Congenital Factor XI Deficiency, Diagnosis and Management

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Simon Davidson

13.1 Introduction

Factor XI (FXI) deficiency, or hemophilia C, is a rare autosomal recessive injury-related hemorrhagic disorder, but some dominant cases have also been reported. Worldwide prevalence of the disorder is 1:1,000,000, but this rate is higher in some ethnicities such as Ashkenazi and Iraqi Jewish populations with a prevalence of 1:11 for heterozygotes and 1:450 for homozygotes or compound heterozygotes, affecting both males and females similarly [1–4]. The clinical symptoms are highly heterogeneous, and there is no direct relationship between bleeding tendency and residual plasma FXI level. Bleeding is usually observed in homozygotes and combined heterozygotes, while heterozygotes are generally asymptomatic. Sometimes, patients never experience any bleeding diathesis in their life. Bleeding is usually post-traumatic, postsurgical, postpartum, and in areas with high fibrinolytic activity such as the mucosal surface of the oral cavity [3–6]. FXI deficiency is classified into two phenotypes: in type I or CRM−, both FXI coagulant activity (FXI:C) and FXI antigen (FXI:Ag) is low; in type II or CRM+, FXI:Ag is normal, while the FXI:C is lower than the normal range. A wide spectrum of mutations has been identified within the *F11* gene. FXI deficiency in Ashkenazi Jews is mostly due to four common causative mutations that were categorized as types I to IV. The disorder based on FXI:C level is classified into three types, including mild, moderate, and severe [7–9]. FXI level is less than 20 U/dL in severe deficiency, while in mild and moderate deficiency, FXI level is usually between 20 and 70 U/dL. Due to the mild phenotype of the disorder, in most cases, the disorder is diagnosed based on family history or randomly in routine workup [3, 10, 11]. The main therapeutic strategy in

S. Davidson (✉)
Division of Medicine, University College London, London, UK
e-mail: Simon.davidson@ucl.ac.uk

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these patients is on-demand therapy, and the main therapeutic products that can be used to raise FXI levels in these patients are fresh frozen plasma (FFP) preferably solvent detergent treated (FFP-SD), FXI concentrate, and desmopressin [1, 2, 4, 12].

13.2 Factor XI Structure

F11 gene is located at the distal end of the long arm of chromosome 4 (4q35) and consists of 15 exons and 14 introns that span a 23-kb region. FXI protein has an 18 amino acid-leader peptide. Exon 1, exon 2, exons 3–10, and exons 11–15 encode the promoter region, signal peptide, 4 N-terminal apple domains, and C-terminal catalytic domain, respectively [10, 13–16]. FXI has a distinguished structure from other coagulation proteases. It is a 160-kDa homo dimer with a similar 607 amino acid sequence in both subunits. These two subunits are joined together by covalent and noncovalent bonds. Each subunit has four 90–91 amino acid sequences at the N-terminal, named apple domain (Ap1–Ap4) and a catalytic domain with 238 amino acids at the C-terminal with serine protease (SP) role that is homologous to the serine protease domains of other coagulation factors (Fig. 13.1) [15–19].

Apple domains form a disk structure in each subunit that provides a surface for the binding of platelet, high-molecular-weight kininogen (HMWK), and factor IX. Prekallikrein has a homolog structure with FXI in their apple domains. In the monomeric structure of FXI, each apple domain contains seven β-strands that form an antiparallel sheet and an α-helix is attached to the concave side of the sheet by two disulfide bonds. Another bond connects N-terminal to C-terminal. These four apple domains are adhesion sites for other molecules [16, 19, 20]. These include Ap1 and Ap2, which are adhesion sites for thrombin and HMWK, respectively, and Ap3 is a binding site for glycoprotein Ib, factor XI, and heparin. An Ap4 is an adhesion site for activated FXII (FXIIa) [14, 15, 21]. Ap4 is also necessary for

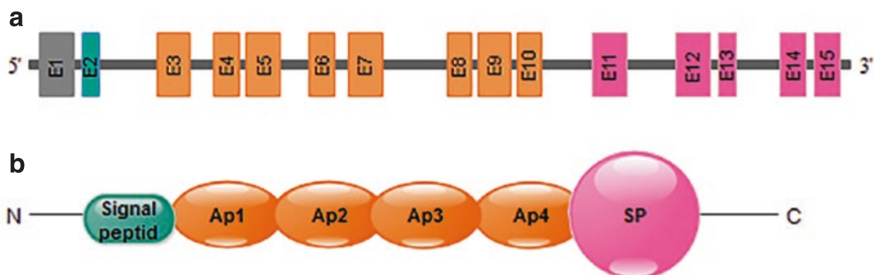

Fig. 13.1 (**a**) Factor XI gene structure: *F11* gene consists of 15 exons and 14 introns. Exons 1 and 2 encode promoter and signal peptide, respectively. Exons 3–10 encode 4 apple domains and exons 11–15 encode the serine protease domain. (**b**) Schematic structure of factor XI protein: Factor XI protein is composed of a signal peptide, 4 apple domains (Ap1–4) at the N-terminal, and a catalytic domain with serine protease activity at C-terminal. *N* N-terminal; *C* C-terminal; *Ap* apple domain; *SP* serine protease domain; *E* exon

dimerization. Catalytic domain that is in C-terminal site on the apple domains in a "cup and saucer" arrangement comprised of His413, Asp462, and Ser557 in a trypsin catalytic domain [20–22].

In circulation, FXI is the only SP in dimeric structure that is active in this form. Ap4 is a major domain for dimerization. Two Ap4 domains develop an interface disulfide bond between two monomer subunits. This bond is formed through Cys321 at a finger-like loop in Ap4 domain. Moreover, hydrophobic interaction between residues including Tyr329, Ile 290, and leu284 of the A4 domain interface and a salt bridge between Lys331 in one subunit and Glu 287 in another subunit is necessary for dimerization [16]. FXI has 5 N-linked glycosylation sites including Asn residues: at positions 72, 108, and 335 in heavy chain and Asn residues at 432 and 473 of light chain. Almost all circulating FXI is in complex with HMWK. HMWK is a multifunctional plasma protein with 6 domains (D1–D6). The D6 domain is the binding site of FXI Ap2 domain that is necessary for optimal binding to the platelet surface and efficient activation of FXI. Optimal binding of FXI to platelet occurs through Ap3 domain, GPIb, and HMWK in the presence of zinc ions [13, 17, 18].

13.3 Factor XI Activation and Function

Plasma activators of FXI are FXIIa, thrombin, meizothrombin, and FXIa (auto-activation). Platelet polyphosphate affects FXI activation via α-thrombin, β-thrombin, and FXIa, and all of them cleave FXI monomers at Arg369-Ile370 site between Ap4 and catalytic domain. The dimeric form of FXI is necessary for the effective activation and function of FXI. FXI activators bind to one monomer and activate another subunit [19]. HMWK is required for optimal FXI binding to GPIb on activated platelet. Indeed, FXI binds to platelet, as a negative charge surface, through one monomer and binds to substrate (FIX, FV, and FVIII) through another subunit, an explanation for its dimeric structure. FXIa is composed of 2 N-terminal heavy chains containing 4 apple domains and 2 C-terminal light chains containing catalytic domains. Dimeric structure of FXIa is maintained by 3 disulfide bonds: heavy chains bind to light chains by 2 bonds in each of the monomers and the two monomers connect together by 1 disulfide bond [20].

