Congenital Factor VII Deficiency, Diagnosis, and Management

11

Mahmood Shams

11.1 Introduction

Congenital factor (F) VII deficiency is a rare autosomal recessive bleeding disorder with an estimated prevalence of 1 per 500,000 in the general population, with ethnic or gender predilection [1, 2]. However, the prevalence is higher in regions with a high rate of consanguinity marriage [1, 3, 4]. Clinical pictures in these patients ranging from asymptomatic condition to severe, life-threatening hemorrhages [5, 6]. There is a relatively poor correlation between FVII coagulant activity (FVII:C) and bleeding tendency, as well as mutation profile in congenital FVII deficiency [3, 7]. Severe clinical symptoms usually present in patients with less than 1% FVII:C; however, some patients with severe deficiency do not experience severe bleeding episodes. The complete absence of functional FVII in knock-out mice is incompatible with life, suggesting FVII deficiency is not associated with complete absence of functional FVII. In line with this issue, patients with residual FVII level can survive and able to prevent lethal bleeding [8, 9]. The disorder is accompanied with a wide spectrum of bleeding problems, including mild symptoms such as mucous membranes and skin hemorrhages and life-threatening hemorrhages such as central nervous system (CNS) bleeding. Iron deficiency due to menorrhagia is common in women with FVII deficiency [3]. In aggregate, it seems that patients with FVII deficiency experience substantial psychosocial impact due to the high disease burden, however, the generally held view is that the majority of patients maintain optimism regards to their disease management and thereby the quality of their living [10]. This disorder can be managed by different therapeutic options including fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), plasma-derived FVII (pd-FVII) products, and recombinant activated FVII (rFVIIa).

M. Shams (✉)
Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

A. Dorgalaleh (ed.), *Congenital Bleeding Disorders*,
https://doi.org/10.1007/978-3-031-43156-2_11

11.2 Factor VII Structure and Function

FVII is a low molecular weight protein (50 kDa) composed of 406 amino acids, synthesized as single-chain molecule in the endoplasmic reticulum of hepatocytes. FVII has homology with FIX, FX, and protein C at the catalytic site and the amino-terminal region [11]. Within hepatocytes, FVII has a signal peptide that is required for secretion and a propeptide (removed intracellularly) that is necessary for γ-carboxylation of all glutamate residues within ~45 amino acids at the N-terminus site of the FVII protein [12]. Coagulation FVII consists four domains, including a gamma-carboxyglutamate acid (**Gla**) domain on the N-terminal with ten glutamic acid residues (at residues 6, 7, 14, 16, 19, 20, 25, 26, 29, and 35). These ten glutamic acid residues undergo post-translational modification; convert to γ-carboxyglutamic acid (Gla) with calcium-binding capacity. Binding of calcium to the Gla domain induces conformational changes and exposure of new epitopes that facilitate its subsequent binding to tissue factor and phospholipid [13]. The other FVII domains are two epidermal growth factor-like domains (**EGF1**, **EGF2**) and a catalytic serine protease (**SP**) domain at the C-terminal of the FVII protein [1, 12] (Fig. 11.1).

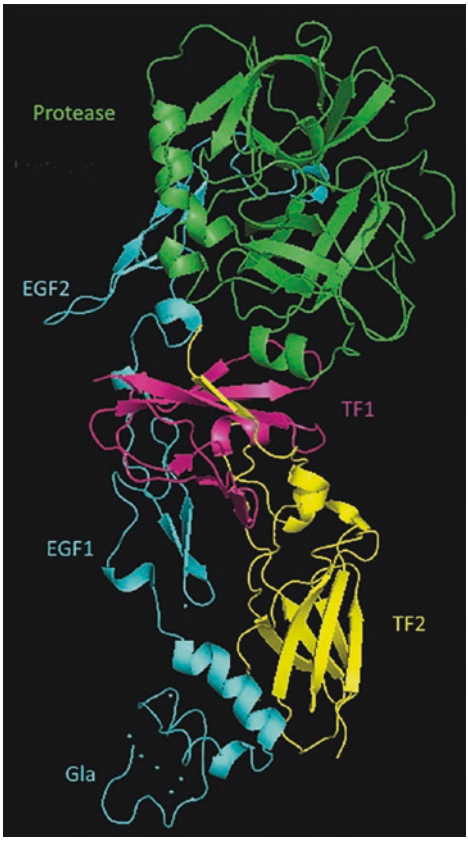

Fig. 11.1 The cartoon representation of activated factor VII (FVIIa)/soluble tissue factor (sTF) complex. FVII has four domains including a gamma-carboxyglutamate acid (Gla), two epidermal growth factor-like (EGF1, EGF2) domains, and serine protease domain. sTF contains two fibronectin type III domains (TF1 and TF2 represent of N- and C-terminal of sTF, respectively)

Zymogen form of FVII with the plasma half-life of 5 h has the shortest half-life among the clotting factors; however, the half-life of free activated FVII (FVIIa) is 2 h, whereas the plasma half-life of most other activated coagulation factors is very short [1, 12].

FVII reversibly in a Ca2+ dependent manner can bind to membranes with negatively charged phospholipids such as phosphatidylserine or phosphatidic via Gla domain [14, 15]. The majority of plasma FVII circulates as the single-chain inert zymogen (10 nmol/L (0.5 μg/mL)) and the minority circulates in the plasma as two-chain active protein ~10–110 pmol/L) [1, 3, 12, 16]. The key event in the activation of FVII is proteolysis of a single peptide bond between Arg-c15 (amino acid 152) and Ile-c16 (amino acid 153) in the connecting region of EGF2 and SP domains. This results in formation of two polypeptide chains: heavy chain with 254 amino acids (30 kDa) (residues 153–406), comprised of serine protease domain with Trypsin homology at C terminus, and light chain with 152 amino acids (20 kDa) (residues 1–152), composed of a Gla and two EGF-like domains [1, 12, 17–19].

FVII, chiefly interacts with TF via the Gla and EGF1 domains, however, it's worth noting that two other domains can also interact with TF [18, 20]. FVII/TF complex is necessary for restructuring the active site and achieving full enzymatic activity of FVIIa, because free FVIIa exhibits very weak catalytic activity [17, 18]. In addition to FVII/TF complex, several other coagulation factors, including FXa, FIIa, FIXa, FXIIa, and FIXa contribute to FVII activation, however it seems that membrane-bound FXa is the most effective activator [21]. Once the TF/FVIIa complex is formed, it results in proteolytic activation of FIX and FX to FIXa and FXa, respectively, generating few amount of thrombin that able to produce a strong feedback amplification of coagulation cascade [12, 22].

Tissue factor pathway inhibitor (TFPI) and antithrombin (AT) are inhibitors of FVIIa, but they can only inhibit FVIIa after the formation of the FVIIa/TF complex [23, 24]. TFPI is a Kunitz-type proteinase inhibitor that attaches to the membrane surface via glycophosphatidylinositol (GPI)-linkage. TFPI is mainly expressed by endothelial cells and, to a lesser extent, by platelets [25]. The TFPI/FXa complex can inhibit the FVIIa/TF complex and prevents further FX activation via the formation of inactivated tetra-molecular (TF-FVIIa-TFPI-FXa) complex, causing rapid inhibiting of the extrinsic coagulation pathway [23, 25]. Inhibitory function of TFPI/FXa, at last partly, is inducing TF-expressing cells to internalize the TF/FVIIa complexes, leading to degradation of the majority of FVIIa [26]. TFPI is synthesized by microvascular endothelial cells, megakaryocytes, and the liver [3, 27]. Heparin and various platelet agonists can increase the release of TFPI from the surface of endothelial cells. AT reaction is heparin-dependent and its reactivity with FVIIa is increased after FVIIa/TF complex formation. Following the binding of AT to the FVIIa/TF complex, the affinity of FVIIa to TF decreases, and the FVIIa/AT complex is then released into the blood-stream [3, 23] (Fig. 11.2). The FVIIa/AT complex is increased in many prothrombotic situations and seems to be an early marker of coagulation cascade activation [23].

