Congenital Factor II Deficiency

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7.1 Introduction

Coagulation factor (F) II (prothrombin) is a vitamin K-dependent coagulation factor which plays a pivotal role in blood coagulation cascade. Prothrombin is a 72-kDa glycoprotein that synthesized as an inactive zymogen by hepatocytes [1]. Prothrombin is activated to thrombin by prothrombinase complex which consists of the activated FX (FXa), FVa, and calcium [2] (please refer to Chap. 1). Thrombin is a multifunctional enzyme which converts fibrinogen to fibrin in the blood coagulation cascade. In addition, this protein has different roles including platelet, FXIII, FV, FVIII, and protein C activation [3, 4]. Congenital FII deficiency which was first reported by Quick in 1947 is the rarest autosomal recessive coagulation disorder (similar to FXIII deficiency, please refer to Chap. 13) with incidence of 1:2 million in the general population. This disorder mostly presents in regions which parental consanguinity is commonly practiced [5]. Based on the FII activity level, prothrombin deficiency is classified into three groups including mild, moderate, and severe forms with >10%, <10%, and undetectable level of FII activity, respectively [6]. Moreover, this disorder can be classified into four main phenotypes including hypoprothrombinemia (type I deficiency) with concomitant decrease of both FII activity and antigen levels, dysprothrombinemia (type II deficiency) with normal or slightly decreased level of FII antigen, but low level of activity, hypo–dys or dys–dys forms, and combined deficiency of prothrombin and other γ-carboxylated coagulation factors [6, 7]. The most common clinical manifestations of this disorder are mucosal

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bleeding, hematoma, and post-surgical bleeding [3]. Life-threatening bleedings including central nervous system (CNS) bleeding, gastrointestinal (GI) bleeding, and umbilical cord (UC) bleeding are rarely presentations in affected patients [3, 8]. About 60 causing mutations in *F2* gene are identified; 80% of them are missense. Other mutations are insertion/deletion (10%), nonsense (6%), and splice site mutations (4%) [9]. FII deficiency is diagnosed based on family history, clinical manifestations, and routine and specific coagulation laboratory tests. FII deficiency is suspected through prolonged prothrombin time (PT) and activated thromboplastin time (aPTT) and confirmed by FII assays. The one-stage PT-based assay is the most commonly used FII activity assay technique [3]. Since there is no specific available prothrombin concentrates, prothrombin complex concentrate (PCC) and fresh frozen plasma (FFP) are the treatments of choice [10].

7.2 Coagulation Factor II (Prothrombin) Structure

Coagulation FII (prothrombin) is a vitamin K-dependent glycoprotein which plays a pivotal role in blood coagulation system. Prothrombin is a 72-KDa glycoprotein and synthesized in the liver and needs posttranslational carboxylation to become active. This protein circulates in blood stream at the concentration of 0.1 mg/mL with half-life of 60 h [6].

Prothrombin mapped to centromeric region of 11p11-q12 with length about 21 kb and composed of 14 exons and 13 introns (Fig. 7.1). This protein consists of 4 fragments and 579 amino acid residues including gamma carboxyglutamic (Gla) domain (residues 1–46), kringle-1 (residues 65–143), kringle-2 (residues 170–248), and protease domain (residues 285–579). Protease domain includes chain A (residues 285–320) and chain B (residues 321–579) [1].

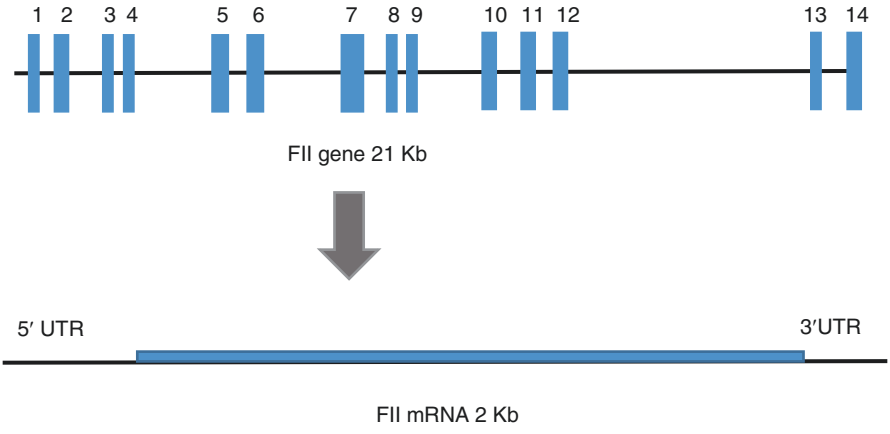

Fig. 7.1 Factor (F) II structure. The schematic presentation of *F2* gene. The *F2* gene composed of 14 exons and 13 introns covering a 21 kb region. This protein is mapped on short arm of chromosome 11 (11p11-q12)

Thrombin is highly homologous with serine proteinases including chymotrypsin. Crystal structure of thrombin shows that this protein consists of the active site, exosites I and II, and different loops including γ-loop, 60-loop, and loop which contains Na^+ binding site [11]. Thrombin-like chymotrypsin has serine residue (Ser195) which in conjugation with other residues including His57 and Asp189 forms the active site that is necessary for target peptide bond's nucleophilic attack [12]. In addition, surface of this thrombin is exposed to loops and is charged patched which has known as exosites around various residues in the active site. Thrombin contains two exosites including I and II which centered different residues including Lys36, His71, Arg73, Arg75, Tyr76, Arg77a, Lys109/110 and Arg93, Lys236, Lys 240, Arg101, Arg233, respectively. The roles of exosites are interaction with thrombin's cofactor and substrates [13, 14].