Along with the activation of FXI by thrombin and FXII, an intermediate form is generated that has one activation subunit. This intermediate is named 1/2FXIa. Inhibition of FXI by antithrombin in the presence of 1/2 molar heparin shows that each of the FXI subunits has an independent catalytic function, so 1/2FXIa can be the main form of activated FXIa (Fig. 13.2) [21–23].

In the initiation phase of the coagulation cascade, the tissue factor (TF)/FVII complex activates FX and produces a small amount of thrombin. Then a tissue factor pathway inhibitor (TFPI) inhibits TF and blocks the extrinsic coagulation pathway. At this time, the amplification phase begins with the conversion of FXII to FXIIa. Then FXI is converted to FXIa in the intrinsic pathway. Since there is no bleeding tendency in patients with FXII deficiency, this fact demonstrates that an alternative mechanism is present for FXI activation [24]. Thrombin-mediated

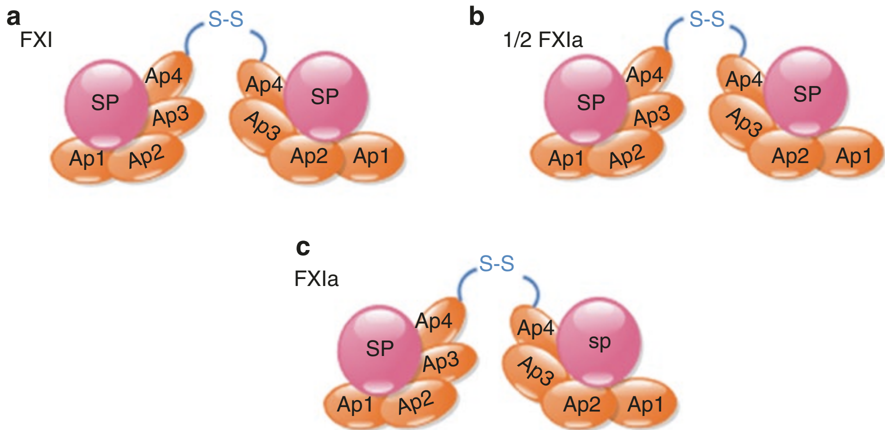

Fig. 13.2 (**a**) Each monomer of factor XI consists of 4 apple domains (Ap1–Ap4) and a serine protease domain (SP). The factor XI dimer is linked by Cys321 interchain disulfide bond between 2 subunits. (**b**) All factor XI activators cleavage factor XI between Arg369-Ile370 site and develop an intermediate form named 1/2FXIa. (**c**) With cleavage in another subunit, 1/2FXIa is changed to activated factor XI. *FXI* factor XI; *FXIa* activated factor XI; *Ap* apple domain; *SP* serine protease domain

activation of FXI reinforces the coagulation common pathway with this thrombin feedback loop [24]. Since in the initiation phase of the coagulation cascade, TF/FVII complex is inhibited by circulating TFPI, only a small amount of thrombin can be produced, and the extrinsic pathway is stopped. At this time, generated thrombin activates platelet, FXI, and FV-FVIII. FXI dimeric structure is necessary because FIX cannot bind to monomeric FXI on the platelet surface; therefore, one of the Apple3 domains is required to bind to the surface of the platelet leaving the AP3 domain on another monomer available for binding of FIX [20]. Therefore, after dimerization and conversion of FXI to FXIa, FXI binding site—amino acids 183–191 at Ap3—is exposed and FXIa can bind to FIX. Then FXIa cleaves FIX at Arg145–Ala146 and Arg180-Val181 sites and releases active peptide (Ala146–Arg180); at this time, FIX converts to its active forms (FIXaα and FIXβ). Thrombin generation will continue even after clot formation. This additional thrombin activates a thrombin-activable fibrinolysis inhibitor that protects the clot from fibrinolysis. Therefore, FXI has both procoagulant and antifibrinolytic activities (refer to Chap. 1) (Fig. 13.3) [9, 19, 20].

Antithrombin, protease nexin-2 (PN-2), C1 inhibitor, aprotinin, leupeptin, P-aminobenzidamin, and protein Z-dependent protease inhibitors are FXI inhibitors. Heparin also binds to serpin and A3 domain of FXI and inhibits FXI. Heparin also inhibits FXI via binding to the catalytic domain through a charge neutralization mechanism. PN-2 that is released from active platelets inhibits FXI through the binding of its Kunitz-type domain to FXI catalytic domain [20, 22].

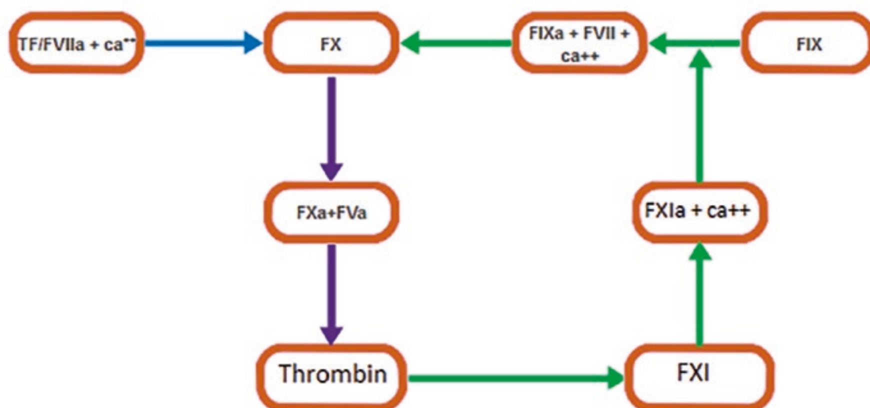

Fig. 13.3 Thrombin feedback loop. In the extrinsic coagulation pathway, a small amount of thrombin is generated by tissue factor (TF)/factor (F) VII + Ca^{++} complex in the initiation phase. Then, tissue factor pathway inhibitor (TFPI) inhibits TF/FVII. In the amplification phase, thrombin activates the intrinsic pathway through the conversion of FXI to FXIa. FXIa cleaves FIX to FIXa, and then FIXa activates FX. At this time, a large amount of thrombin is generated in the common pathway. *TFPI* tissue factor pathway inhibitor; *FXI* factor XI; *FXIa* activated factor XI; *FXa* activated factor X; *FIX* factor IX; *FIXa* activated factor IX; *TF* tissue factor; *FVII* factor VII; *FV* factor V

13.4 Factor XI Deficiency

FXI deficiency, hemophilia C, plasma thromboplastin antecedent deficiency, or Rosenthal syndrome is a rare hemorrhagic disorder with variable clinical symptoms that was first described by Rosenthal in 1953 [2]. The incidence of the disorder is equal in both genders and is ~1 per one million in the general population, but it is more common among Ashkenazi Jews with ~5% carriers and 1 per 450 homozygotes [3–5]. The bleeding tendency is mild in this disorder. Patients with FXI deficiency may not experience abnormal bleeding in their lives. Although severe bleeding is rare, menorrhagia and epistaxis are relatively common. Due to high fibrinolytic activity in the oral and nasal cavities or the urinary tract, bleeding risk is higher in these areas. Some patients may experience bleeding after some surgeries such as tooth extraction and tonsillectomy. Postpartum hemorrhage may occur in this disorder [2, 4–7].

