy before delivery.
- Based on the recommendations from UK guidelines, a level of FX:C >30 IU/dL should be achieved and maintained following delivery in women with a history of bleeds and low FX:C level, and also for women who need a cesarean operation [13, 40, 62].

12.15 Prophylaxis

As FX deficiency is one of the most severe rare bleeding disorders, prophylaxis is recommended for those patients at risk of severe bleeding including CNS, GI bleeding, hematoma, and hemarthrosis particularly in patients with FX <0.05 IU/mL. Previous data on thrombin generation in patients with FX deficiency showed that roughly 5% of residual FX in plasma might be enough to obtain at least 50% of thrombin generation and to prevent severe bleeding. Based on these data, an individual prophylaxis could be planned targeting this level. According to a case series data from the Greifswald registry, nine FX-deficient patients took a dose of 15–20 IU/kg Factor IX HS concentrate (once a week in seven patients and every other in two patients) leading to a significant reduction of hemorrhage, especially in children. Another case series showed that the bleeding episodes were significantly reduced in 10 patients with severe FX deficiency who received 20 IU/kg Behring Factor X P concentrate per week as prophylaxis [13, 14, 62, 69]. If PCC is used, doses of 15–40 IU/mL twice or three times a week are shown as more effective than 20–70 IU/mL weekly. Also, four neonates and three children with severe FX deficiency presenting with umbilical cord bleeding and ICH achieved hemostasis by receiving a dose of 50–70 IU/kg PCC once or twice a week [62, 69]. As described before, the efficacy of pdFX for prophylactic treatment was evaluated in two TEN02 and TEN05 studies (please refer to Sect. 12.12.3.3 and 12.12.3.5). Similar to on-demand treatment, no serious complications and inhibitor development were

reported in patients receiving prophylaxis [62]. The different prophylactic strategies described in these studies demonstrate clearly that the clinical levels of FX required to achieve adequate hemostasis, and the dose and frequency of factor administration, remain to be determined.

A prophylactic approach could also be crucial to prevent CNS bleeding at birth in families already with one severely affected child.

12.16 Intracranial Hemorrhage (ICH)

It is one of the most challenging bleeding sites in patients (generally neonates) diagnosed with severe congenital FX deficiency **(53% had FX activity <0.01 U/mL)**. 9–26% of cases, especially with a Gly380Arg mutation developed cerebral bleeding or ICH. Some cases received prophylactic therapy in the form of FFP and PCC. Dosages of 40–70 IU/kg FIX once or twice per week in some patients and 50 IU/kg activated PCC twice a week in another report have been shown promising, though the infusion of FFP has been insufficient. There is a report of successful treatment with pdFX in a young male patient with severe FX deficiency presenting with subdural hematoma [62].

12.17 Prognosis

By early identification and diagnosis of patients, bleeding complications of FX deficiency would be effectively managed. Special consideration is also required for affected women and children. On the other hand, in heterozygous cases, genetic counseling may be greatly helpful to prevent the birth of affected offspring [18].

12.18 Acquired Factor X Deficiency

In addition to an inherited form of the disease, FX deficiency may occur secondary to other disorders such as AL amyloidosis, myeloma, tumors, infections, and drug consumption as well as liver diseases [3]. Acquired deficiency of FX may present either as an isolated deficiency or along with other factors deficiencies, as FV deficiency [13, 20].

1. **Liver disease or vitamin K deficiency**

   The deficiency of vitamin K due to its malabsorption or oral anticoagulants may result in FX deficiency. On the other hand, affected patients with dysfunctional liver or hepatocellular damages such as hepatocellular carcinoma (HCC) are also at risk of acquired FX deficiency. However, in such disorders, there is a concomitant decrease of other hepatic-derived or vitamin K-dependent coagulation factors [13, 26].

2. **AL amyloidosis**

For the first time, Korsan-Bengsten et al. reported a case of amyloidosis with concomitant FX deficiency. They described an association between amyloidosis and FX deficiency. Since then, more similar cases were reported in several studies, and the correlation between systemic amyloidosis and FX deficiency became clearer [3].