Moreover, thrombin contains different loops such as γ-loop and 60-loop that surround the active site. The 60-loop, which causes structural rigidity, interacts with residues in substrate's amino-terminal side, while the γ-loop which is more mobile interacts with substrate's residues in the carboxyl-terminal side. In addition, thrombin has another loop which contains Na^+ binding sites. This loop influences thrombin function allosterically by promoting the binding and hydrolysis of its substrates. These substrates include fibrinogen, FV, FVIII, and PAR1 [11, 15].

7.3 Synthesis of Prothrombin

Initially prothrombin is produced in the liver as a prepro-prothrombin which consists of signal peptide. Following the removal of signal peptide by signal peptidase, pro-prothrombin is generated. Pro-prothrombin has a Gla domain which contains ten glutamic acid residues in the N-terminal region [16]. Vitamin K-dependent carboxylase catalyzes the conversion of all ten glutamic acid residues of pro-prothrombin to Gla (γ-carboxylation) and produces the prothrombin (Fig. 7.2). Gla domain is involved in binding of prothrombin to the anionic phospholipid surfaces on the activated platelets and also on vascular injuries in the presence of Ca^{++} [1, 17, 18]. Following the γ-carboxylation, the propeptide is removed and the mature zymogen is generated. Then three N-linked carbohydrates were added which are located in kringle-1 and serine protease domain [16, 19].

7.4 Thrombin Generation

Prothrombin activation which leads to α-thrombin generation is a critical step in coagulation cascade. α-thrombin generation is mediated by prothrombinase complex which is composed of FXa and its cofactor FVa that are assembled in negatively charge surface provided by activated platelet in the presence of Ca^{++} ions (please refer to Chap. 1) [2].

Although FXa is capable to catalyze this process, the rate of prothrombin activation is markedly low. When prothrombinase complex is formed, the rate of

prothrombin activation is raised about 10^5-fold [20]. Prothrombin activation occurs by cleavages in two sites. The first cleavage occurs in Arg271 residue (between kringle-2 and A chain) and generates inactive intermediate fragment 1.2 and prothrombin-2. The second cleavage occurs at Arg320 residue and leads to conversion of prothrombin-2 to α-thrombin. In an alternative pathway, the first cleavage occurs in Arg320 residue which leads to generation of active intermediate meizothrombin (mIIa) followed by cleavage at Arg271 and generation of α-thrombin [1, 21]. The rate of prothrombin activation is controlled through cofactor Va and phospholipids. In the absence of FVa, prothrombin activates via prothrombin-2 pathway, while in the presence of FVa, the preferred pathway is meizothrombin intermediate (Fig. 7.2) [22].