The normal range of FXI coagulant activity (FXI:C) is 70–150 U/dL. Severe FXI-deficient patients have 1–20 U/dL of FXI:C level, while those with partially deficient FXI have 20–60 U/dL FXI:C levels and patients with mild deficiency have 61–70 U/dL FXI plasma levels. Individuals with severe FXI deficiency are homozygous or compound heterozygous for causative mutations, while those with partial

deficiency are heterozygous, with one mutated allele [1, 2, 6]. Activated partial thromboplastin time (APTT) is the screening test for FXII-mediated FXI activation and is prolonged in FXI deficiency. Thrombin-mediated FXI activation is assayed via FXIIa-inhibited diluted thromboplastin time (FXIIai DTT). FXI antigen level (FXI:Ag) is measured via enzyme-linked immunosorbent assay (ELISA). Generally, the mainstay of treatment in these patients is on-demand therapy. Replacement therapy is used in invasive operations, but it is not required for minor surgeries such as tooth extraction. Desmopressin elevates endogenous FXI levels and can be used for severe FXI deficiency. Recombinant FVII can be used in patients with inhibitor (plasma FXI level <1%). Oral antifibrinolytic agents such as tranexamic acid or ε-aminocaproic acid can be used in pregnant women and for minor surgeries such as tooth extraction [2, 6, 7]. However, adverse reactions such as thrombosis can occur, and these antifibrinolytics should be avoided in genitourinary tract bleeding [4].

13.5 Molecular Basis

F11 gene is located at the distal end of the long arm of chromosome 4 (4q35) and consists of 15 exons and 14 introns that span a 23-kb region. This gene is expressed in hepatocytes and regulated via transcription factor hepatocyte nuclear factor-4a (HNF4-α). Blood mononuclear cells, granulocytes, pancreas, and kidney also express a little *F11* gene. FXI deficiency is mostly an autosomal recessive injury-related hemorrhagic disorder, but autosomal dominant forms of the disorder have also been reported [25].

FXI deficiency is due to mutation in *F11* gene, and most mutations are associated with CRM− phenotype and fewer associated with CRM+ phenotype. In type I, CRM−, FXI activity (FXI:C), and antigen (FXI:Ag) levels are decreased [3, 5, 19]. In this type, the mutant protein level is low or absent, which can be due to reduced translation, secretion, or stability of the protein. There are three subgroups in type I (CRM−):

1. Mutations that affect protein synthesis; therefore, the production of polypeptide is decreased or stopped. Glu117stop mutation is common in this group.
2. Mutations that disrupt dimerization within two Ap4 domains; therefore polypeptide remains in monomeric form in the intracellular that results in decreased plasma level of FXI. Phe283Leu mutation is common in this group.
3. Mutations that cause the production of nonsecretable homodimers. Mutations that decrease homodimer secretion of wild-type FXI also result in decreased plasma levels of FXI. Ser225Phe and Trp569Ser mutations are common in this group (Fig. 13.4).

In type II, CRM+, the FXI:C is low, but FXI:Ag is normal. In other words, although the activity is decreased, the plasma protein level is normal or close to normal. A total of 8 CRM+ variants, 5 in catalytic domain and 3 in apple domain, have been identified up to now [6, 15].

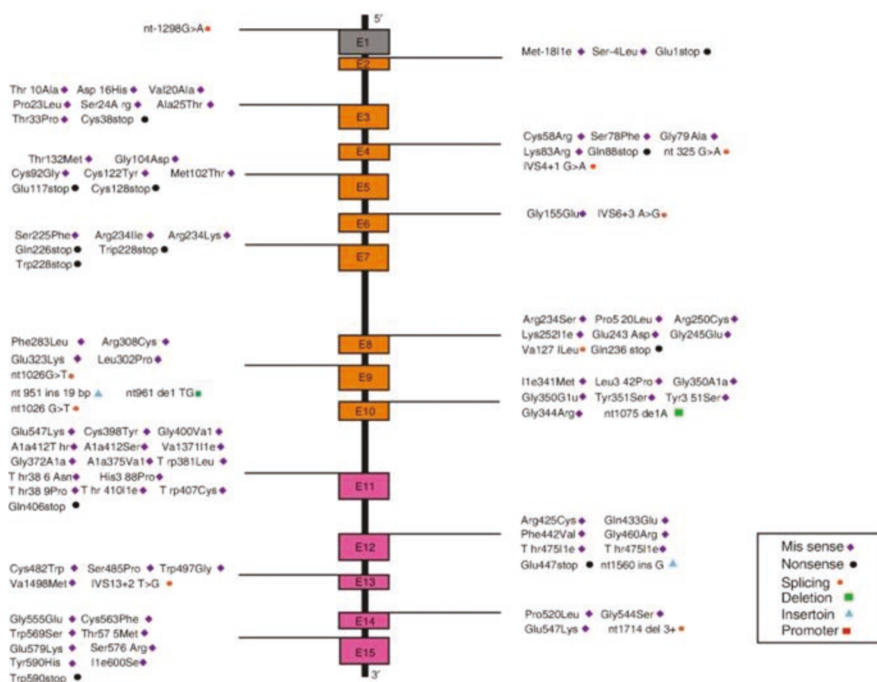

Fig. 13.4 A number of *F11* gene mutations. Missense mutations are the most common mutations within *F11* gene. Most of the mutations occur in the catalytic domain. *E* exon

Inhibitor formation against exogenous FXI is another problem in patients with FXI deficiency, mostly in severely affected patients. Patients with severe deficiency are at higher risk of inhibitor generation. FXI inhibitors are polyclonal IgG alloantibodies against various epitopes of the FXI molecule and inhibit FXI activation. The prevalence of antibodies in patients with FXI deficiency is 3–5%. Most patients with inhibitors are homozygotes, with <1% FXI plasma level and a positive history of replacement therapy or injection of RH immunoglobulin. Glu117Stop is the most common mutation in patients with FXI deficiency and inhibitors [8, 15, 19, 26].

FXI deficiency in the Jewish population is due to four common causative mutations that are categorized as types I to IV. Type I, a point mutation, occurs at the donor splice site of the last intron (-intron N) that is a G → A substitution. Type II, a nonsense mutation, GLU117stop in exon 5 results in early chain termination. Type III, a missense mutation, Phe283Leu substation in exon 9 results in the partial defect in dimerization and intracellular retention of FXI monomers [9]. Type IV is caused by a 14 bp deletion in exon 14/intron N splice site. Types II and III account for >90% of causative mutations in the Jewish population [10]. Other frequent mutations were observed in other populations including type II in Iraqi, Arab, and other Middle Eastern Jews. Type III mutation more frequently is found in recent European origin, Cys88Stopp mutation in French Basques, and the Cys128Stop mutation in the United Kingdom [11, 27]. More than 220 mutations were observed in *F11* gene.