FVII with the initiation of coagulation pathway following complex formation with TF at injury site has a critical role in the coagulation cascade. This complex is an important activator of both extrinsic and intrinsic coagulation pathways by activation of FVII, FIX, and FX [5]. It was shown that complete deletion of FVII gene

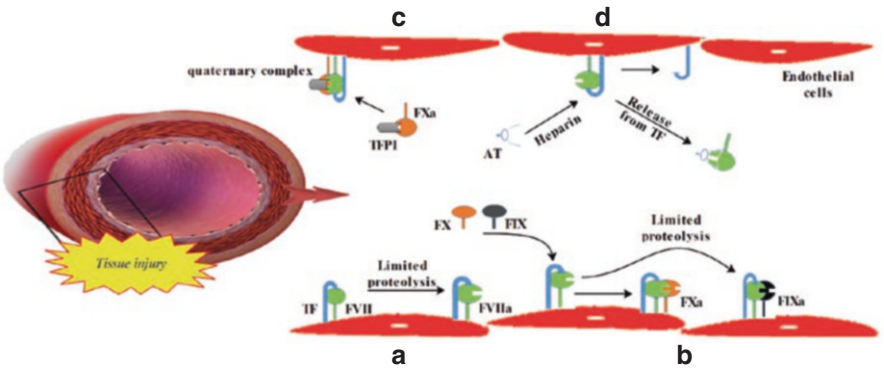

Fig. 11.2 In vivo activation and inhibition of factor VII (FVII). (**a**, **b**) FVII binds to tissue factor and is converted to its active form, FVIIa, by minor proteolysis and then activates factor X (FX) and factor IX (FIX). (**c**, **d**) Tissue factor pathway inhibitor (TFPI) and antithrombin (AT) are inhibitors of FVII/TF complex. TFPI/FXa complex can form an inactive tetra-molecular (TF-FVIIa-TFPI-FXa) complex that results in extrinsic coagulation pathway inhibition. In addition to direct deactivation of FXa, TFPI can prevent further FX activation after TFPI/TF/FVIIa complex formation. TFPI/FXa can induce internalization of TF/FVIIa complex, resulting in degradation of the majority of FVIIa (not shown). TFPI can also inhibit the early forms of prothrombinase (Not shown). AT directly attaches to TF/FVIIa complex and causes segregation of FVIIa from TF by losing affinity of FVIIa for TF, resulting in the release of FVIIa/AT into blood-stream and therefore causes extrinsic coagulation pathway inhibition. AT activity can be strengthened by heparin

leads to mouse perinatal death, while mice and humans with very low FVII levels could be survived [8, 28, 29]. Although the normal perinatal course was observed in FVII knockout mice, major abdominal and intracranial hemorrhages (ICH) lead to death in such cases at birth or shortly after birth [9]. Generally, it is accepted that the absence of FVII is incompatible with life [3, 22, 30].

TF known as thromboplastin, coagulation FIII, or CD142 is a glycosylated, transmembrane protein that doesn't require proteolysis for activation [12]. It is well known that normal hemostasis process in some tissues with high TF-expression such as the brain, bowel, uterus, placenta, lungs, and heart depends mainly on the extrinsic pathway, therefore, reduced or absence of FVII can result in bleeding in some of these tissues [5, 8]. In addition to well-known role of TF in the coagulation process, in complex with FVIIa other functions including embryonic angiogenesis, oncogenic angiogenesis, tumor progression, leukocyte diapedesis, and regulation of inflammation and sepsis are described. This complex can also change cellular physiology in the TF-expression cells [31, 32].

11.3 Congenital Factor VII Deficiency

Congenital FVII deficiency (OMIM 227500) is an autosomal recessive bleeding disorder, for the first time described by Alexander in 1951 in a 4-years old white girl who experienced prolonged umbilical cord bleeding at birth [33]. This bleeding disorder with a prevalence of 1 per 500,000 individuals is the most common among rare

Table 11.1 Classification of congenital factor VII deficiency and results of coagulation tests

	APTT	PT	FVII:C	FVII:Ag
Normal people	Normal	Normal	Normal	Normal
FVII deficiency (type I)	Normal	Prolonged	Decreased	Decreased
FVII deficiency (type II)	Normal	Prolonged	Decreased	Normal or nearly normal

APTT activated partial thromboplastin time; *PT* prothrombin time; *FVII:C* factor VII coagulant activity; *FVII:Ag* factor VII antigen

bleeding disorders (RBDs) [1, 6, 33]. While this disorder is distributed worldwide, it is more frequent in some areas such as the United Kingdom, United States, Brazil, Turkey, Italy, Slovak Republic, and Iran, as reported in the annual global survey of World Federation of Hemophilia (WFH). Although consanguinity is the main cause for high rate of disorder, in some countries like the United Kingdom, this increae has also been attributed to noticeable grow up of hygienic surveillance and enhanced overall quality of life. The number of patients with congenital FVII deficiency might be underestimated probably due to undiagnosed asymptomatic patients and those with the only mild bleeding tendencies. FVII deficiency is categorized into two groups, including type I (quantitative deficiencies), which is characterized by simultaneous decreases in FVII activity and antigen levels, and type II (qualitative defects) with only decreases in FVII activity with normal or near normal FVII antigen level (Table 11.1) [6]. It should be noted that type II is divided into two distinct groups including cases with no variation in the FVII:C levels, regardless of the type of thromboplastin used, and those with different FVII:C levels when different origins of the thromboplastin are used. Clinical manifestations of the disorder are highly variable both in severity and type of bleeding, with poor correlation between residual plasma factor activity and severity of bleeding [5, 34]. The FVII reference range is between 70% and 140% and usually, less than 2% FVII activity (FVII:C) is related to an increased risk of severe bleeds during the newborn and young childhood periods [1, 7]. The disorder is due to mutations in *F7* gene, and a wide spectrum of mutations has been identified within this gene. Most of the identified mutations are new and restricted to the special area or specific family and could be used for carrier detection, precise diagnosis, and prenatal diagnosis in affected families.

The presence of abnormal bleeding, accompanied by isolated prolonged PT, serve as the first clue for suspicion to FVII deficiency, but in general, a combination of clinical presentations, physical examination, family history, and laboratory assessments can be used for precise diagnosis of the disorder. Several therapeutic options such as fresh-frozen plasma (FFP), plasma-derived FVII (pd-FVII), prothrombin complex concentrate (PCC), activated PCC (aPCC), and more recently recombinant FVIIa (rFVIIa) are available for patients with FVII deficiency.

11.4 Acquired Factor VII Deficiency

Acquired FVII deficiency can be present either in isolation or as a part of a deficiency in vitamin-K dependent coagulation factors [1, 35, 36]. Acquired isolated FVII deficiency is an extremely rare condition, with only few reported cases, so far

[37]. However, the frequency was underestimated, because clinical symptoms of the disorder can be moderate with only slightly prolonged PT [35, 36, 38]. Isolated acquired FVII deficiency should be considered a potential medical condition in patients with prolonged PT and no personal or family history of bleeding tendency. In such cases, FVII:C should be repeated after resolving the acute phase of the illness. If persistently high levels of FVII are observed, genetic testing is indicated to investigate the possibility of congenital FVII deficiency [39]. Different conditions such as malignancies, severe systemic sepsis, infectious agents, drugs (penicillin), aplastic anemia, stem cell transplantation, and presence of an inhibitory antibodies, may be accompanied by acquired isolated FVII deficiency [1, 36, 40, 41]. In this setting, an interesting study showed a mild FVII deficiency (<60%, ranged 35–56%) with no bleeding tendency in a majority of patients (89.6%) who received chronically the packed cell transfusions [42]. In some cases, no underlying condition of FVII deficiency (idiopathic) was identified [36]. The exact pathophysiological basis and the possible mechanism of FVII deficiency are not clear in these situations [36].

Simultaneous deficiency of FVII with other coagulation factors may arise in different conditions including [1]:

- Problem in synthesis, particular in liver failure that leads to decrease of all coagulation factors.
- A defective synthesis, especially during hypovitaminosis K syndrome caused by insufficient intake, malabsorption, or anticoagulant therapy with vitamin K antagonists such as Warfarin, Acenocoumarol (Sinthromin), and Phenprocoumon (Marcoumar). These conditions only lead to vitamin-K dependent coagulation factors deficiency (FII, FVII, FIX, and FX) and the decrease of protein C and protein S levels. Warfarin inhibits the vitamin K-dependent reductase and the vitamin K-dependent quinone reductase and leads to disturbing in the recycling of vitamin K to its enzymatically active form and its carboxylation activity.
- Consumption syndromes, especially disseminated intravascular coagulation (DIC) or hyperfibrinolysis that leads to consumption of all coagulation factors.

As mentioned, FVII has the shortest plasma half-life among clotting factors, thus, the decrease in plasma level of FVII occurs faster than other coagulation factors. Therefore, the diagnosis of isolated FVII deficiency should be made with caution [1].

11.5 Clinical Manifestations

Patients with congenital FVII deficiency have variable bleeding diathesis with poor correlation between FVII activity and bleeding tendency [1, 36]. The clinical phenotype is very heterogeneous and ranging from asymptomatic conditions to life-threatening diathesis. The clinical phenotype of these patients could be categorized into two main categories [1, 3, 43]:

- Asymptomatic composed about one-third of patients.
- Symptomatic with two subgroups:
  - Nonsevere: Mild to moderate with mucocutaneous bleeding (mimic platelet disorders) including approximately two-third of affected patients. These patients usually don't require medical intervention.
  - Severe with life- or limb-threatening hemorrhages (central nervous system (CNS) bleeding, gastrointestinal (GI) bleeding, or hemarthrosis) that composed about 10–15% of patients [1, 3, 43].