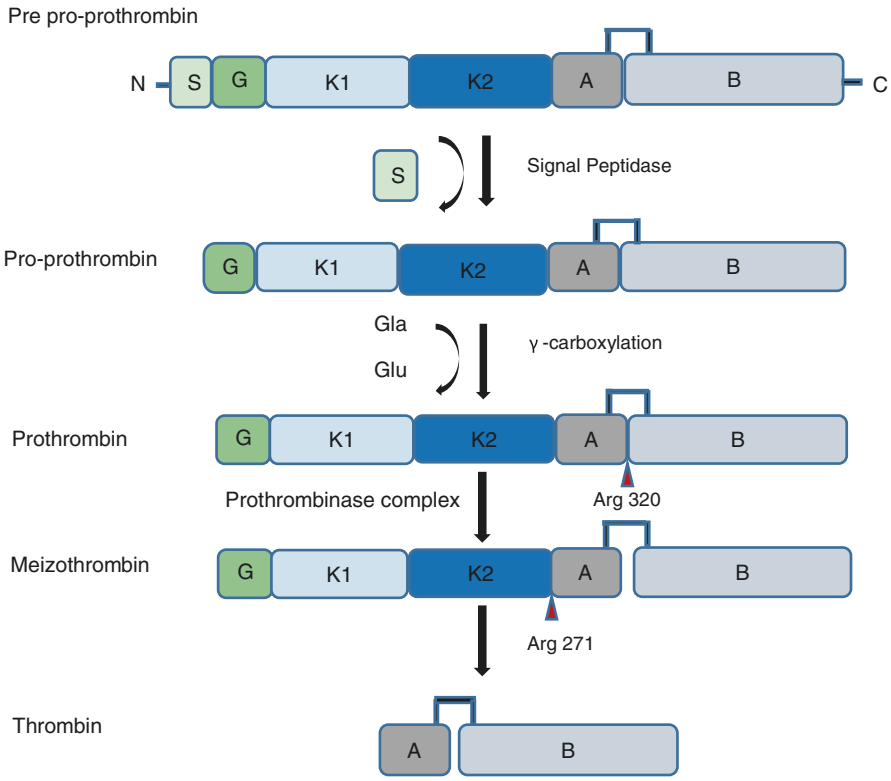

Fig. 7.2 Prothrombin synthesis and thrombin generation. Prepro-prothrombin is a precursor of prothrombin which synthesized in the liver. Following the removal of signal peptide by signal peptidase, the pro-prothrombin which consists of Gla domain is generated. The vitamin K-dependent carboxylase catalyzes the conversion of glutamic acid residues of pro-prothrombin to Gla (γ-carboxylation), and prothrombin is produced. Prothrombinase complex which consists of FXa and its cofactor (FVa) cleaves prothrombin and thrombin is produced. In an alternative pathway, prothrombinase complex cleaves prothrombin in two sites; the first cleavage occurs at Arg320 residue, and active intermediate meizothrombin is generated and the second cleavage occurs at Arg271 residue that results in conversion of meizothrombin to α-thrombin. *S* signal peptide, *G* gamma-carboxyglutamic acid-rich domain, *K1* kringle-1 domain, *K2* kringle-2 domain

The proteolytically active thrombin comprised of two polypeptides, the A-chain (36 residues) and the B-chain (259 residues) which are covalently linked by single disulfide bond through the Cys^{1}-Cys^{122} [23]. Autoproteolysis of thrombin in the A-chain at position of Arg284-Thr285 leads to removal of 13-residue N-terminal peptide of A-chain and generation of α-thrombin. In solution, α-thrombin automatically undergoes cleavage of the Arg62-Ile and Arg73-Asn bonds in B-chain and subsequently in the Arg123-Glu and Lys154-Gly bonds, giving rise to formation of β-thrombin and γ-thrombin, respectively. These forms of thrombin are much less active in comparison with α-thrombin [12, 16, 24].

7.5 Hemostatic Roles of Thrombin

Thrombin is a multifunctional serine protease which is involved in regulation of numerous pathophysiological coagulation and inflammation processes [4].

7.5.1 Fibrin Formation

The primary role of thrombin is conversion of soluble fibrinogen into insoluble fibrin. During this reaction, thrombin binds to the central E nodule of fibrinogen and cleaves four specific Arg-Gly bonds at the N-terminal of both Aα and Bβ chains, leading to the release of fibrinopeptide A (FPA) and B (FPB), respectively (Fig. 7.3). Following the cleavage of FPA, a fibrin monomer which is also termed as fibrin I is formed. Then fibrin I spontaneously polymerizes to protofibrils. Cleavage of FPB results in generation of fibrin II protofibrils. The deposition of fibrin leads to formation of extensive meshwork which by surrounding the platelet forms the stabilized clot [25, 26].

7.5.2 Factor XIII Activation

In the final stage of blood coagulation, thrombin activates FXIII by cleavage of an activating peptide on the A subunit of FXIII (FXIII-A) after Arg37 (Fig. 7.4). Following the cleavage, the activation peptide is released and the active site is exposed. Therefore, the transglutaminase cross-linked fibrin fibrils and increases clot's mechanical strength (please refer to Chap. 13) [27].

7.5.3 Factor V and Factor VIII Activation

A small amount of thrombin which is generated on the tissue factor-bearing cell acts as a positive feedback of coagulation cascade via activation of FV and FVIII. Following the activation of these two factors, the function of FXa and FIXa is enhanced and therefore leads to increased and more sustained thrombin generation and FXa formation. Thrombin activates these two factors by cleavage of them which is followed by removal of B domain [28]. The