Amyloidosis is a general name to define some clinical disorders in which insoluble abnormal fibril proteins are aggregated extra- or intracellularly on the affected tissues leading to functional defects of involved organs. Primary or systemic light-chain amyloidosis that is also called AL amyloidosis is a myeloma-associated disorder. It is characterized by the deposition of immunoglobulins' abnormal light chains that involves variable organs and may lead to thrombotic and hemorrhagic complications [70]. Isolated FX deficiency is the most clinically significant coagulopathy in association with primary amyloidosis that occurs in 6.3–14% of cases [59]. Several mechanisms are accounted for the underlying pathophysiology of acquired FX deficiency including the irreversible binding of FX to the abnormal paraproteins or adsorption by amyloid fibrils with subsequent sequestration in the liver, spleen, and vasculature leading to rapid clearance of both endogenous and exogenous FX from plasma. FX activation may also be interrupted by a specific FX inhibitor (IgG4-mediated). Therefore in these situations, either antigenic or functional levels of FX are reduced, although the reduction in the level of FX:C is more profound than FX:Ag [13, 20, 57]. The most common clinical manifestations in acquired isolated FX deficiency similar to the congenital form are epistaxis, easy bruising, and menorrhagia. Amyloidosis is usually accompanied by other hemostatic disorders such as other clotting factor deficiencies due to liver damage, vascular fragility because of amyloid infiltration, and defects in platelet aggregation as well as fibrin polymerization. Therefore, it is not practically possible to determine an obvious correlation between the severity of the disorder and the FX:C level. Acquired FX deficiency in relation to secondary or AA amyloidosis is very rare [3, 13, 59].

3. **Miscellaneous**

Besides acquired FX deficiency due to AL amyloidosis, there are few reports of non-amyloid deficiency of FX [20]. Some malignancies such as myeloma without amyloidosis, acute myeloid leukemia, thymoma, as well as renal, adrenal, and gastric carcinoma may lead to a reduction in FX level. However, the exact mechanism is not clear. In this scenario, **Reynolds et al.** reported a rarely acquired FX deficiency in a patient with multiple myeloma in **2019.** The coagulation profile of the case revealed prolonged PT and aPTT that were corrected in the mixing study with normal plasma. There were reductions in both FX:Ag and FX:C levels, <2% and <10% respectively. This survey highlighted several significant points in these specific situations, for instance, the consideration of acquired coagulopathy in patients with multiple myeloma with or without amyloidosis, or conversely, attention to underlying multiple myeloma and/or amyloidosis in a patient with known or new-onset coagulopathy. It should be noted that the presence of amyloidosis is related to serious prognostic and therapeutic con-

sequences; so thorough evaluations for possibly underlying amyloidosis are valuable.

In addition to malignancies, a transient FX deficiency has been reported about viral or bacterial upper respiratory tract infections, especially due to mycoplasma pneumonia. It is probably due to similar antigenic determinants of FX and infectious agents. Therefore, circulatory FX is cleared by the patient's immune system [3, 13, 20, 57, 59]. Some medicines may also be in association with isolated reduction of FX as sodium valproate or treatment with fungicides and amsacrine [59]. Warfarin and other oral anticoagulants could be a cause of acquired FX deficiency along with the reduction of other coagulation factors [13].