Asymptomatic patients might be randomly diagnosed or identified during family studies, especially in cases with other affected family member (s). According to a large study, 71% of homozygous and 50% of compound heterozygous patients are symptomatic, while only 19% of heterozygous subjects are symptomatic [2]. Based on another large study, the most common bleeding features among patients with FVII deficiency are epistaxis, easy bruising, gum bleeding, hematoma, hemarthrosis, postoperative bleeding and menorrhagia, and less common bleeding features are hematuria, GI bleeding, and CNS bleeding (Table 11.2) [2, 7, 30, 43–46]. Severe clinical presentations generally occur at young ages (soon after birth or when they are toddler) in severely affected patients [11, 43]. Severe chronic iron deficiency due to menorrhagia is common in women with FVII deficiency [3]. In this regard, due to the high rate of FVII deficiency in patients with unexplained heavy menstrual bleeding, alongside evaluation of the von Willebrand factor, evaluation of FVII

Table 11.2 Clinical manifestations of patients with congenital factor VII deficiency

	Mariani et al. (n:174[a])	G Mariani et al. (n:139[b])	G Mariani et al. (n:228)	F. H. Herrmann et al. (n:217)	F. Peyvandi et al. (n:28)	G Mariani et al. (n:24)
CNS bleeding	4.6%	6.5	7%	1%	17%	n.r.
GI bleeding	13.8%	14.4	14%	9%	n.r.	17%
Hemarthrosis	16.1%	21.6	22%	12%	21%	67%
Epistaxis	56.3%	66.2	83%	58%	64%	62%
Easy bruising	47.7%	43.2	62%	37%	32%	29%
Gum bleeding	33.9%	25.9	42%	25%	n.r.	33%
Menorrhagia	62.9%	–	15.6	57% (of 106 female)	60% (of 10 female)	90% (of 10 female)
Hematomas	16.1%	20.9	21%	20%	12%	46%
Hematuria	5.2%	12.2	12%	7%	10%	29%
Postoperative bleeding	29.8%	30.4	34%	–	55%	–
Thrombosis	3%	–	–	–	–	–

CNS central nervous system; *GI* gastrointestinal

[a] The incidence of menorrhagia has been reported in females aged >10 and <50 years and all of patients are female

[b] Only male

deficiency as part of the initial congenital bleeding disorders investigations is recommended [47]. Patients with plasma FVII levels <2% may present severe bleeding, while those with >20% are generally asymptomatic. Interestingly, bleeding can be observed among patients with plasma levels between 20% and 50%, while asymptomatic subjects with plasma levels <1% were also reported [11]. Prediction of hemorrhagic risk may not be possible, even in presence of laboratory assays such as thrombin generation test, FVIIa, and FVII antigen level (FVII:Ag) assays, and TFPI measurement [48]. CNS bleeding is a less common condition and is an important problem, mainly in children under 6 months with severe FVII deficiency and is associated with a high rate of morbidity and mortality [3, 11]. Bleeding episodes in FVII deficiency may mimic hemophilia (hemophilia type) with the presence of hemarthrosis and hematoma or may mimic primary hemostasis defects with menorrhagia, epistaxis or ecchymosis [11]. Based on available data, FVII deficiency does not provide protection against thrombotic events [49]. Accordingly, in addition to bleeding episodes, thrombotic events with the unknown mechanism (particularly deep vein thrombosis) also may occur in ~3% of patients with severe FVII deficiency, especially in the presence of prothrombotic risk factor such as recent surgical interventions, replacement therapy, immobilization, or trauma; however spontaneous thrombosis also may occur [49–51]. Although severe clinical events have been observed in homozygous or compound heterozygotes, the heterozygous are usually asymptomatic [2, 30].

11.6 Laboratory Approach to Congenital Factor VII Deficiency

The first case with congenital FVII deficiency was described by prolonged PT in 1951 [33]. The diagnosis is based on clinical presentations, physical examination, family history and laboratory assessments [44]. Occasionally, the disorder could be identified during routine work up. For a more precise diagnosis and to confirm the presence of the disease, the molecular diagnosis is strongly recommended [1].

11.6.1 Primary Tests for Diagnosis of FVII Deficiency

FVII deficiency is usually suspected by the presence of isolated prolonged PT which is corrected by 50:50 mixing of patient's plasma with normal pooled plasma. In this setting, the activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen concentration and platelet count are usually normal. Evaluation of FVII coagulant activity (FVII:C) (with twice repetition) leads to confirmation of disorder [3, 44]. In addition, unlike FX, the normal result of Russell viper venom (RVV) clotting time is also observed. Nonetheless, the normal result of RVV in FX Friuli (a variant of FX deficiency) should be considered [30, 52]. In general, the mainstay in the diagnosis of FVII deficiency is FVII:C assay.

11.7 Differential Diagnosis

FVII deficiency can be readily differentiated from acquired FVII deficiency due to the absence of family history in patients with acquired FVII deficiency. Moreover, patients with acquired FVII deficiency may also exhibit a history of normal PT results. Acquired combined FVII is usually observed during excessive consumption and/or inadequate production of FVII, resulting in a concomitant decrease in other vitamin K-dependent clotting factors. In such cases, in addition to PT, APTT would also be increased. Assessment of other coagulation factors (FII, FIX, and FX) may be useful for excluding cases with acquired combined FVII deficiency [35].

Exclusion of vitamin K deficiency or other acquired causes of clotting factor deficiencies is useful, but it is not necessary because concomitant prolongation of APTT is observed [3].

In patients with isolated FVII deficiency, isolated prolongation of PT occurs, and APTT is normal. However, a prolonged APTT might occur in some patients with the presence of lupus anticoagulant (LA). In this situation, an isolated FVII deficiency should be diagnosed by assessing FVII: C levels. In addition, acquired isolated FVII deficiency, may develop in the presence of autoantibody against FVII, severe infections, or malignancy. In such cases, prolonged PT is observed to be the same as congenital FVII deficiency. In this regard, family history, the absence of bleeding episodes, FVII: C assessment post-treatment, and the presence of severe infections or malignancy can help in the differential diagnosis [48, 53].

Evaluation of other vitamin-K dependent clotting factors might be helpful to rule out other disorders. For further investigation, a mixing study should be performed. After the mixing study, if PT was prolonged, the presence of a specific FVII inhibitor is suspected, and the Bethesda assay could confirm the presence of specific FVII inhibitor [36]. In this way, the absence of bleeding history, the presence of malignancy (or other underlying conditions), and the absence of a family history of congenital FVII deficiency could all be useful during the process of diagnosis. In order to rule out transient FVII deficiency, the test should be repeated in a couple of weeks. On the whole, clinical manifestation, family history, and laboratory investigation constitute the main basis of FVII diagnosis [1].

11.8 Functional Assays of FVII

In order to confirm FVII deficiency, the FVII activity level must be determined. FVII activity assays can be measured through clotting- or chromogenic-based methods.

11.8.1 The One Stage PT-Based FVII Activity Assay

FVII:C usually determined by one-stage prothrombin time-based assay [54].

Box 11.1 The Principle of the One-Stage PT-Based FVII Activity Assay
The assay relies upon measuring the extent of PT correction in FVII deficient-plasma, following the addition of the test plasma. In this assay, a serial dilutions of standard and patients' plasma (1/10, 1/20, 1/40, 1/80, etc.) are prepared. Typically, the 1/10 dilution is considered as having 100% factor activity. Standard curves are usually prepared using seven or eight dilutions. The equal volume of each dilution is then mixed with substrate plasma (FVII deficient-plasma) and incubated at 37 °C. Following the addition of the pre-warmed thromboplastin containing calcium, the clotting time for each dilution is recorded. The clotting times of both standard and patients are plotted on logarithmic paper (on Y-axis, the clotting time (seconds). On the X-axis, the FVII activity level (IU/dL)) is drawn. All the points of standard and patient are connected by drawing a line (Fig. 11.3) [4].

Interfering variables: The accuracy and precision of the test depend on the FVII-deficient plasma, calibration materials, and type of thromboplastin. It is recommended that the mean FVII activity in FVII-deficient plasma should be less than 1%. FVII-deficient plasma with more than this threshold results in the overestimation of the FVII:C, especially in patients with low FVII level. Indeed, even slight contamination of thromboplastin with FVIIa can decrease sensitivity to a patient's plasma FVII:C, while increasing sensitivity to the patient's plasma level of FV, FX, and prothrombin [1, 55]. In spite of the presence of certified reference materials for the accuracy of calibrators, the different calibration materials may exhibit variable inter-laboratory precision, especially in cases with an FVII level below 20% [1, 56, 57].

Three types of thromboplastin reagents with different sensitivity including rabbit brain, ox brain or human recombinant thromboplastin are available [54, 58].