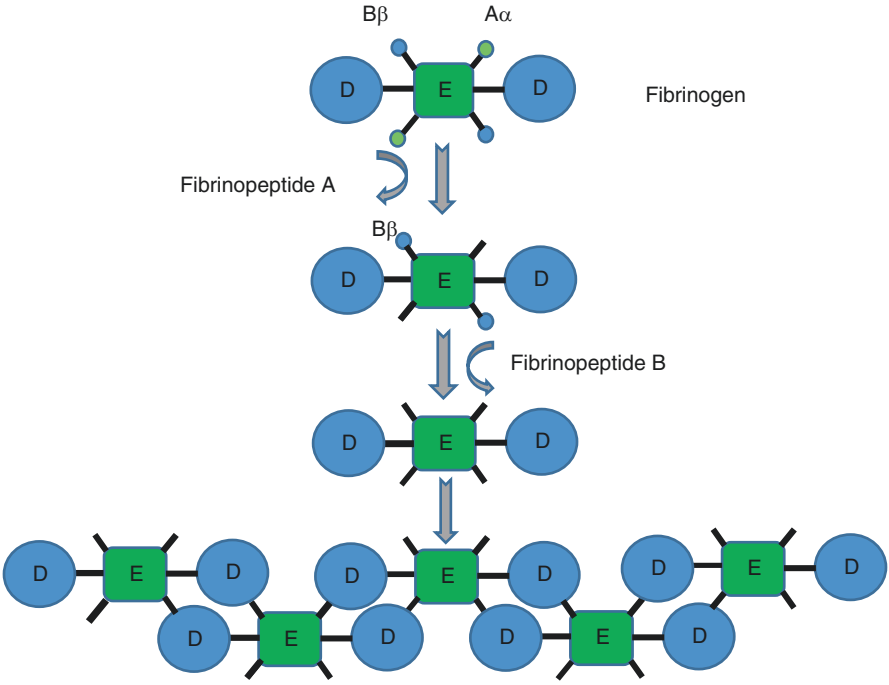

Fig. 7.3 Thrombin binds to the central E nodule of fibrinogen and cleaves this protein at four specific Arg-Gly bonds at the N-terminal of Aα and Bβ chains. This reaction leads to release of fibrinopeptide A (FPA) and B (FPB), respectively. Following the cleavage of FPA, a fibrin monomer which is also termed as fibrin I is formed. Fibrin I polymerizes to protofibrils. The deposition of fibrin leads to extensive meshwork formation (please refer to Chap. 6)

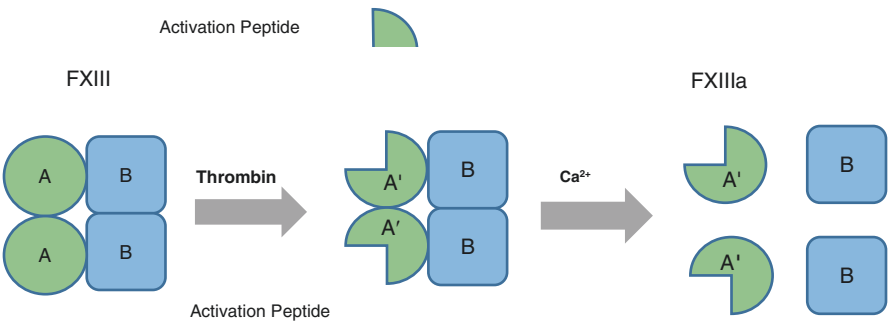

Fig. 7.4 FXIII activation. Thrombin cleaves FXIII-A subunit (Arg37). Following the cleavage, the activation peptide is released and the active site is exposed. FXIII-A and FXIII-B subunits are separated from each other in the presence of Ca^{++}, and therefore FXIII became activated

cleavage sites in FV are Arg709, Arg1018, and Arg1545. Following the cleavages, the A1-A2 domain ionically binds to A3-C1-C2 (Fig. 7.5a). FVIII is cleaved in Arg740, Arg1649, and Arg1689 residues (Fig. 7.5b). Then the A1-A2 fragments associated noncovalently with A3-C1-C2. The residues Lys70, Arg73, and Trp76 are important for binding of thrombin to FV and