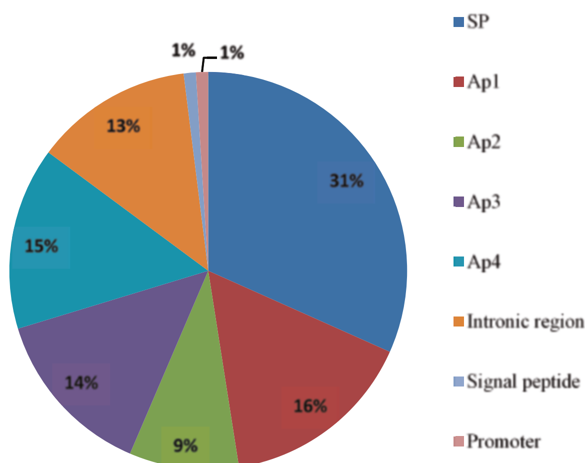

Fig. 13.5 Distribution of different mutations within *F11* gene. *Ap* apple domain, *SP* serin protease domain

FXI mutations include missense (67%), nonsense (11.9%), splice site (10.6%), deletion/insertion (9.4%), and promoter mutations (0.8%) [9, 28].

Most mutations occur at the catalytic domain with 32% frequency and then Ap1, Ap4, Ap3, intronic region, Ap2, signal peptide and linker region, respectively (Fig. 13.5) [19, 21, 22].

13.6 Clinical Manifestations

Patients with FXI deficiency present with variable clinical phenotypes. Patients with homozygote and compound heterozygote mutations usually have less FXI less than 15–20 U/dL, while heterozygotes usually have FXI levels between 20 and 70 U/dL (mild to moderate deficiency). Generally, there is no direct correlation between bleeding tendency and FXI plasma level, bleeding tendency is more profound in homozygotes and compound heterozygotes than heterozygotes, and heterozygotes are usually asymptomatic. Some homozygotes may not experience abnormal bleeding in their lives. FXI deficiency is usually detected in preoperative patient blood workup, in hemostatic challenges, and in patients with a positive family history of FXI deficiency. Although spontaneous bleeding is rare in this disorder, life-threatening bleeds after surgery or post-trauma may occur [28–30]. Excessive bleeding often occurs in tonsillectomy, dental extraction, and sinus surgery. There is a mild to moderate bleeding tendency in hemostatic fluctuations at the sites with high fibrinolytic activity such as oral cavity, nasal cavity, or genitourinary tract. Postpartum hemorrhage only occurs in ~20% of affected women. Abnormal bleeding in women due to obstetric and menstruation can occur [29–33]. Other contributing factors for bleeding tendency are type of mutations, plasmatic factors, platelet

Table 13.1 Clinical manifestations of patients with congenital factor XI deficiency

Bleeding symptom	Santoro et al. (N:95)	Shao et al. [34] (N:57)	Peyvandi et al. [35] (N:18)*
Ecchymosis	28		–
Epistaxis	24	5.3	27
Gastrointestinal bleeding	15	–	–
Hematuria	4	–	0
Hematoma	2	–	22
Menometrorrhagia	7 of women	7.5 of women	0.3 of women
Post-traumatic intracranial hemorrhage	1	–	–
Pulmonary hemorrhage	1	–	–
Gum bleeding	1	–	–
Bleeding from minor wounds	–	1.8	–
Postdental extraction bleeding	–	3.5	–
Postsurgical bleeding	–	2.5 of women	66
Hemarthrosis	–	–	27
Oral cavity bleeding	–	–	78
Easy bruising	–	8.8	–

*Severe/moderate factor XI deficiency

and endothelial disturbance, von Willebrand disease, hemophilia A or B, and other bleeding disorders [33]. These variable clinical symptoms make the diagnosis and management of FXI potentially difficult [36] (Table 13.1).

13.7 Laboratory Diagnosis of Factor XI Deficiency

13.7.1 Overview

Due to the mild phenotype of the disorder, diagnosis of FXI deficiency is difficult, and in most cases, diagnosis is made based on unexplained bleeding episodes, family history, or presurgery laboratory workup. Routine and specific coagulation tests can be used for an appropriate and timely diagnosis of the disorder. These tests include PT, APTT, FXI:C, and FXI:Ag assays. FXIIai DTT can determine thrombin-mediated FXI activation [19, 37, 38]. Other factor deficiencies, specific XI inhibitors, lupus anticoagulants, and other factors in the APTT assay that are commonly found as interferents, e.g., heparin should be excluded.

- Factor XI deficiency should be suspected when PT is normal and APTT is prolonged, and other intrinsic coagulation pathway factor deficiencies have been excluded. Differential diagnosis can be made by FXI:C and FXI:Ag assays.
- Factor XI activity, FXI:C, can be performed using a one-stage factor assay or chromogenic assay.
- FXI plasma level (FXI:Ag) assay can be performed by ELISA [39].
- FXI activity can be measured via a modified APTT and FXIIai DTT [38].

APTT reflects the FXII-mediated FXI activation, and this test is prolonged in FXI deficiency. Patient's plasma is mixed with normal plasma in a 1:1 ratio. The correction percentage shows the amount of FXI in patient's plasma [4].

Factor XIIa-inhibited dilute thromboplastin time (FXIIa iDTT): Rabbit Thromboplastin is diluted with normal saline to make different dilutions. Corn trypsin inhibitor (CTI) is an FXII inhibitor that is added immediately before the assay. Diluted thromboplastin and plasma are mixed in identical proportions and incubated at 37 °C for 2 min. Then, prewarmed $CaCl_2$ is added to the mixture and clotting time is measured. FXIIai DTT is markedly prolonged in severe FXI deficiency at 1:1000 concentration of thromboplastin and has a good correlation with the severity of bleeding [19, 38].

Thrombin generation assay and thromboelastometry can be used to measure the efficacy of treatment with FFP-SD or FXI concentrate in patients with FXI deficiency undergoing surgery and monitor the treatment with recombinant activated FVII (FVIIa) [40–43].

APTT mixing study is used to detect inhibitors in patients with severe FXI deficiency (<1%). The lack of APTT correction after mixing of patient and control plasma suggests the presence of an inhibitor. Incubation of patient and control plasma at varying dilutions from 100% patient plasma to 0% at 37 °C up to 2 h can be required. Repeating the APTT at 30-min intervals.

A specific inhibitor assay should be performed, e.g., modified Nijmegen Bethesda assay [44–46].

13.7.1.1 Functional Assay

The One-Stage APTT-Based FXI Activity Assay

A one-stage APTT-based FXI activity assay is used for the measurement of FXI activity in plasma. In this assay, the FXI activity level is measured based on the ability of test plasma to correct or shorten the APTT of FXI-deficient plasma. One-stage factor assay is normally fully automated even on small coagulation instrumentation.

Box 13.1 The Principle of the One-Stage APTT-Based FXI Activity Assay

Serial dilutions (1/10, 1/20, 1/40, etc.) of the standard plasma (a value of 100% of FXI) are prepared and mixed with an equal volume of substrate plasma (FXI-deficient plasma) and the APTT is measured for each dilution. Then the APTT values are plotted against the dilutions in the semi-log paper either manually or via computer. The test plasma (patient plasma) is also treated in the same way as standard plasma (i.e., preparation of the serial dilutions followed by mixing with substrate plasma). Following the addition of phospholipids and an activating agent, the calcium ions are added in order to start the coagulation reaction and the APTT is measured (Fig. 13.6). The results of APTT are plotted on the graph. Since the substrate plasma lacks the FXI, the difference between the dilutions of standard plasma and the test plasma is determined as the FXI activity level [46, 47].