According to the type of thromboplastin, different results might be obtained, however, variability that caused by qualitative FVII defects such as FVII Padua (Arg304Gln), FVII Nagoya (Arg364Trp), and FVII Tondabayashi, or Shinjo (Arg79Gln) are more profound. For example, in Padua variant which usually associates with no bleeding history and the normal range of FVII:Ag, disparate results toward different thromboplastins could be obtained, so that normal results using ox brain thromboplastin and abnormal results by use of rabbit brain thromboplastin might be obtained [1, 59]. It should be noted that variable reactivity of different thromboplastins only occurs in type II deficiency such as Padua, not type I deficiency. In FVII Padua or Nagoya, the FVII:C, when measured by rabbit brain thromboplastin and ox brain thromboplastin, are around 5% and 100% of normal, respectively [1, 59]. In this framework, based on structural similarity of recombinant thromboplastins and human TF, use of this product is more reliable than other thromboplastins for FVII assay. Although this approach can be suitable in certain situations, such as control of coumarin treatment, it is not an absolute rule. In cases of FVII Padua and FVII Nagoya, the level of FVII: C with human-origin

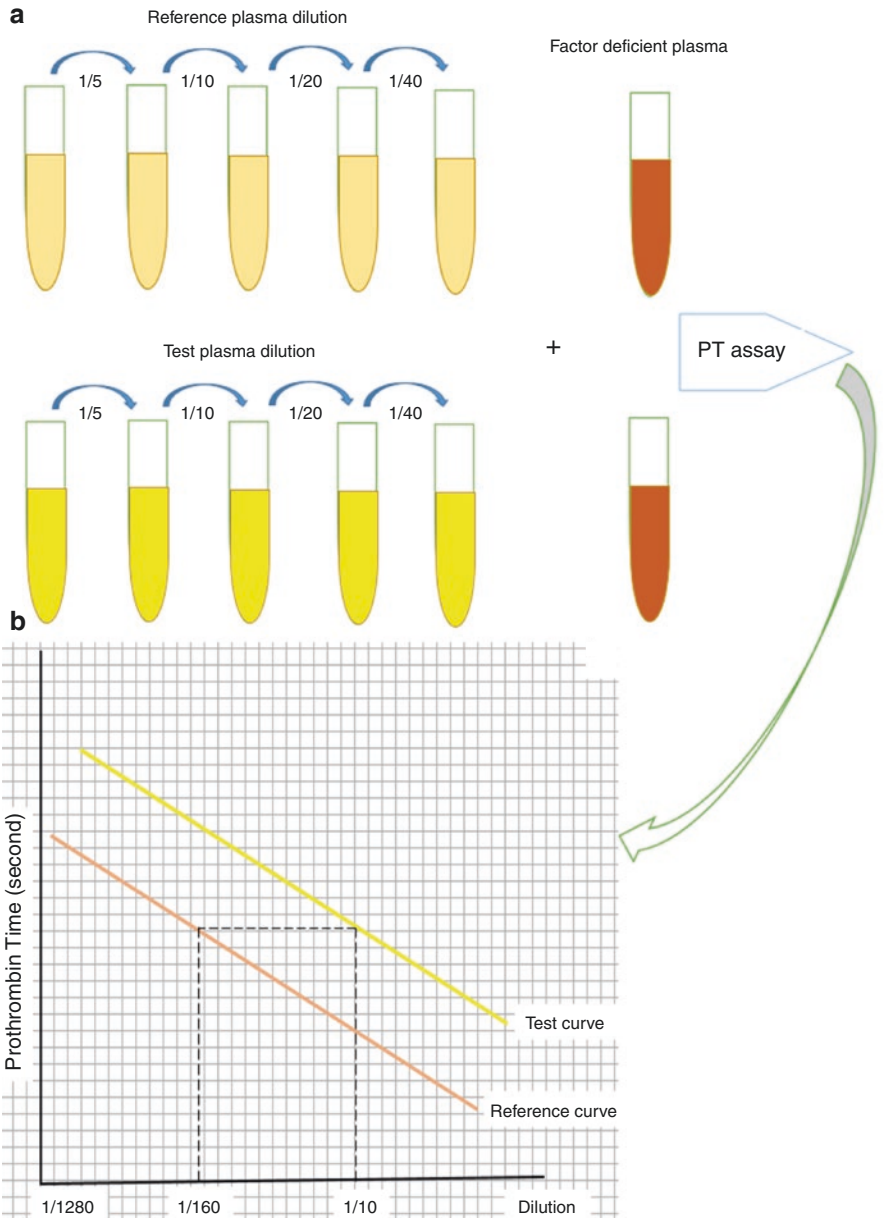

Fig. 11.3 One-stage PT-based factor VII activity assay. (**a**) Preparation of the serial dilutions of the standard and test plasma with factor VII deficient plasma. (**b**) Assessment of factor VII activity based on the reference curve

thromboplastin are about 35% of the normal levels which is different compared to other origins of thromboplastin [52, 60]. It seems that using a three-panel thromboplastin including rabbit brain, ox brain, and human origin thromboplastin is a robust approach to accurately identify all patients, especially those with qualitative FVII defect. Nevertheless, due to the high sensitivity of rabbit brain thromboplastin, it is recommended as a first-line screening test in cases with FVII defect [61]. More recently, a new variant of FVII (c.194C>G; p.Ala65Gly) has also found with discrepancies in its results when tested with different thromboplastin sources. In the corresponding study, FVII:C activity levels were found to be 4%, 28%, and 21%, respectively, using rabbit brain, recombinant human tissue factor, and human placental thromboplastin [62].

Finally, it should be noted that plasma FVII level is raised in some circumstances, such as female gender, increasing age and hyperlipidemia, especially hypertriglyceridemia [63].

Interpretation of the results: FVII deficiency is one of the RBDs with a poor correlation between FVII level and clinical picture. But on the whole, FVII activity less than 55% is considered a boundary for FVII deficiency. The relevant clinical picture usually develops in FVII:C <30% while FVII:C <2% is associated with an increased risk of severe hemorrhages. However, there are some cases with FVII<1% without spontaneous or provoked bleeding manifestations. In contrast, there are some patients with FVII:C >20% who experience severe bleeding [1, 64].

11.8.2 The Chromogenic Factor VII Assay

The chromogenic FVII assay is another approach for quantitatively measuring of FVII activity levels in citrated plasma.

Box 11.2 The Principle of Chromogenic Factor VII Assay
In this method, activated factor VII (FVIIa) triggers the activation of FX to FXa. A captured specific FVII antibody is coated onto wells. The test plasma is added to the microplate well, followed by an incubation period and subsequent washing. Captured FVII is activated to FVIIa upon the addition of a reagent containing TF (typically recombinant human TF), calcium, and FX. FX in the mixture is activated to FXa via TF/FVIIa complex during incubation. After the washing step, the specific FXa chromogenic substrate is added. The intensity of the FXa substrate, which is releases a yellow paranitroaniline (pNA) chromophore, is directly proportional to FVII activity.

Sample requirement: Blood samples should be collected into tube containing 3.8% citrate anticoagulant. The collected plasma could be stored in a plastic anticoagulant coated tube for up 4 h at room temperature and up to 24 h at 4 °C. The plasma sample can be kept at −20 °C for short and at −70 °C for long-term

storage. It should be considered that frozen plasma should be immediately thawed at 37 °C before testing.

11.9 Activated Factor VII Assay

In addition to the pivotal role of TF-FVIIa pathway in the initiation of the coagulation cascade, it has the important effect on the inflammatory pathway, regulation of inflammation and sepsis [31].

When recombinant activated FVII (rFVIIa) was introduced for the treatment of patients with inhibitor-complicated hemophilia, FVII deficiency, and also several other off-label indications such as retropubic prostatectomy bleeding, the interest for concentrates FVIIa assay was increased [1, 65, 66]. Although PT and FVII:C assay could also be used for monitoring of rFVIIa treatment, but as mentioned above, both of these tests have some limitations such as variability in results (mostly due to the source of thromboplastin), especially in a cross-reacting material-positive variants [67], even if different assays used thromboplastins with similar sensitivity, and high cost. Therefore, FVIIa assay may be more effective than PT and FVII:C for monitoring of these patients [1, 65]. FVIIa assay is not recommended for diagnosis of FVII deficiency [1, 65]. It should be noted that the level of FVIIa can be increased in some pathological conditions that are associated with coagulation activation, while it decreases during heparin therapy. The assessment of FVIIa could be performed by two methods including clotting-based and chromogenic-based assays.

11.9.1 The Clotting-Based Activated Factor VII Assay

The first method relies on PT-based clotting-based assay using recombinant soluble mutant TF molecule (sTF1–219), a TF without transmembrane and cytoplasmic domains. STF cannot activate FVII, but its FVIIa cofactor activity conserved [68, 69].