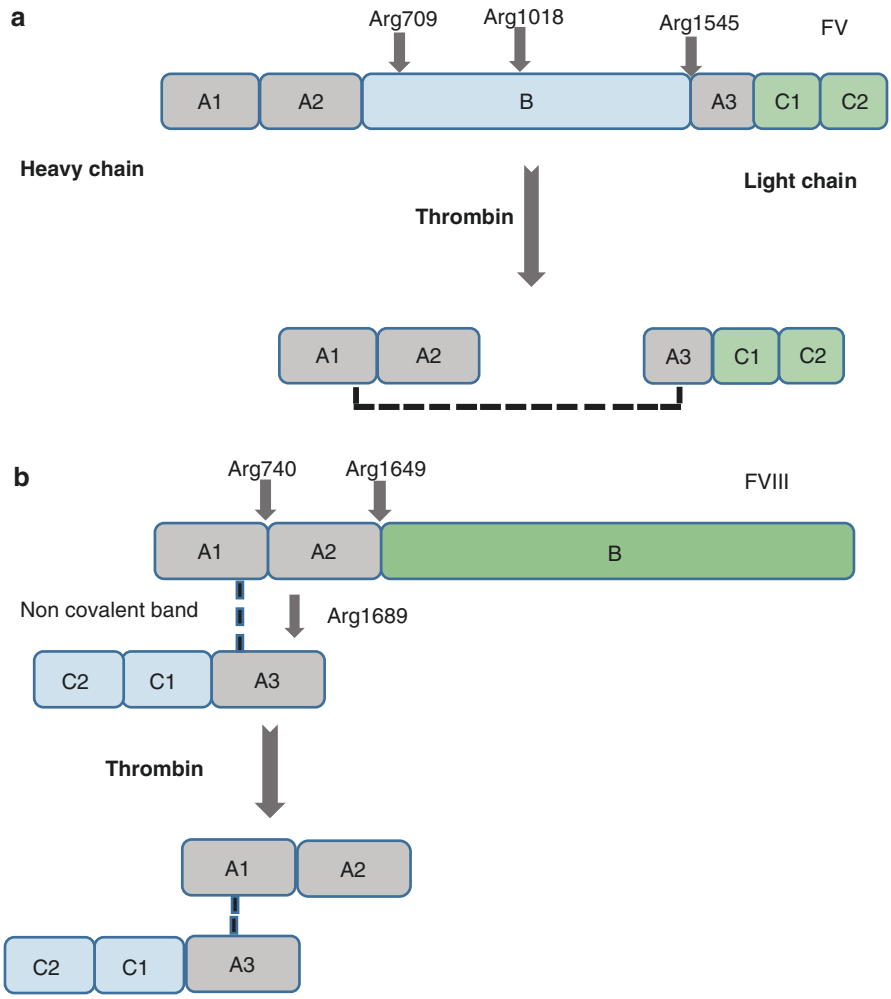

Fig. 7.5 FV and FVIII activation. (**a**) FVIII consists of the heavy and light chains (A1, A2, and B domains) and light chains (C1, C2, and A3) that noncovalently linked to each other. Thrombin activates FVIII through cleavage between A1-A2, A2-B, and at the A3 domains (shown by arrows). Following the cleavages, the B domain is released and FVIII became activated. (**b**) Thrombin cleaves FV in three sites including Arg709, Arg1018, and Arg1545 that leads to FV activation. Activated FV (FVa) consists of two chains including heavy (A1 and A2 domains) and light (A3, C1, and C2 domains) chains

FVIII, whereas Arg101 is important only for binding to FV (please refer to Chaps. 4 and 8) [29, 30].

7.5.4 Thrombin-Activated Fibrinolysis Inhibitors

Thrombin-thrombomodulin (TM) complex inhibits fibrinolysis through activation of thrombin-activatable fibrinolysis inhibitor (TAFI). The activation of TAFI by thrombin occurs via single cleavage at Arg92 and subsequently release of a

glycosylated activation peptide. This process requires high concentration of thrombin and is stimulated about 1250-folds by the TM. Activated TAFI (TAFIa) suppresses fibrinolysis by removing C-terminal lysine residues of fibrin that results in disruption of fibrinolytic protein binding sites [31].

7.5.5 Platelet Activation

Thrombin plays an essential role in platelet activation. It causes shape change and release of platelet activators including adenine di-phosphate (ADP), serotonin, thromboxane 2, cytokines, and growth factors [32]. Thrombin-mediated effects are preceded by activation of G protein-coupled PAR. Among the PAR, PAR1, PAR3, and PAR4 are activated by thrombin; however, PAR1 and PAR4 are the major human platelet thrombin receptors [33].

PAR1 is activated when thrombin cleaves its amino-terminal exodomain to unmask a new receptor amino terminus. Then the new N-terminus serves as a tethered peptide ligand, binds intramolecularly to the receptor, and causes receptor activation. PAR1 activation leads to rapid increase in intracellular calcium concentration, while PAR4 induces a more slowly increased and prolonged response in low and high thrombin concentrations, respectively [32].

7.5.6 Protein C Activation

Thrombin binds to the transmembrane protein TM on the surface of endothelial cells and represents its anticoagulant role in coagulation system. In fact, formation of the thrombin-TM complex results in change of substrate specificity of thrombin from procoagulant to anticoagulant reactions by activation of protein C. Procoagulant activity of thrombin stopped by occupancy of exosite I, while cleavage of protein C (PC) at

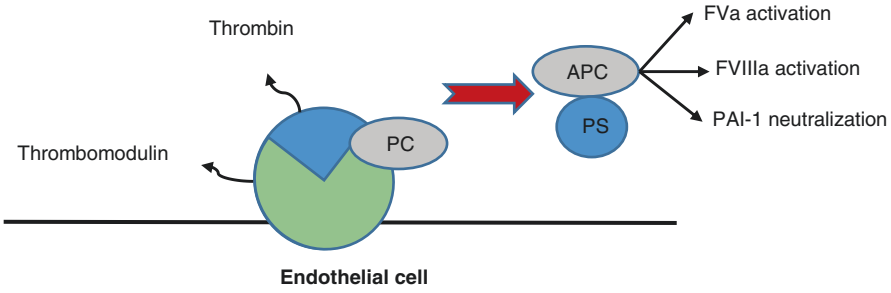

Fig. 7.6 Role of TM complex. Thrombin binds to the TM on the endothelial cells and shows its anticoagulant roles. In fact, thrombin-TM complex formation results in activation of PC. Activated PC (APC) with its cofactor, protein S (PS), inactivates the factor (F) V and FVIII and therefore inhibits the further thrombin formation. In addition, APC inhibits the function of plasminogen-activated inhibitor-1(PAI-1). *PC* protein C, *PS* protein S, *APC* activated protein C, *FVa* activated factor V, *FVIIIa* activated factor VIII, *PAI-1* plasminogen activator inhibitor-1

Arg169 leads to activation of this protein. Activated PC (APC) in association with its cofactor, protein S (PS), inactivates the FV and FVIII and therefore inhibits the further thrombin formation. APC also neutralizes the plasminogen-activated inhibitor-1(PAI-1) and therefore inhibits fibrinolysis (Fig. 7.6) (please refer to Chap. 1) [14, 34].