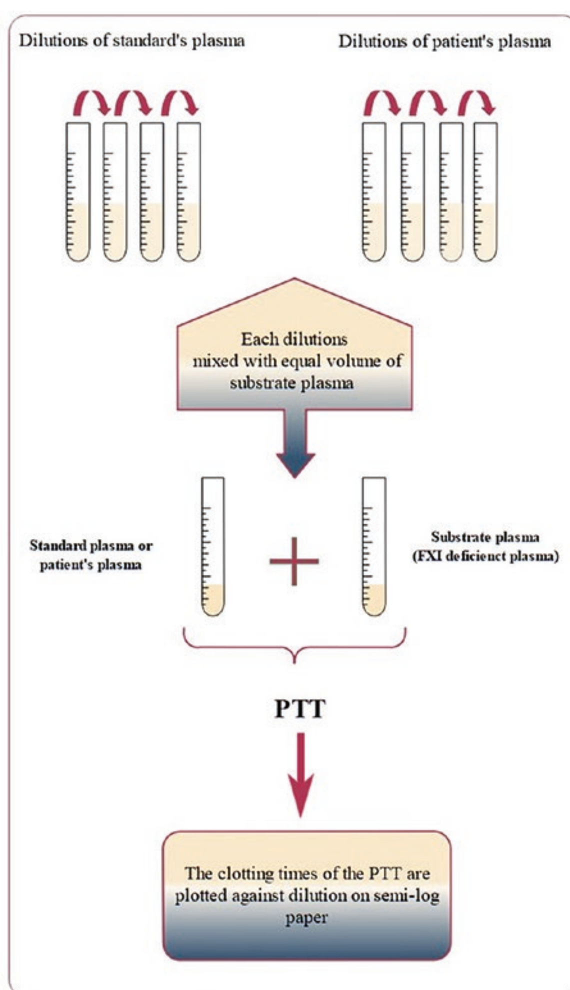

Fig. 13.6 The one-stage APTT-based FXI activity assay. In this assay, serial dilutions of standard and test plasma are prepared and mixed with equal volumes of substrate plasma. Following the mixing, the APTT is measured, and the results are plotted in the semi-log paper

Sample requirements: Blood should be collected into trisodium citrate anticoagulant with a concentration of 105–109 mmol/L (3.1–3.2%) and 1:9 ratio of blood: citrate. The collected blood was transported to the laboratory at a temperature of 18–25 °C in 4 h of collection. The samples can be kept at −20 °C for 2 weeks or at −40 to −70 °C for 3–6 months. Since thawing the frozen samples results in precipitation of the coagulation factors and abnormal results, the frozen sample should be thawed at 37 °C and inverted gently [47].

The interfering variables: This assay can be influenced by all the pre-analytical variables, which affect the APTT. Hemolysis, lipemia, icterus samples, underfilling tube, the presence of clot, and the use of incorrect sample tubes [48].

The Chromogenic Factor XI Assay

The chromogenic assay for measurement of the FXI:C in plasma is based on the two-stage assay and can be fully automated [49].

> **Box 13.2 The Principle of the Chromogenic Factor XI Assay**
> In this assay, plasma is treated with acetone to destroy inhibitors against FXIIa and FXIa in the plasma. Then the contact system is activated with an activating agent (kaolin), which results in the activation of FXI by FXIIa. Following the activation step, the FXIIa is inhibited with CTI. Then the FXI level in the plasma is determined by the ability of FXI to cleave the chromogenic substrate (substrate-pNA) and release p-nitroaniline (pNA). This can be measured photometrically; the intensity is proportional to the FXI concentration (Fig. 13.7).

Sample requirement: The plasma is collected in the 105–109 mmol/L (3.1–3.2%) concentration of Trisodium citrate. The plasma samples should be assayed within 2 h of blood collection or stored frozen at −20 °C.

Factor XII-Inhibited Dilute Thromboplastin Time

The FXIIai DTT-based assay reflects the thrombin generation of FXIa and is used for the determination of FXI:C [38].

> **Box 13.3 The Principle of the Factor XII-Inhibited Dilute Thromboplastin Time**
> In this assay, different dilutions of thromboplastin in saline are prepared. Before starting the assay, in order to inhibit FXII, CTI is added. The diluted thromboplastin and plasma are mixed and incubated for 2 min at 37 °C. Following the incubation, the prewarmed Cacl2 is added and the clotting time is recorded. In patients with FXI deficiency, the FXII ai DTT is prolonged at a 1:1000 concentration of thromboplastin and has a better correlation between FXI level and the severity of bleeding episodes.

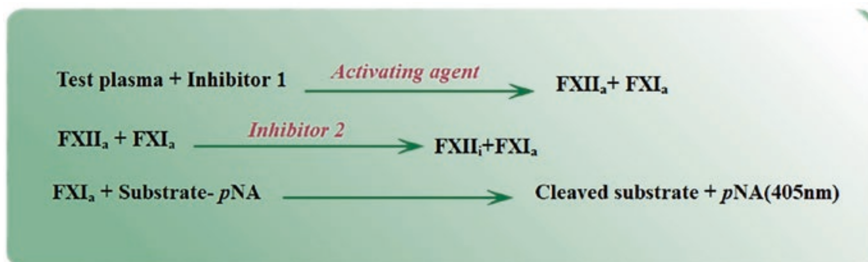

Fig. 13.7 The principle of the chromogenic factor XI assay

13.7.1.2 Immunological Factor XI Assay

Although measurement of FXI antigen level is not a routine assay in most of the laboratories, distinguishing between the quantitative and qualitative defects of FXI deficiency is necessary. The concentration of FXI antigen in plasma, serum, or other biological fluids is estimated with an ELISA assay.

Box 13.4 The Principle of Factor XI Immunological Assay

In this test, a specific monoclonal antibody against FXI is adsorbed onto the wells of the 96-well microtiter plate. Following the preparation of dilution of the plasma sample and addition to the wells, the FXI antigen in the plasma binds to the precoated antibody. After appropriate washing steps for omitting the unbound conjugates, the peroxidase-labeled detecting antibody is added and binds to the captured FXI. Following another washing step, a solution of TMB (the peroxidase substrate, tetramethylbenzidine) is applied. TMB is catalyzed by streptavidin-peroxidase and develops blue-colored products that change to yellow upon quenching the reaction by acid (Fig. 13.8).

The color developed is measured spectrophotometrically at 450 nm. The absorbance at 450 nm is directly proportional to the quantity of FXI antigen captured on the plate (Fig. 13.9) [19, 49].

Sample requirement: The required samples are in a standard 105–109 mmol/L (3.1–3.2%) concentration of citrate anticoagulants. The assay should be done immediately or in 5 days when the samples are stored at 2–8 °C. Otherwise, the samples should be kept at −20 °C (≤1 month) or −80 °C (≤2 months). Before performing the assay, samples should be defrosted at 37 °C for 5 min and gently inverted to mix.

Interfering variables: Hemolysis, Lipemia, and Icterus blood samples can affect the result. However, the conditions of the performing assay, including the operator, pipetting and washing, and temperature of the laboratory, can affect the results of the test.