12.19 Management of Acquired Factor X Deficiency

Usually acquired FX deficiency is a transient and short-lived condition, so that coagulation test may turn to normal without any special treatment. Treatment of the underlying cause of acquired FX deficiency is the first step in the management of acquired FX deficiency. Then based on the severity of the deficiency, a suitable therapeutic choice should be selected. However, due to shortened half-life of infused FX, as it is adsorbed by amyloid fibrils, administration of FFP, PCC, or other products increases FX level transiently and so improves the bleeding tendency just for short time; therefore, they are not actually useful. Nevertheless, infusion of human FX concentrate is preferred to FFP and PCC owing to possible side effects (thrombosis, hepatitis, allergic reactions, fluid overload, and TRALI). Administration of vitamin K is recommended in case of severe liver dysfunction. Antifibrinolytic agents may also be useful to improve the hemostatic system in some cases [40, 57, 59, 62]. Recombinant FVII (rFVII) is another useful product for primary amyloid-associated FX deficiency [26]. It is suggested that the level of FX:C be maintained at $\geq$10% to limit the risk of bleeding. For patients with life-threatening hemorrhage, the source of bleeding should be controlled immediately, and then, in the following step, the FX:C level must be kept above 50% by administration of FX concentrates twice weekly. Given that, a large amount of amyloid deposition fibrils aggregate in the spleen, splenectomy could be a highly beneficial therapeutic choice for AL amyloid-related FX deficiency that provides a long-term recovery of FX deficiency by restricting the FX rupture in the RES [40, 57, 59]. In cases of Al amyloidosis and plasma cell dyscrasias, a combination of induction chemotherapy (melphalan), followed by autologous stem cell transplant has been reported highly promising for long-lasting improvement of FX deficiency without the need for FX infusion.

Hemostatic coverage by using FFP, PCC, and vitamin K administration for AiFXD patients has been reported in the literature, though FFP and vitamin K had no true effect to arrest the bleeding episodes; it must have been due to the small amount of FX in FFP and also that the deficiency in vitamin K is not the cause of symptoms. As there is no hyper-fibrinolytic state in AiFXD subjects, opposing Al amyloidosis, antifibrinolytic agents do not have therapeutic potency for these patients. There are some reports of successful management of acquired FX

deficiency associated with inhibitors by using plasmapheresis and steroid therapy as well as intravenous immunoglobulins [3, 10, 58, 71]. It must be implied that there is no standardized therapy for acquired FX deficiency in general and infusion of pdFX is not yet licensed in these conditions [62].

12.20 Autoimmune Factor X Deficiency and Factor X Inhibitors

Although rare, coagulation factors may turn into targets for the immune system with the formation of autoantibodies resulting in bleeding; an extremely rare condition referred to as autoimmune coagulation factor deficiency (AiCFD). Noteworthy that, autoimmune FX deficiency (AiFXD) is less frequent than the others (AiFVIIID>AiFVD>AiFXIIID>AiVWFD). Some patients may be diagnosed with no underlying condition, whereas anti-FX autoantibodies in others might be either specific or non-specific due to the presence of lupus anticoagulant or may occur as a result of burns, leprosy, and infection disease mainly involving the respiratory tract. In the case of infectious diseases, treatment with antibiotics could be accounted for anti-FX auto-antibodies development. According to a report by **Akitada Ichinose et al. in 2021**, both neutralizing and non-neutralizing anti-FX antibodies may be detected in the patients. Neutralizing antibodies or FX-inhibitors bind to the FX in a Ca2+-dependent manner and inhibit the functional activity of FX leading to an extreme reduction in the specific activity of FX (FX:C <1%). Patients with non-neutralizing anti-FX antibodies also have extremely low FX:C levels though in the absence of FX inhibitors (presenting with an undetectable or invaluable titer of FX inhibitor). Reported patients in this study demonstrated soft tissues and intramuscular bleeding, and as many as 70% and 22% of the patients indicated Grade III and Grade II bleeding, respectively, emphasizing that AiFXD could be even more severe than congenital FX deficiency.

When a symptomatic patient presents with prolonged PT and aPTT as well as low FX activity regardless of the correction of mixing tests based on PT or aPTT, without a personal or family history of bleeding and taking any anticoagulant, AiFXD should be considered by the clinicians. Of great concern, since both neutralizing and/or non-neutralizing autoantibodies can cause AiFXD, a functional FX inhibitor assay is not sufficient to certainly diagnose antibodies and immunological examination should be performed. Moreover, AL-amyloidosis is the most important condition that should be concerned for differential diagnosis [3, 11, 14, 58, 59].

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