7.6 Congenital Factor II (Prothrombin) Deficiency

Congenital FII (Prothrombin) deficiency is a very rare autosomal recessive coagulation disorder with estimated incidence of 1 per 2 million in the general population. The incidence of FII deficiency is higher in regions with high rates of parental consanguinity. Prothrombin deficiency was described by Quick et al. in 1947, while the first case of this disorder was reported by Shapiro in 1969 [5]. Prothrombin deficiency is classified in two main phenotypes including hypoprothrombinemia which is also known as a type I deficiency with low levels of both FII activity and antigen (homozygotes and compound heterozygotes) and dysprothrombinemia (type II) characterized by normal or slightly decreased level of FII antigen, but low level of FII activity (homozygotes or heterozygotes), hypo–dys or dys–dys forms (compound heterozygotes), and combined deficiency of prothrombin and other γ-carboxylated coagulation factors [6, 7]. The ratio of type I which is also known as a true deficiency to type II is 1/2 to 1/3. The complete prothrombin deficiency is incompatible with life. This issue is confirmed in knockout mouse models with experimental prothrombin gene inactivation which results in embryonic lethality. Moreover, based on FII activity, this disorder is classified in three forms. The severe form of diseases is characterized by undetectable FII activity, while the mile and moderate forms of disease are characterized by FII activity >10% and <10%, respectively. Congenital FII disorders represent approximately 3% of all rare bleeding disorders (RBD) [35, 36].

7.7 Acquired FII Deficiency

Acquired FII deficiency is a rare disorder which presents with various clinical manifestations. In acquired FII deficiency, antibodies act direct against FII and cause degradation or activity's blocking of this protein. FII inhibitors occur in different conditions including lupus anticoagulant, hypoprothrombinemia syndrome, liver disease, use of anticoagulant drugs, vitamin K deficiency, hematological malignancies, nephritic syndromes, and infections. To evaluate the causes of FII inhibitor, an extensive workup is recommended [37].

7.8 Clinical Manifestations

The clinical manifestations among patients with FII deficiency are highly variable. Patients affected by severe FII deficiency presented with more severe bleeding episodes. The most common bleeding features in patients affected by FII deficiency are

Table 7.1 Clinical manifestations of patients affected by congenital prothrombin deficiency

Bleeding features	Girolami et al. (*n*: 26) (%)	Acharya et al. (*n*: 16) (%)		Stefano Lancellotti et al. [39] (%)
Umbilical cord bleeding	15.4	Homozygous subject (*n*: 10)	Heterozygous subject (*n*: 6)	–
Epistaxis	53.8			–
Hematoma and ecchymosis	68	40[a]	83[a]	60
Gingival bleeding	12			–
Post tooth extraction bleeding	36	–	–	36
Gastrointestinal bleeding	11	13	–	12
Hemarthrosis	44	26	–	42
Hematuria	8	–	–	–
Menorrhagia	100[b]	–	–	20
Postpartum hemorrhage	100[b]	–	–	–
Intracranial hemorrhage	12	20	–	12

[a]This study does not report the frequency of mucocutaneous bleeding (ecchymosis, epistaxis, and gingival bleeding) individually
[b]These bleeding features were observed in all three women who were included in the study

mucosal bleeding, post-trauma bleeding, and hematoma. Life-threatening episodes including gastrointestinal bleeding and central nervous system (CNS) bleeding were reported in few patients (Table 7.1) [3, 8]. Heterozygote patients (with plasma thrombin level of 40–60%) are usually asymptomatic and in some occasions present with postsurgical bleeding. Patients with dysprothrombinemia usually show milder bleeding episodes in comparing with true FII deficiency (type I deficiency) [3]. There is no exact correlation between coagulation test results and clinical manifestations in patients with dysprothrombinemia. However severity of bleeding episodes depends on FII activity, type of FII defect, and FII mutation [3].

Based on different studies, homozygous cases with Arg382His mutation show prothrombin activity lower than 20% with minimal bleeding tendency [38]. Moreover, prothrombin Salakta or Himi is not associated with bleeding tendency [39]. Patients with dysprothrombins Yukuhashi and Scranton have mutation in Na^+ binding loop and do not show hemorrhagic phenotypes [40, 41]. Some cases of FII with mutations of C20209T in 3′ UTR present with thrombosis [42].

7.9 Molecular Basis

Congenital prothrombin deficiency results from different mutations in *F2* gene. These causing mutations are missense mutations (80%), insertion/deletion (10%), nonsense (6%), and splice site mutations (4%) [9].