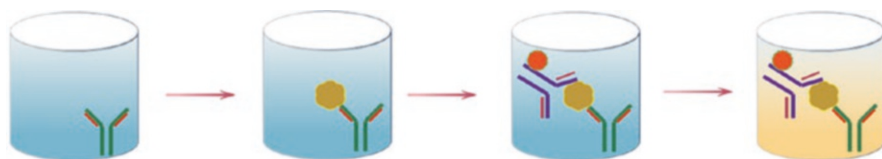

Fig. 13.8 The principle of the FXI antigen assay. The antibodies against FXI is precoated. Following the addition of the test plasma, the FXI binds to precoated antibodies. Then the labeled binds to the captured FXI. Following the addition of TMB solution, the yellow color developed, which directly related to FXI

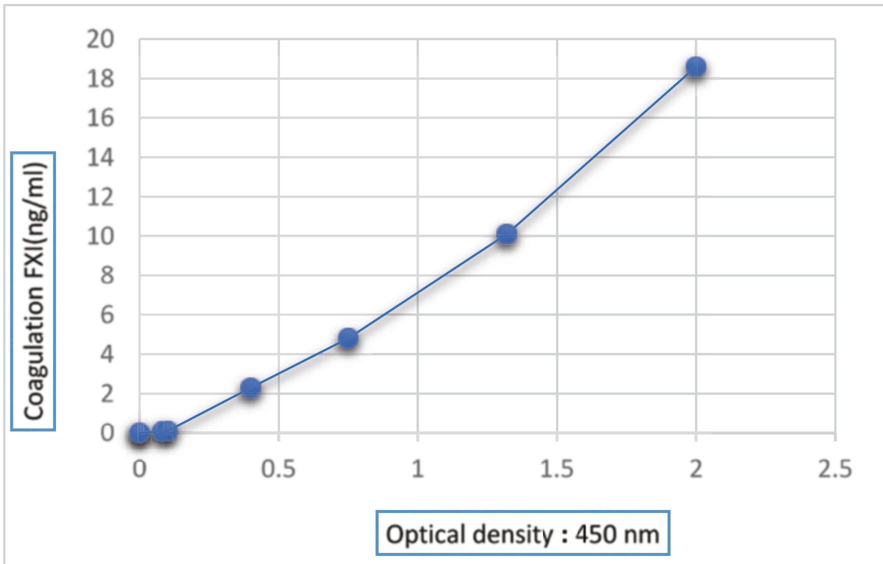

Fig. 13.9 Typical standard curves for measurement of FXI antigen level. In order to determine the FXI antigen level in the plasma sample, the OD values of the standard are plotted against the known concentration of the standard plasma

13.8 Global Hemostasis Assays

Since the bleeding tendency in patients with FXI deficiency is unpredictable and there is a lack of relationship between the FXI activity level and the severity of bleeding episodes, the use of reliable assays is required to correctly identify patients who are prone to bleeding [49]. Thrombin generation assay and thromboelastography/rotational thromboelastometry are widely used for the assessment of bleeding tendency in congenital FXI deficiency. Furthermore, these assays are used for the determination of the treatment's effectiveness, while the coagulation assays are not able to correlate with the severity of bleeding. However, due to the small sample size, the variable FXI activity level, and conflicting results in different studies, the ability of these assays to predict the bleeding tendency is a matter of debate depending on the sample conditions [49].

13.9 Molecular Diagnosis

FXI deficiency can be affected by some specific mutations including heterozygous large deletions mutations which are located in regions that cannot be detected by conventional mutational screenings (promoter region far from the transcription start site or deep intronic mutations). The other mutations in *F11* gene are the nucleotide

variations including those that occur in untranslated regions of the mRNA and alter the regulation of the gene expression; therefore, molecular diagnosis of FXI deficiency is a challenge [49]. However, more than 220 mutations were reported in *F11* gene. These mutations include missense as the most common type of mutation (67%) followed by nonsense (11.9%), splice site (10.6%), deletion/insertion (9.4%), and promoter mutations (0.8%) [49]. There are four common types of causative mutations in the Jewish population that are commonly affected by FXI deficiency. Type I is a point mutation (G → A substitution) that occurs at the donor splice site of the last intron. Type II is a nonsense mutation in exon 5 (Glu117stop) that results in early termination. Type III is another missense mutation that occurs in exon 9 (Phe283Leu) causing a partial defect in dimerization [49]. Type IV which results from a 14-bp deletion in exon 14/intron N splice site. Among all, types II and III accounted for more than 90% of the causative mutations in the Jewish population. Moreover, type II mutation is reported in Iraqi and Arab populations [10, 49]. Type III mutation, Cys88Stopp, and Cys128Stop are more frequently reported in the population of European origin, French Basques, and United Kingdom, respectively [11, 27].

13.10 Recommendations and Precautions in Laboratory Investigation

Hemolytic, Icterus, and lipemic plasma may affect some coagulation tests in a number of automated analyzers. Fasting is not necessary for routine coagulation tests. Patients should avoid stressful conditions, strenuous exercise, and some drugs that can affect the results before blood samples are taken for investigation. Anticoagulant drugs can also affect the results of coagulation tests [50].

Different APTT reagents have been shown to give rise to variations in the APTT sensitivity; therefore, reference ranges should be established locally [51–53].

Some mutations such as large deletions or mutations in those regions that are not covered by conventional mutational screening may not be detected and should be analyzed either by reverse transcription–PCR on suitable patient specimens (when available) or by using in vitro approaches based on the transfection of appropriate minigene construct containing the nucleotide variation under investigation [25, 54].

Differential diagnoses should exclude the presence of lupus anticoagulant, liver dysfunction, heparin contamination, von Willebrand disease, and other coagulation factor deficiencies or coagulation factor inhibitors [19].

13.11 Treatment

Most of the patients with FXI deficiency have few bleeding problems during their life. Spontaneous bleeding except for menorrhagia is rare and usually subsides with treatment. The mainstay of treatment in FXI deficiency, like most other rare bleeding disorders, is on-demand treatment to stop the bleeding as soon as possible. The

presence of FXI inhibitors should be excluded in patients with severe deficiency (<1%), particularly those that have received plasma, FXI concentrates, or immunoglobulin. Assessment of thrombotic risk in specific types of surgeries should carefully be evaluated when selecting specific treatment options. Age and underlying diseases such as heart and renal disorders can also affect the treatment [4, 55, 56].

For patients undergoing major surgery, replacement therapy with FFP-SD or FXI concentrate is used to achieve trough levels of 45 U/dL for 5–7 days prior to surgery. In areas with high fibrinolytic activity such as nose, tonsils, oral cavity, and urinary tract the use of antifibrinolytics should be used. The use of FFP-SD may result in circulatory overload in patients with congestive heart failure and chronic renal failure. **FXI concentrates** have several advantages including shorter infusion times, the level of other coagulation factors is not increased, and a lower rate of transfusion-related reactions. Special precautions should be considered when these products are used in elderly adults and patients with cardiovascular disease because of their potent thrombotic risk. The dose should not exceed 30 U/kg, and peak FXI levels should not exceed 70 U/dL. Tranexamic acid should be avoided in patients receiving FXI concentrates because it may pose a thrombotic risk [55, 56]. **Recombinant FVII (rFVII)** can be used in patients with FXI deficiency and inhibitors (see below). Replacement therapy is not necessary for minor surgeries. For minor surgeries such as tooth extraction and cataract extraction, antifibrinolytic agents such as **tranexamic acid** at a dose of 1 g daily for 12 h before surgery until 7 days after surgery are suitable. Epsilon aminocaproic acid can be used at 5–6 g four times daily for the same period of time [55, 56]. **Fibrin glue** can be used in resection of skin lesions and local hemostasis; however, there is a lack of clinical trials showing efficacy and risk [55, 56]. Prophylactic treatment is not necessary in patients without a bleeding history. **Desmopressin (DDAVP)**, a synthetic analog of the natural antidiuretic hormone vasopressin, as a supplementary agent can elevate endogenous FXI level that can be used in patients with a bleeding history (refer to Chap. 3). Indeed, desmopressin can normalize coagulation parameters in heterozygous patients with FXI deficiency by slightly increasing both FXI activity and antigen levels. Replacement therapy is not necessary in patients undergoing surgery without a history of bleeding tendency, but tranexamic acid and/or fibrin glue is used in high-risk surgeries such as prostatectomy [12, 56].