Box 11.3 The Principle of the Clotting-Based FVII Activity Assay
The truncated mutant TF molecule (sTF1–219) is the main material in the clotting-based FVIIa assay. The sTF1–219 is a recombinant TF without transmembrane and cytoplasmic domains, which is not able to activate FVII into FVIIa. However, it retains cofactor activity for FVIIa. Therefore, the presence of FVII in the plasma could not interfere with the assay. The citrated test plasma is diluted/mixed with FVII-deficient plasma. For the clotting assay, a reagent containing sTF, phospholipids, and CaCL2 is added, and the clotting time is recorded. The clotting time is converted to the FVIIa level using the standard curve. The clotting time is directly proportional to the FVIIa level. Recently, using highly purified and homogeneous sTF, a new modified method (HemoclotTM FVIIa), is developed. This method is more sensitive for FVIIa assay and can measure FVIIa in very low concentrations [1, 4].

Normal plasma FVIIa level using this technique is 0.5–8.4 ng/mL (mean 3.6 ng/mL), encompasses 1–3% of the total inactive zymogen form [3, 69], based on the specificity of the FVIIa assay [70].

Interfering variables: As mentioned above, in physiologic states, a small amounts of FVIIa are circulated in plasma. This quantity can increase in hypercoagulable states and certain pathological conditions associated with coagulation activation. However, during heparin therapy, FVIIa concentration can diminish [25, 53].

11.9.2 Chromogenic-Based Activated FVII Assays

The activated FVII on chromogenic-based assays is measured using enzyme-linked immunosorbent assay (ELISA) which is highly sensitive compared to clotting-based assays. When using this method, lower FVIIa concentration was reported compared to activity-based assay. However, a strong correlation is observed between the two methods. This method, use a high specific antibody against two-chain FVIIa that cannot reactive with FVII [69, 70]. The newly introduced chromogenic assay, BiophenTM FVIIa, utilizes sTF, which can attach specifically to FVIIa and then activate the FX to FXa. Following the addition of the chromogenic substrate releasing para-nitro-aniline (pNA), the FVIIa concentration is measured at 405 nM [53].

Reference range: approximately 0.0125 ng/mL (±0.01 ng/mL) [3].

11.10 Immunological Assay of Factor VII

The FVII:Ag can be determined by different methods including ELISA or immunoturbidimetric assays (IRMAs) using polyclonal or epitope-specific monoclonal antibodies against free-circulating FVII. Distinguishing between type I and II defects is feasible, using the FVII:Ag assay. The FVII:Ag level is not a good predictor of the severity of bleeding tendency, but it can aid in understanding of mutational mechanisms of FVII deficiency [1, 44].

Box 11.4 The Principle of Immunological Assay

Free-circulating FVII is sandwiched by the immobilized antibody specific for FVII. The biotinylated polyclonal antibody specific for another epitope of FVII is then added and followed by streptavidin-peroxidase conjugate that can be attached to the biotin antibody. Following the washing step, the OD is measured by a spectrophotometer after adding the peroxidase enzyme substrate.

11.11 Molecular Basis

Molecular studies have received noticeable attention in recent years and can reveal the disease's causative mutations. The poor correlation between the clinical picture and the FVII activity level makes molecular diagnosis useful for the exact diagnosis of disease as well as for prenatal diagnosis (PND) [71]. Moreover, molecular diagnosis can be used for the classification of FVII deficiency, especially in patients with qualitative defects. The *F7* gene spans 12.8 kb on chromosome 13q34 and contains nine exons and five short tandem repeats. These minisatellite DNA sequences cover more than a quarter of the introns sequence and more than one-third of 3′ untranslated region (UTR (of mRNA. The *F7* gene is located approximately 2.8 kb upstream of the *F10* gene and located near another vitamin K-dependent protein *Z* gene [5, 19, 30, 72]. The *F7* gene and protein are structurally homologous to other vitamin K-dependent coagulation factors, particularly FIX, FX, and protein C. The overall base compositions of the *F7* gene in exons and introns are similar (60% G-C and 40% A-T), which is similar to the protein C and the *F10* genes [19]. *F7* gene consists of nine exons. Exon 1 and 2 (classically exon 1b; an alternatively spliced target in 90% of factor VII mRNA transcripts) and a part of exon 3 encode 5′ UTR and a main part of the pre-pro leader. The Gla domain is encoded by exon 3. Exon 4 encodes the hydrophobic aromatic stack, exons 5 and 6 encode two epidermal-like growth factor (EGF) domains, exons 7 and 8 responsible for the encoding of the activation region, and finally, exon 9 encodes the catalytic domain as well as 3′ UTR including poly (A) tail (Fig. 11.4) [3].

A wide spectrum of mutations have been identified within the *F7* gene, and whole gene sequencing including exons, introns, boundaries, and promoter regions is recommended for mutation detection in patients with congenital FVII deficiency. This is mostly due to a large number of identified mutations within the *F7* gene, the short length of the gene and merely the possibility of the detection of a recurrence mutation [1]. In general, 90–92% of mutated alleles could be identified with the current routine direct sequencing methods, while ~10% of gene mutation could not be found. Although new techniques, such as next-generation sequencing (NGS) certainly can improve this situation, however, some of the cases with congenital FVII deficiency may result from mutations in other genes, which can make FVII deficiency still an open question [1, 22]. A wide spectrum of normal gene variations and disease-causing mutations, including missense, nonsense, splice site mutations, and insertions/deletions have been observed in the *F7* gene. Several functional and nonfunctional polymorphisms have also been observed (Table 11.3) [3, 22, 73]. For example, functional promoter polymorphism at position −402 (G > A) of the ATG codon leads to increased FVII:C, while promoter polymorphism at position −401 (G > T) decreases plasma FVII level [74]. Arg413Gln substitution in exon 8 (classically known as R353Q variant) arises from polymorphisms including G to A substitution at nucleotide 10,976 and is commonly found in association with the polymorphism of decanucleotide (10-bp sequence) insertion at position −323 in the 5′-flanking region of the *F7* gene. Arg403Gln, result in 30% and 23% of the variance in FVII:C and FVII:Ag, respectively, while the decanucleotide polymorphism

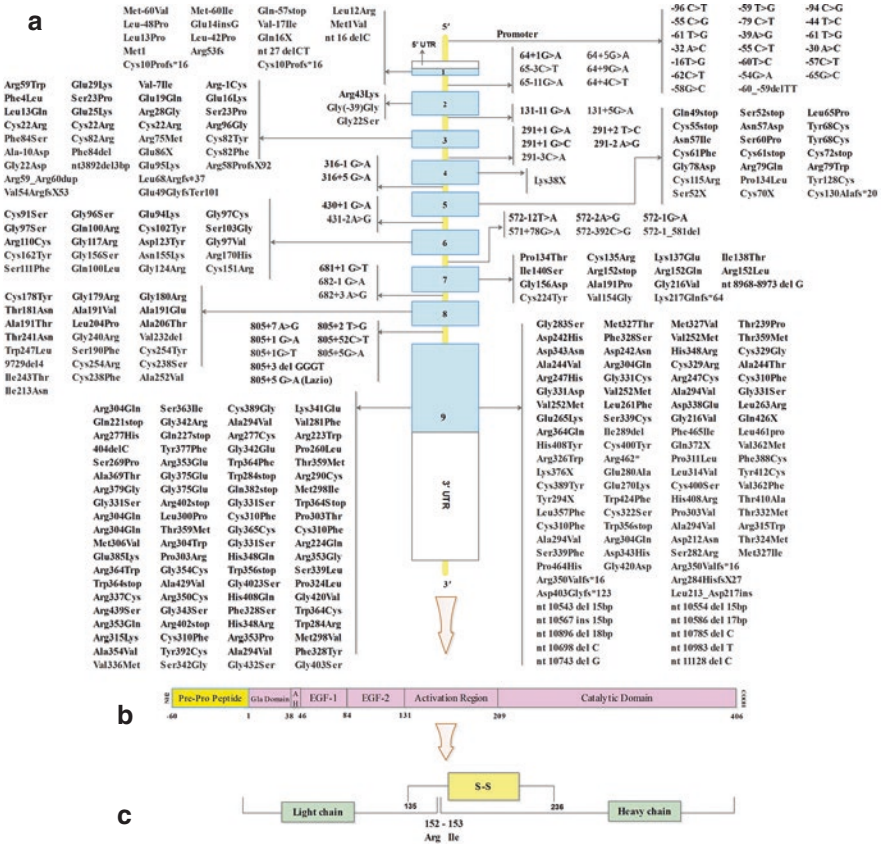

Fig. 11.4 *F7* gene and FVII protein structures and spectrum of gene mutations in *F7* gene. (**a**) *F7* gene contains nine exons that encode FVII protein. Exon 2 is usually alternatively spliced in 90% of the FVII mRNA transcript. (**b**) The FVII protein contains pre-pro sequence, Gla, EGF1, EGF2, and catalytic serine protease domains. (**c**) Cleavage at Arg152-Ile153 location leads to generation of a two-chain molecule that joins together by a disulfide bond between Cys135 and Cys236. Light chain contains residues +1 to 152 and heavy chain contains residues 153–406. *FVII* factor VII; *Gla* gamma-carboxyglutamate acid; *EGF* epidermal growth factor1

brings about 26% and 23% of the variance in FVII:C and FVII:Ag, respectively [3, 74, 75]. The *F7* gene has five short tandem repeats (STR) that span a quarter of the intron sequence and more than one-third of the 3′ untranslated region of mRNA. A small part of one of these STRs is located on the end of exon 8 (including 11 nucleotides "CGCGGTGCTGG"). This sequence in wild-type exon has 6 repeats with 26 spaces between them (795_805 + 26 [6]). Three polymorphisms with 6, 7, and 8 sequence repetition may be seen during direct sequencing in this location. The presence of STR, repeated regions, and polymorphism variation of repeats within the *F7* gene may be associated with misdiagnosis that requires special attention to design the PCR primers and the use of other ways than conventional polymerase chain reaction (PCR)-based techniques such as semi-quantitative multiplex