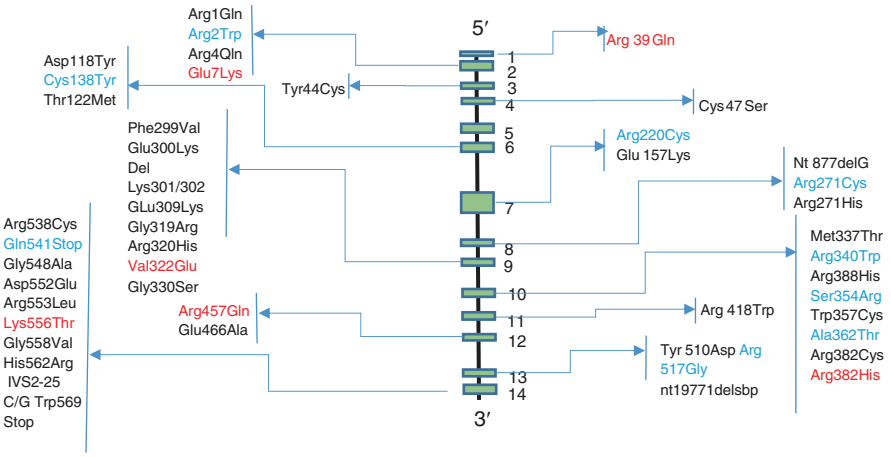

Fig. 7.7 The mutational spectrum of factor II deficiency. Almost all 60 variants (46 missenses, 3 splicing, 4 regulatory, and 7 frameshift) are represented. Exons and introns are shown by boxes and lines, respectively

Approximately 60 variants (46 missense, 3 splicing, 4 regulatory, and 7 frameshift) are accompanied with prothrombin deficiency that listed in the Human Gene Mutation Database (HGMD) http://www.hgmd.cf.ac.uk/ac/all.Php [9]. These mutations are shown in (Fig. 7.7). Although mutations involved different exons, the prevalence of them is higher in exon 8 to exon 14 [3]. Dysfunctional defects are classified in two groups including activation mechanism defects such as FII Barcelona (Arg271Cys) and Padua (Arg271His) or thrombin's protease activity defects [43–45].

Defects in protease activity of thrombin result from:

(1) Amidolytic activity defects for both low and macromolecular substrates which result from impaired catalytic activity of thrombin. FII Molise (Arg418Trp and Stop codon 174) and FII Vellore (Ala362(56)Thr) cause this type of defect.
(2) Defective interaction of thrombin with macromolecular substrates including fibrinogen, TM, and PAR1 which occurs due to mutations in molecular recognition domain of enzyme or insertion loop. FII Quick (Arg382(67)Cys and stop codon Gln541(209)), FII Salakta (Glu466(146)Ala), FII Himi II (Arg388(73) His), and Arg382(67)His lead to this type of defect [3].

Heterozygous prothrombin mutations are found in approximately 2% of US white population, while it is rare in other populations (African-Americans, Asian, and Native American) [46]. Some polymorphisms in *F2* gene lead to increases of FII level. The most common polymorphism of this type is G20210A in the 3′ UTR of gene. This substitution (G to A) is associated with higher prothrombin levels. The prevalence of FII G20210A heterozygotes is higher in Caucasian population. This substitution alters processing, stability, and translocation of prothrombin mRNA. Another

polymorphism is A19911G polymorphism located within the last intron of prothrombin [47–49]. 19911G allele is associated with mildly elevated plasma prothrombin level (4 UdL^{-1} higher than A allele) and increases the venous thrombosis risk [50].

7.10 Laboratory Diagnosis

In general, family history, clinical manifestations, and screening laboratory tests are pivotal for diagnosis of FII deficiency. The presence of family history for bleeding disorders can be helpful, but their absence does not exclude the existence of these disorders. FII deficiency is suspected in the presence of prolonged PT and aPTT. For confirmation of FII deficiency, FII activity and antigen level should be performed [51, 52].

7.10.1 Measurement of Factor II Activity

Following the prolonged PT and aPTT, specific assays should be performed. Several assays including PT-based one-stage assay, tiger snake venom assay, taipan viper venom assay, Textarin time, *Echis carinatus* venom assay, and staphylocoagulase assay are available which today the PT-based one-stage assay is the most widely used method. Each assay has an especial activating substance which converts prothrombin to thrombin in different ways. In classical one-stage assay, the presence of FV, phospholipids, and calcium is necessary, and tissue thromboplastin acts as an activating agent. Tiger snake venom acts as an activating agent in the presence of FV, calcium, and phospholipids while *Echis carinatus* venom activates prothrombin without need of other factors. Taipan viper venom activates the prothrombin to thrombin in the presence of calcium and phospholipids [53, 54].

7.10.2 PT-Based One-Stage Assay

PT-based one-stage assay is a specific test which determines the FII activity. This assay is based on PT test and compared the ability of patient's plasma with standard plasma to correct the PT of substrate (prothrombin-depleted plasma). In this assay equal volume of dilution of patient's plasma and standard's plasma (1 in 5, 1 in 10, 1 in 20, and 1 in 40) is mixed with substrate plasma and is warmed in 37 °C. Then by adding the equal volume of diluted thromboplastin (recombinant and re-lipidated tissue factor), the clotting time is recorded. For calculation of the FII activity, the clotting time of each dilution of test plasma and standard plasma is plotted against concentration of FII [3, 10].