Menorrhagia: *Oral antifibrinolytic agents* such as **tranexamic acid** are used in women with prolonged menorrhagia [57].

Pregnant women: Women with partial FXI deficiency without previous bleeding history do not mean that they will not bleed during pregnancy. Bleeding assessment tools should be used and can help in targeting women with potential bleeding risk. In women with a significant bleeding history, tranexamic acid is used for 3 days with the first dose being administered during vaginal delivery. FXI concentrate can be prescribed for women with severe FXI deficiency during vaginal delivery or cesarean section. Recombinant VIIa can also be used for severe FXI deficiency. Tranexamic acid can be used in FXI deficiency patients with levels 15–40% either having a vaginal or c-section birth. As with all of the potential treatments, there is a

potential thrombotic risk in this patient group. FFP-SD is advisable only when there is excessive bleeding during cesarean or vaginal delivery [30, 32, 36].

Circumcision: FXI deficiency may be observed first in this procedure. It is important to check newborn males in areas with high rates of FXI deficiency such as Ashkenazi and Iraqi Jewish populations. For diagnosis of FXI deficiency in infants, a factor FXI level of <10 U/dL is indicative of deficiency. FXI level should be rechecked until 6 months. If the FXI level remained low, the procedure should be performed by cover of either FXI concentrate or FFP-SD. The boys with FXI levels >10 U/dL should receive tranexamic acid at a dose of 15 mg/kg every 8 h for 3 days under circumcision procedures [30, 36, 58].

Patients with an inhibitor: Patients with severe FXI deficiency may develop an inhibitor after replacement therapy. **RFVIIa** or **prothrombin complex** is used in these patients RFVIIa is effective at a low dose of 15–30 μg/kg with **oral tranexamic acid** for major surgeries. It is also used in patients with a history of allergic reactions to replacement therapy or those with IgA deficiency. **Epsilon aminocaproic acid** is used at a dose of 5–6 g four times daily [59, 60].

References

1. O'Connell NM. Factor XI deficiency-from molecular genetics to clinical management. Blood Coagul Fibrinolysis. 2003;14:S59–64.
2. Peyvandi F, Lak M, Mannucci PM. Factor XI deficiency in Iranians: its clinical manifestations in comparison with those of classic hemophilia. Haematologica. 2002;87(5):512–4.
3. Asakai R, Chung DW, Davie EW, Seligsohn U. Factor XI deficiency in Ashkenazi jews in Israel. N Engl J Med. 1991;325(3):153–8.
4. Lewandowska MD, Connors JM. Factor XI deficiency. Hematol Oncol Clin. 2021;35(6):1157–69.
5. Lupo H, Lanir N, Brenner B, Shpilberg O, Seligsohn U, Peretz H, et al. The two common mutations causing factor XI deficiency in Jews stem. Blood. 1997;90(7):2654–9.
6. Bolton-Maggs PH. Factor XI deficiency—resolving the enigma? ASH Educ Program Book. 2009;2009(1):97–105.
7. Saunders RE, O'Connell NM, Lee CA, Perry DJ, Perkins SJ. Factor XI deficiency database: an interactive web database of mutations, phenotypes, and structural analysis tools. Hum Mutat. 2005;26(3):192–8.
8. Salomon O, Zivelin A, Livnat T, Dardik R, Loewenthal R, Avishai O, et al. Prevalence, causes, and characterization of factor XI inhibitors in patients with inherited factor XI deficiency. Blood. 2003;101(12):4783–8.
9. Hancock JF, Wieland K, Pugh RE, Martinowitz U, Schulman S, Kakkar VV, et al. A molecular genetic study of factor XI deficiency. Blood. 1991;77(9):1942–8.
10. Shpilberg O, Peretz H, Zivelin A, Yatuv R, Chetrit A, Kulka T, et al. One of the two common mutations causing factor XI deficiency in Ashkenazi Jews (type II) is also prevalent in Iraqi Jews, who represent the ancient gene pool of Jews. Blood. 1995;85:429.
11. Zivelin A, Bauduer F, Ducout L, Peretz H, Rosenberg N, Yatuv R, et al. Factor XI deficiency in French Basques is caused predominantly by an ancestral Cys38Arg mutation in the factor XI gene. Blood. 2002;99(7):2448–54.
12. Franchini M, Manzato F, Salvagno GL, Montagnana M, Lippi G. The use of desmopressin in congenital factor XI deficiency: a systematic review. Ann Hematol. 2009;88(10):931–5.
13. Emsley J, McEwan PA, Gailani D. Structure and function of factor XI. Blood. 2010;115(13):2569–77.

14. Jin L, Pandey P, Babine RE, Gorga JC, Seidl KJ, Gelfand E, et al. Crystal structures of the FXIa catalytic domain in complex with ecotin mutants reveal substrate-like interactions. J Biol Chem. 2005;280(6):4704–12.
15. Berber E. Molecular characterization of FXI deficiency. Clin Appl Thromb Hemost. 2011;17(1):27–32.
16. Cheng Q, Sun MF, Kravtsov D, Aktimur L, A, Gailani D. Factor XI apple domains and protein dimerization. J Thromb Haemost. 2003;1(11):2340–7.
17. Gailani D, Smith S. Structural and functional features of factor XI. J Thromb Haemost. 2009;7:75–8.
18. Bouma BN, Griffin JH. Human blood coagulation factor XI. Purification, properties, and mechanism of activation by activated factor XII. J Biol Chem. 1977;252(18):6432–7.
19. He R, Chen D, He S. Factor XI: hemostasis, thrombosis, and antithrombosis. Thromb Res. 2012;129(5):541–50.
20. Sinha D, Marcinkiewicz M, Lear JD, Walsh PN. Factor XIa dimer in the activation of factor IX. Biochemistry. 2005;44(30):10416–22.
21. McVey JH, Lal K, Imanaka Y, Kemball-Cook G, Bolton-Maggs PH, Tuddenham EG. Characterisation of blood coagulation factor XIT475I. Thromb Haemost. 2005;93(06):1082–8.
22. Zucker M, Zivelin A, Landau M, Rosenberg N, Seligsohn U. Three residues at the interface of factor XI (FXI) monomers augment covalent dimerization of FXI. J Thromb Haemost. 2009;7(6):970–5.
23. Smith SB, Verhamme IM, Sun M-f, Bock PE, Gailani D. Characterization of novel forms of coagulation factor XIa: independence of factor XIa subunits in factor IX activation. J Biol Chem. 2008;283(11):6696–705.
24. Hoffman M. A cell-based model of coagulation and the role of factor VIIa. Blood Rev. 2003;17:S1–5.
25. Dai L, Rangarajan S, Mitchell M. Three dominant-negative mutations in factor XI-deficient patients. Haemophilia. 2011;17(5):e919–e22.
26. Zucker M, Zivelin A, Teitel J, Seligsohn U. Induction of an inhibitor antibody to factor XI in a patient with severe inherited factor XI deficiency by Rh immune globulin. Blood. 2008;111(3):1306–8.
27. Bolton-Maggs P, Peretz H, Butler R, Mountford R, Keeney S, Zacharski L, et al. A common ancestral mutation (C128X) occurring in 11 non-Jewish families from the UK with factor XI deficiency. J Thromb Haemost. 2004;2(6):918–24.
28. Moser SS, Chodick G, Ni YG, Chalothorn D, Wang M-D, Shuldiner AR, et al. The association between factor XI deficiency and the risk of bleeding, cardiovascular, and venous thromboembolic events. Thromb Haemost. 2022;122(05):808–17.
29. Bolton-Maggs P, Patterson D, Wensley R, Tuddenham E. Definition of the bleeding tendency in factor XI-deficient kindreds–a clinical and laboratory study. Thromb Haemost. 1995;73(02):194–202.
30. Santoro C, Di Mauro R, Baldacci E, De Angelis F, Abbruzzese R, Barone F, et al. Bleeding phenotype and correlation with factor XI (FXI) activity in congenital FXI deficiency: results of a retrospective study from a single Centre. Haemophilia. 2015;21(4):496–501.
31. Salomon O, Steinberg DM, Tamarin I, Zivelin A, Seligsohn U. Plasma replacement therapy during labor is not mandatory for women with severe factor XI deficiency. Blood Coagul Fibrinolysis. 2005;16(1):37–41.
32. Wheeler AP, Hemingway C, Gailani D. The clinical management of factor XI deficiency in pregnant women. Expert Rev Hematol. 2020;13(7):719–29.
33. Bolton-Maggs P, Perry D, Chalmers E, Parapia L, Wilde J, Williams M, et al. The rare coagulation disorders–review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. Haemophilia. 2004;10(5):593–628.
34. Shao Y, Cao Y, Lu Y, Dai J, Ding Q, Wang X, Xi X, Wang H. Clinical manifestations and mutation spectrum of 57 subjects with congenital factor XI deficiency in China. Blood Cells, Molecules, and Diseases. 2016;1:58:29–34.