Table 11.3 Factor VII gene polymorphisms>

Polymorphism type	Location	MAF	Effect on FVII:C	Comment
Decanucleotide [CCTATATCCT] inser[a]	5′ region (−323)	0.23	Decrease	In linkage disequilibrium with p.Arg413Gln
G/T dimorphism	5′ region (−401)	0.2041	Decrease	In complete allelic association with c.-323ins10 and c.-122C
G/A dimorphism	5′ region (−402)	0.2326	Increase	–
c.64+9G>A (G73A)[a]	Intron 1	0.2096	Decrease	In linkage disequilibrium with p.Arg413Gln
c.525C>T (his 115=)	Exon 6	0.1419	–	–
VNTR repeat (37 bp monomer repeat, 9716ins)[b]	Intron 8	–	Decrease	VNTR[6] is the Wild Type
c.806-20G>A (G/A dimorphism)	Intron 8	0.1342	–	
c.1238G>A (Arg353Gln polymorphism)[a]	Exon 9	0.80 0.20	Decrease	In linkage disequilibrium with the c.-401 T/c.-325_-324ins10/c.-122C haplotype

MAF minor allele frequency; *NM* not mentioned. [a] The 10 bp insertion and the Arg353Gln polymorphism indicate a strong linkage disequilibrium, and therefore it is not clear whether the 73A allele or 10 bp insertion contributed per se to lowering FVII:C. [b] The high mRNA expression in quantitative mRNA analysis has shown that this polymorphism probably is associated with increased plasma FVII level, although there are contradictory results in this regard

fluorescent-PCR (SQF-PCR), multiplex ligation-dependent probe amplification or multiplex amplification and probe hybridization, especially in patients who have a discrepancy between genotype and FVII:C level or those that present the discrepancies in the inheritance pattern [76, 77]. For instance, in another study, two patients with the previously known homozygous mutation were further studied by SQF-PCR and were revealed that they carry a novel heterozygous large genomic rearrangement. As mentioned above, in cases with confounding results, these uncommon techniques may be useful [76].

According to databases available at https://f7-db.eahad.org/, and http://www.factorvii.org/index.php as well as our literature review [78], most of the mutations in the *F7* gene, similar to other congenital bleeding disorders are point mutation., however, in spite of the presence of some minor different in mutations number and percentage, in general, missense mutations are the most frequent while nonsense mutations are the rarest mutations. Exon 9 as the largest exon (1.6 kb) [19] in the *F7* gene that is responsible for encoding the catalytic domain has a considerable number of mutations (Fig. 11.5).

Prenatal diagnosis (PND) can be used in patients with congenital FVII deficiency, but it is more suitable for those families with severe factor deficiency and a history of life-threatening bleeding such as ICH [1, 79].

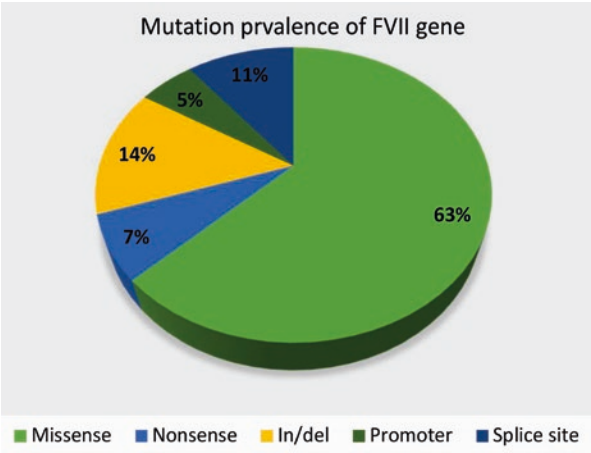

Fig. 11.5 Mutation prevalence of factor VII (FVII) gene in its exons. Most common mutations of FVII gene are missense mutations (79%), while small Ins/Del (9%), splice sites (8%) and nonsense (4%) mutations form others mutations in *F7* gene

11.12 Management

Due to highly variable clinical presentations and the low correlation between the severity of clinical presentations and FVII:C level, bleeding risk prediction and management of these patients remains controversial. The mainstay of treatment in patients with congenital FVII deficiency is on-demand replacement therapy which means the stop of bleeding as soon as possible after the occurrence of bleeding. It should be borne in mind that in the setting of high-risk bleeding conditions such as childbirth or surgery, this rule still holds, and thereby clinical history should be considered as an indicator of the need for prophylactic treatment. Nonetheless, there are very low reports about the use of rFVIIa in women with FVII deficiency who experienced vaginal delivery or cesarean and therefore don't exist definite guidelines to help caregivers to manage this process. However, in pregnant women with known severe FVII deficiency, rFVIIa administration is suggested during delivery (both vaginal and cesarean) and the decision about prophylaxis in cases with mild-moderate FVII deficiency is depending on bleeding tendency, multiple gestations, type of delivery, and PT or FVII levels in the third trimester. On the whole, management of delivery should be addressed on a case-by-case. In these situations, the efficient use of rFVIIa (with different treatment protocols) before delivery to prevent postpartum hemorrhage, has been shown. Due to the lack of specific guidelines to point to the specific dose and duration of treatment during delivery, the same dose of hemorrhage treatment is used for hemorrhagic events [80, 81].

In patients with a history of life-threatening bleeding such as ICH, secondary prophylaxis is recommended. Primary prophylaxis could be used for those patients

Table 11.4 Available therapeutic options for patients with factor VII deficiency

Factor	Advantage	Disadvantage
FFP	Easily available, cheap	Limited effectiveness, need to high volumes for treatment, fluid overload, risk of viral transmission
Pd-FVII	Effective; suitable for surgery	Unavailable in some countries, other vitamin K-dependent factors concentrations are higher than factor VII, risk of viral transmission, risk of TE
rFVIIa	Very effective Low dosage requirement for treatment No risk of viral transmission Not immunogenic in patients with hemophilia Not produces an anamnestic response in hemophilia patients with inhibitors Very low thrombogenicity	Risk of TE, expensive
PCC	Suitable for surgery	Risk of TE, concentration of other vitamin K-dependent factors is higher than factor VII and presence of activated factors. Variable amount of factor VII

Pd-FVII plasma-derived FVII; *FFP* fresh frozen plasma; *rFVIIa* recombinant FVIIa; *TE* thrombotic events; *PCC* prothrombin complex concentrates

with severe factor deficiency and risk of life-threatening bleeding. Different therapeutic choices including FFP, pd-FVII, PCC, aPCC, and rFVIIa are available for patients with FVII deficiency (Table 11.4) [1, 30]. In this setting, Pd-FVII and rFVIIa are the most commonly used treatments. However, in low-incoming countries, where access to these products is limited, FFP and PCC are also used, although they contain low levels of FVII [82].