7.10.3 Chromogenic Assay

The chromogenic/fluorogenic assays can also be used for measurement of thrombin level. In this assay the amidolytic activity of thrombin in presence of chromogenic

substances such as anilides or fluorogenic peptide including 7-amino-4-methylcoumarin amides is measured. These chromogenic/fluorogenic substances interact with thrombin's active side pocket. This method is able to detect any difference between the level of FII in this assay and the level of it in the coagulation assay. Therefore, the result of chromogenic activity is not always the same as coagulation activity. Any difference between this assay and PT-based one-stage assay suggests the presence of dysprothrombin which results from mutation of recognition domain of thrombin especially fibrinogen recognition domain [37].

7.11 Determination of Factor II Antigen Level

Enzyme-linked immunosorbent assay (ELISA) is used conventionally for measurement of FII antigen level. In this assay, a specific antibody is coated on the plate. Following the addition of standard and patient's plasma, antihuman prothrombin primary antibody binds to captured protein. Then unbounded antibodies are washed away, and the secondary antibody which is conjugated with horseradish peroxidase (HRP) is added. By adding the substrate, the color change is spectrophotometrically assessed at 450 nm. The concentration of FII in sample is determined by comparing the optical density (OD) of sample via standard curve [10, 55].

7.12 Factor II Inhibitor Assay

For detection of inhibitor against FII, the patient's plasma is mixed with normal pooled plasma (50:50). When the results of PT and aPTT do not normalize, the presence of an inhibitor is suspected. Then inhibitor should be titrated by Bethesda method.

For this method, two tubes are prepared for normal plasma and patient's plasma, respectively, and two tubes are prepared for equal mixture of patient and normal plasma (time-dependent and immediate inhibitors, respectively). APTT and PT should be performed duplicate for all tubes (please refer to Chap. 8) (Table 7.2) [56].

7.12.1 Bethesda Assay

For inhibitor titration, the Bethesda method is used. The Bethesda unit is defined as amount of inhibitor which neutralized 50% of 1 unit of FII in normal plasma. In this method two dilution series are prepared as follows and incubated at 37 °C for 0 hour (h), 1 h, or 2 h:

(1) Dilutions of patient's plasma with an equal volume of normal plasma (normally contain 100% FII)
(2) Dilutions of control plasma which contain no inhibitors (equal volume of normal plasma and buffer)

The residual FII is determined based on one-stage PT assay for each mixture. The dilution with residual FII activity about 50% is considered for determination of

Table 7.2 Interpretation of the inhibitor screen for factor II deficiency based on PT and aPTT

Tube	Content	The results of clotting time		
		FII deficiency	Immediate inhibitors	Time-dependent inhibitors
1	Normal plasma	Normal	Normal	Normal
2	Patient's plasma	Prolonged	Prolonged	Prolonged
3	Equal volume of normal and patient's plasma (2 h incubation)	Normal	Prolonged	Prolonged
4	Equal volume of normal and patient's plasma (without incubation)	Normal	Normal	Prolonged

APTT activated partial thromboplastin time
PT prothrombin time

inhibitor strength. For calculation of FII inhibitor, the standard graph of residual FII activity versus inhibitor units is used [10, 55].

7.13 Treatment

Replacement therapy is required in the homozygous cases with bleeding or prior to surgical procedures. The minimum level of FII needed for hemostasis is >10% [57].

As no prothrombin-specific concentrate is available for replacement therapy of FII, PCC or FFP is currently used for on-demand therapy and long-term prophylaxis in patients with FII deficiency. Moreover in cases with mild bleeding, antifibrinolytic agents (tranexamic acid and epsilon-aminocaproic acid) are also recommended (Table 7.3) [10].

Although PCC is a mainstay of therapy in prothrombin, high or repeated doses of PCC have been associated with arterial and venous thrombosis; therefore patients require close monitoring [10]. PCC contains three factors including FII, FIX, and FX. This product is known as three-factor PCC. If the amount of FVII is more than 10%, this product is labeled as a four-factor PCC (please refer to Chap. 10) [58].

As the incidence of severe bleeding is not high during the neonatal periods, prophylactic replacement therapy is not routinely recommended for this group. On the other hand, prophylactic replacement therapy should be used according to the frequency and type of bleeding [59]. Although it is difficult to make firm recommendations on pregnancy management, it had been suggested that a FII level up to 25 IU/dL minimizes the bleeding complications during labor and delivery [60].

Table 7.3 Doses and target therapeutic levels in patients with factor II deficiency

Kind of treatment	Recommend level	Dosage of therapeutic level
On-demand therapy	>10%	15–25 mL/kg: FFP
		20–40 U/kg: PCC
Long-term prophylaxis	>10%	20–40 U/kg once a week
Major surgery	>20% (maintaining level)	20–40 IU/kg (before surgery)
		10–20 IU/kg every48 h

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