35. Peyvandi F, Lak M, Mannucci PM. Factor XI deficiency in Iranians: its clinical manifestations in comparison with those of classic hemophilia. Haematologica. 2002;1;87(5):512–4.
36. Mumford AD, Ackroyd S, Alikhan R, Bowles L, Chowdary P, Grainger J, et al. Guideline for the diagnosis and management of the rare coagulation disorders: a United Kingdom Haemophilia Centre Doctors' Organization guideline on behalf of the British Committee for Standards in Haematology. Br J Haematol. 2014;167(3):304–26.
37. Wheeler AP, Gailani D. Why factor XI deficiency is a clinical concern. Expert Rev Hematol. 2016;9(7):629–37.
38. He R, Xiong S, He X, Liu F, Han J, Li J, et al. The role of factor XI in a dilute thromboplastin assay of extrinsic coagulation pathway. Thromb Haemost. 2001;85(06):1055–9.
39. Wilmot H, Hockley J, Rigsby P, Gray E. Establishment of the World Health Organization 2nd international standard for factor XI, plasma, human. Front Med. 2017;4:28.
40. Pike G, Cumming A, Thachil J, Hay C, Bolton-Maggs P, Burthem J. Evaluation of the use of rotational thromboelastometry in the assessment of FXI deficency. Haemophilia. 2017;23(3):449–57.
41. Pike GN, Cumming A, Thachil J, Hay CR, Burthem J, Bolton-Maggs PH. Evaluation of the use of global haemostasis assays to monitor treatment in factor XI deficiency. Haemophilia. 2017;23(2):273–83.
42. Désage S, Dargaud Y, Meunier S, Le Quellec S, Lienhart A, Negrier C, et al. Report of surgeries, their outcome and the thrombin generation assay in patients with factor XI deficiency: a retrospective single-Centre study. Haemophilia. 2022;28(2):301–7.
43. Riddell A, Abdul-Kadir R, Pollard D, Tuddenham E, Gomez K. Monitoring low dose recombinant factor VIIa therapy in patients with severe factor XI deficiency undergoing surgery. Thromb Haemost. 2011;106(09):521–7.
44. Franchini M, Marano G, Mengoli C, Piccinini V, Pupella S, Vaglio S, et al., editors. Inhibitors in patients with congenital bleeding disorders other than hemophilia. Seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2018.
45. Franchini M, Lippi G, Favaloro EJ, editors. Acquired inhibitors of coagulation factors: part II. Seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2012.
46. Bortoli R, Monticielo OA, Chakr RM, Palominos PE, Rohsig LM, Kohem CL, et al., editors. Acquired factor XI inhibitor in systemic lupus erythematosus—case report and literature review. Seminars in arthritis and rheumatism. Elsevier; 2009.
47. Kitchen S, Adcock DM, Dauer R, Kristoffersen AH, Lippi G, Mackie I, et al. International Council for Standardization in Haematology (ICSH) recommendations for processing of blood samples for coagulation testing. Int J Lab Hematol. 2021;43(6):1272–83.
48. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med. 2008;46(6):764–72.
49. Rodgers S, Duncan E. Chromogenic factor VIII assays for improved diagnosis of hemophilia A. Hemostasis and thrombosis. Springer; 2017. p. 265–76.
50. Lippi G, Salvagno GL, Montagnana M, Lima-Oliveira G, Guidi GC, Favaloro EJ, editors. Quality standards for sample collection in coagulation testing. Seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2012.
51. Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, et al. WFH guidelines for the management of hemophilia. Haemophilia. 2020;26:1–158.
52. Lawrie A, Kitchen S, Purdy G, Mackie I, Preston F, Machin S. Assessment of actin FS and actin FSL sensitivity to specific clotting factor deficiencies. Clin Lab Haematol. 1998;20(3):179–86.
53. Salloum-Asfar S, María E, Esteban J, Miñano A, Aroca C, Vicente V, et al. Assessment of two contact activation reagents for the diagnosis of congenital factor XI deficiency. Thromb Res. 2018;163:64–70.
54. Podmore A, Smith M, Savidge G, Alhaq A. Real-time quantitative PCR analysis of factor XI mRNA variants in human platelets. J Thromb Haemost. 2004;2(10):1713–9.
55. Tabatabaei T, Dorgalaleh A. Congenital factor XI deficiency. Congenital bleeding disorders. Springer; 2018. p. 291–306.

56. Duga S, Salomon O, editors. Congenital factor XI deficiency: an update. seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2013.
57. Seligsohn U. Factor XI deficiency in humans. J Thromb Haemost. 2009;7:84–7.
58. Davies J, Kadir R, editors. The management of factor XI deficiency in pregnancy. Seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2016.
59. Kenet G, Lubetsky A, Luboshitz J, Ravid B, Tamarin I, Varon D, et al. Lower doses of rFVIIa therapy are safe and effective for surgical interventions in patients with severe FXI deficiency and inhibitors. Haemophilia. 2009;15(5):1065–73.
60. Livnat T, Tamarin I, Mor Y, Winckler H, Horowitz Z, Korianski Y, et al. Recombinant activated factor VII and tranexamic acid are haemostatically effective during major surgery in factor XI-deficient patients with inhibitor antibodies. Thromb Haemost. 2009;102(09):487–92.