The recommended dose and therapeutic target levels of FVII for on-demand, prophylaxis and surgeries are summarized in Table 11.5 [83].

rFVIIa (eptacog alfa) is a structurally similar product to plasma-derived coagulation factor VIIa but is manufactured using DNA biotechnology [84, 85]. The first report of successful treatment with rFVIIa was in 1988 with Novoseven® (rFVIIa; NovoSeven, Novo Nordisk, Copenhagen, Denmark) in a patient with severe haemophilia A during synovectomy [86]. Another generic rFVIIa, Aryoseven™, is claimed to have bio-similarity with Novoseven® as well as similar clinical safeness and effectiveness [87–89]. rFVIIa has been approved for the treatment of patients with congenital FVII deficiency, congenital hemophilia B with high-responding inhibitors, acquired hemophilia, and Glanzmann thrombasthenia with refractoriness to platelet transfusions, with or without antibodies to platelets and also recommended as the first-line therapeutic option for hemophilia A patients with high-responder inhibitors [84, 85]. rFVIIa has also been used in surgical bleeding caused by dilutional or consumptive coagulopathies or in patients with impaired liver function

Table 11.5 Recommended dose and therapeutic target levels for factor VII on on-demand and prophylaxis approaches in patients with factor VII deficiency

Recommended and Maintaining level	Plasma half-life	On-demand dosages	Major surgery	Minor surgery	Long-term prophylaxis dosages
>20%	2–4 h	Pd-FVII concentrate (30–40 U/kg)	rFVIIa Before surgery: 15–30 μg/kg After surgery: continue the same dose for first day with 4–6 h interval, and then change interval to 8–12 h or Pd-FVII concentrate: 8–40 IU/kg with similar intervals	Tranexamic acid 15–20 mg/kg or 1 g four times daily or Antifibrinolytics[a]	FFP: 10–15 mL/kg two times/week Pd-FVII: 30–40 U/kg three times/week rFVIIa: 20-30 μg/kg two or three times/week or a total weekly dose of 90 μg/kg with three times/week

Pd-FVII plasma-derived FVII; *FFP* fresh frozen plasma; *rFVIIa* recombinant FVIIa
[a] This recommendation need to further research because the quality of evidence is moderate

[90]. In addition, the rFVIIa can be used in various conditions, such as spontaneous bleeding, hemarthrosis, and major surgical procedures. However, it is also necessary to mention that one of the most important challenges of administering rFVIIa is inhibitor development [91]. According to FDA report, the risk of thrombotic events associated with rFVIIa is 2% of treated patients in rFVIIa clinical trials. More recently rFVIIa, eptacog beta (brand name SEVENFACT®, LFB Biotechnologies, Hema Biologics), has been approved by the FDA in 2020 for on-demand treating bleeding episodes in patients >12 to 75 years of age with hemophilia A or B complicated by inhibitors [92, 93]. Marzeptacog alfa (MarzAA), has passed phase 1/2/3, an open-label clinical trial study for the prophylaxis treatment of non-bleeding patients with congenital hemophilia A or B with or without inhibitors [67, 93]. However, the very low frequency of thrombotic events, no virus transmission and scarce production of inhibitory antibodies are advantages of rFVIIa, while expensiveness and short half-life of rFVIIa even than FVII and FVIIa are disadvantages of rFVIIa [1].

PCC is another therapeutic choice for patients with FVII deficiency. It usually includes FII (prothrombin), FIX, FX, and the varying amounts of FVII. In general, 2 commercially types of PCC are available, including 3-factor PCC (with absent or low levels of FVII) and 4-factor PCC (with high level of FVII) (Table 11.6), and also another form is activated PCC (factor eight inhibitor bypass activity; FEIBA), contains 4-factors which includes both inactive (FII, FIX, and FX) and active (FVII) forms [94–96]. The amount of FVII is variable in different manufactured PCC that

Table 11.6 Characteristics of some available four factor prothrombin complex concentrates (PCC)

PCC	Manufacturer/ country	Hemophilia B (factor IX)	Acquired deficiency (e.g., VKA)	Congenital deficiency (specific factor not available)	Other factor deficiency, such as factor II, VII, X	Reference
Beriplex P/N	CSL Behring GmbH/Germany		√	√		[105]
Kcentra[a]	CSL Behring GmbH/Germany		√			[106]
Cofact	Sanquin/ Netherlands		√	√		[107]
Kaskadil	LFB/France		√		√ (FII & FX)	[108]
Octaplex	Octapharma/ Vienna, Austria		√		√ (FII & FX)	[109]
Prothromplex Total 600 IU	Baxalta innovations GmbH/Vienna, Austria		√	√		[110]
Proplex T[b]	Baxter/ Glendale, USA	√			√ (FVII)	[111]

PCC prothrombin complex concentrates; *VKA* vitamin K antagonists; *FIX* factor IX; *FII* factor II; *FVII* factor VII; *FX* factor X

[a] Kcentra is indicated for the urgent reversal of acquired coagulation factor deficiency induced by vitamin K antagonists therapy in adults patients during acute major bleeding (It does not have indication in patients without acute major bleeding)

[b] Proplex T is indicated for treatment of bleeding episodes in patients with factor VIII deficiency with inhibitors

usually indicated by the manufacturer, thus after requirement calculation could be administered [3]. Some PCC may contain additional components such as anticoagulants, protein C, protein S, protein Z, and antithrombin III as well as heparin to mitigate thrombotic risk [95, 97, 98]. Overall clotting factors of these concentrates are approximately 25 times higher than normal plasma [99]. Some advantages of PCC over the FFP include a relatively constant high level of vitamin K-dependent coagulation factors (FII, FVII, FIX, and FX), a more rapid decrease in INR value, and no need for matching the blood groups or thaw the product [96, 100]. Several reports indicated both venous and arterial thrombosis associated with PCC, therefore, these concentrates should not be used in patients with liver disease, major trauma, or neonates (due to their relatively immature livers). The incidence of thrombotic events in patients treated with 4-factor and 3-factor PCC is 1.8% and 0.7%, respectively [96]. Another disadvantage of PCC is a high concentration of other vitamin K-dependent factors than FVII [1, 30, 101–103].

The pd-FVII is a useful product for prophylaxis in children with severe FVII deficiency and for long-term prophylaxis in the range of 30–40 U/Kg, three times a week. Various doses of Pd-FVII have successfully been used for surgery, ranging from 8 to 40 U/Kg every 4–6 h. For major surgeries, FVII level must be kept above

20 U/dL. Similar to PCC, pd-FVII has a lower FVII concentration than other vitamin K-dependent coagulation factors [3, 30, 104]. Acquired FVII deficiency is usually treated as same as inherited FVII deficiency by FFP, PCC, aPCC, and pd-FVII or rFVIIa. However, in these cases, the underlying diseases should be treated.

References

1. Sevenet P-O, Kaczor DA, Depasse F. Factor VII deficiency: from basics to clinical laboratory diagnosis and patient management. Clin Appl Thromb Hemost. 2016;23:1076029616670257.
2. Herrmann F, Wulff K, Auerswald G, Schulman S, Astermark J, Batorova A, et al. Factor VII deficiency: clinical manifestation of 717 subjects from Europe and Latin America with mutations in the factor 7 gene. Haemophilia. 2009;15(1):267–80.
3. Perry DJ. Factor VII deficiency. Br J Haematol. 2002;118(3):689–700.
4. Shams M, Dorgalaleh A. Congenital factor VII deficiency. Congenital bleeding disorders. Springer; 2018. p. 239–59.
5. Lapecorella M, Mariani G, Deficiency IRoCFV. Factor VII deficiency: defining the clinical picture and optimizing therapeutic options. Haemophilia. 2008;14(6):1170–5.
6. Peyvandi F, James P, Salomon O, Mikovic D. Rare bleeding disorders. Haemophilia. 2014;20(4):71.
7. Mariani G, Herrmann FH, Dolce A, Batorova A, Etro D, Peyvandi F, et al. Clinical phenotypes and factor VII genotype in congenital factor VII deficiency. Thromb Haemost. 2005;93(3):481.
8. Mackman N. The role of tissue factor and factor VIIa in hemostasis. Anesth Analg. 2009;108(5):1447.
9. Rosen ED, Chan JC, Idusogie E, Clotman F. Mice lacking factor VII develop normally but suffer fatal perinatal bleeding. Nature. 1997;390(6657):290.
10. Peltier S, Kellum A, Brewer J, Duncan A, Cooper DL, Saad H. Psychosocial impact and disease management in patients with congenital factor VII deficiency. J Blood Med. 2020;11:297.
11. Moerloose P, Schved JF, Nugent D. Rare coagulation disorders: fibrinogen, factor VII and factor XIII. Haemophilia. 2016;22(S5):61–5.
12. Smith SA, Travers RJ, Morrissey JH. How it all starts: initiation of the clotting cascade. Crit Rev Biochem Mol Biol. 2015;50(4):326–36.
13. Hussain MA, Abogresha NM, Hassan R, Tamany DA, Lotfy M. Effect of feeding a high-fat diet independently of caloric intake on reproductive function in diet-induced obese female rats. Arch Med Sci. 2016;12(4):906.
14. Neuenschwander PF, Morrissey JH. Roles of the membrane-interactive regions of factor VIIa and tissue factor. The factor VIIa Gla domain is dispensable for binding to tissue factor but important for activation of factor X. J Biol Chem. 1994;269(11):8007–13.
15. Tavoosi N, Smith SA, Davis-Harrison RL, Morrissey JH. Factor VII and protein C are phosphatidic acid-binding proteins. Biochemistry. 2013;52(33):5545–52.
16. Fair DS. Quantitation of factor VII in the plasma of normal and warfarin-treated individuals by radioimmunoassay. Blood. 1983;62(4):784–91.
17. Vadivel K, Bajaj SP. Structural biology of factor VIIa/tissue factor initiated coagulation. Front Biosci. 2012;17:2476.
18. Eigenbrot C, Kirchhofer D, Dennis MS, Santell L, Lazarus RA, Stamos J, et al. The factor VII zymogen structure reveals reregistration of β strands during activation. Structure. 2001;9(7):627–36.
19. O'Hara PJ, Grant FJ, Haldeman BA, Gray CL, Insley MY, Hagen FS, et al. Nucleotide sequence of the gene coding for human factor VII, a vitamin K-dependent protein participating in blood coagulation. Proc Natl Acad Sci. 1987;84(15):5158–62.

20. Banner DW, D'Arcy A, Chène C, Winkler FK, Guha A, Konigsberg WH, et al. The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. Nature. 1996;380(6569):41–6.
21. Butenas S, Mann KG. Kinetics of human factor VII activation. Biochemistry. 1996;35(6):1904–10.
22. McVey JH, Boswell E, Mumford AD, Kemball-Cook G, Tuddenham EG. Factor VII deficiency and the FVII mutation database. Hum Mutat. 2001;17(1):3–17.
23. Spiezia L, Campello E, Dalla Valle F, Woodhams B, Simioni P. Factor VIIa-antithrombin complex: a possible new biomarker for activated coagulation. Clin Chem Lab Med. 2017;55(4):484–8.
24. Rao L, Nordfang O, Hoang A, Pendurthi U. Mechanism of antithrombin III inhibition of factor VIIa/tissue factor activity on cell surfaces. Comparison with tissue factor pathway inhibitor/factor Xa-induced inhibition of factor VIIa/tissue factor activity. Blood. 1995;85(1):121–9.
25. Monroe DM, Key N. The tissue factor–factor VIIa complex: procoagulant activity, regulation, and multitasking. J Thromb Haemost. 2007;5(6):1097–105.
26. Iakhiaev A, Pendurthi UR, Voigt J, Ezban M, Rao LVM. Catabolism of factor VIIa bound to tissue factor in fibroblasts in the presence and absence of tissue factor pathway inhibitor. J Biol Chem. 1999;274(52):36995–7003.
27. Lwaleed BA, Bass PS. Tissue factor pathway inhibitor: structure, biology and involvement in disease. J Pathol. 2006;208(3):327–39.
28. Rosen ED, Xu H, Liang Z, Martin JA, Suckow M, Castellino FJ. Generation of genetically-altered mice producing very low levels of coagulation factor VII. Thromb Haemost. 2005;94(3):493–7.
29. Tuddenham EGD, Pemberton S, Cooper DN. Inherited factor VII deficiency: genetics and molecular pathology article in thrombosis and haemostasis. Thromb Haemost. 1995;74(1):313–21.
30. Mariani G, Bernardi F. Factor VII deficiency. Seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2009.
31. Versteeg HH, Peppelenbosch MP, Spek CA. Tissue factor signal transduction in angiogenesis. Carcinogenesis. 2003;24(6):1009–13.
32. Krikun G, Schatz F, Taylor H, Lockwood CJ. Endometriosis and tissue factor. Ann N Y Acad Sci. 2008;1127(1):101–5.
33. Alexander B, Goldstein R, Landwehr G, Cook C, Addelson E, Wilson C. Congenital SPCA deficiency: a hitherto unrecognized coagulation defect with hemorrhage rectified by serum and serum fractions. J Clin Investig. 1951;30(6):596.
34. Triplett DA, Brandt JT, Batard M, Dixon J, Fair DS. Hereditary factor VII deficiency: heterogeneity defined by combined functional and immunochemical analysis. Blood. 1985;66(6):1284–7.
35. Girolami A, Santarossa C, Cosi E, Ferrari S, Lombardi AM. Acquired isolated FVII deficiency: an underestimated and potentially important laboratory finding. Clin Appl Thromb Hemost. 2016;22(8):705–11.
36. Mulliez SM, Devreese KM. Isolated acquired factor VII deficiency: review of the literature. Acta Clin Belg. 2016;71(2):63–70.
37. Moosavi L, Bowen J, Coleman J, Heidari A, Cobos E. Acute myelogenous leukemia with trisomy 8 and concomitant acquired factor VII deficiency. J Investig Med High Impact Case Rep. 2019;7:2324709619872657.
38. Toor A, Slungaard A, Hedner U, Weisdorf D, Key N. Acquired factor VII deficiency in hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2002;29(5):403.
39. Zaidi MH, Stanley A, Khan M. Acquired factor VII deficiency–a rare but important consideration. Scott Med J. 2019;64(3):119–22.
40. Biron C, Bengler C, Gris J, Schved J. Acquired isolated factor VII deficiency during sepsis. Pathophysiol Haemost Thromb. 1997;27(2):51–6.

41. Delmer A, Horellou M, Andreu G, Lecompte T, Rossi F, Kazatchkine M, et al. Life-threatening intracranial bleeding associated with the presence of an antifactor VII autoantibody. Blood. 1989;74(1):229–32.
42. Abu-Quider A, Asleh M, Fruchtman Y, Ben-Harosh M, Beck G, Abuhasira R, et al. Factor VII deficiency in patients receiving chronic packed cell transfusions. J Pediatr Hematol Oncol. 2021;43(2):e268–e71.
43. Napolitano M, Giansily-Blaizot M, Dolce A, Schved JF, Auerswald G, Ingerslev J, et al. Prophylaxis in congenital factor VII deficiency: indications, efficacy and safety. Results from the Seven Treatment Evaluation Registry (STER). Haematologica. 2013;98(4):538–44.
44. Napolitano M, Siragusa S, Mariani G. Factor VII deficiency: clinical phenotype, genotype and therapy. J Clin Med. 2017;6(4):38.
45. Mariani G, Mazzucconi M. Factor VII congenital deficiency. Pathophysiol Haemost Thromb. 1983;13(3):169–77.
46. Peyvandi F, Mannucci P, Asti D, Abdoullahi M, Di Rocco N, Sharifian R. Clinical manifestations in 28 Italian and Iranian patients with severe factor VII deficiency. Haemophilia. 1997;3(4):242–6.
47. Trillo A, Kronenfeld R, Simms-Cendan J, Davis JA, Corrales-Medina FF. High prevalence of congenital factor VII (FVII) Deficiency in adolescent females with heavy menstrual bleeding and iron Deficiency anemia. J Pediatr Adolesc Gynecol. 2022;35(6):647–52.
48. Giansily-Blaizot M, Verdier R, Biron-Adreani C, Schved J-F, Bertrand M, Borg J, et al. Analysis of biological phenotypes from 42 patients with inherited factor VII deficiency: can biological tests predict the bleeding risk? Haematologica. 2004;89(6):704–9.
49. Singh B, Modi V, Kaur P, Guron G, Maroules M. Unprovoked pulmonary embolism in factor VII deficiency. Acta Haematol. 2020;143(2):181–3.
50. Mariani G, Herrmann F, Schulman S, Batorova A, Wulff K, Etro D, et al. Thrombosis in inherited factor VII deficiency. J Thromb Haemost. 2003;1(10):2153–8.
51. Marty S, Barro C, Chatelain B, Fimbel B, Tribout B, Reynaud J, et al. The paradoxical association between inherited factor VII deficiency and venous thrombosis. Haemophilia. 2008;14(3):564–70.
52. Girolami A, Cosi E, Santarossa C, Ferrari S, Randi ML. The story of serum prothrombin conversion accelerator, proconvertin, stable factor, cothromboplastin, prothrombin accelerator or autoprothrombin I, and their subsequent merging into factor VII. Seminars in thrombosis and hemostasis. Thieme Medical Publishers; 2015.
53. Amiral J, Dunois C, Amiral C, Seghatchian J. The various assays for measuring activity states of factor VIIa in plasma and therapeutic products: diagnostic value and analytical usefulness in various pathophysiological states. Transfus Apher Sci. 2017;56(1):91–7.
54. Poggio M, Tripodi A, Mariani G, Mannucci PM. Factor VII clotting assay: influence of different thromboplastins and factor VII-deficient plasmas. CISMEL Study Group. Thromb Haemost. 1991;65(2):160–4.
55. Smith S, Comp P, Morrissey J. Traces of factor VIIa modulate thromboplastin sensitivity to factors V, VII, X, and prothrombin. J Thromb Haemost. 2006;4(7):1553–8.
56. Zantek N, Hsu P, Refaai M, Ledford-Kraemer M, Meijer P, Van Cott E. Factor VII assay performance: an analysis of the North American specialized coagulation laboratory association proficiency testing results. Int J Lab Hematol. 2013;35(3):314–21.
57. Takamiya O, Ishikawa S, Ohnuma O, Suehisa H, Iijima K, Kayamori Y, et al. Japanese collaborative study to assess inter-laboratory variation in factor VII activity assays. J Thromb Haemost. 2007;5(8):1686–92.
58. Bleavins MR, Carini C, Jurima-Romet M, Rahbari R. Biomarkers in drug development: a handbook of practice, application, and strategy. Wiley; 2011.
59. Girolami A, Fabris F, Dal Bo ZR, Ghiotto G, Burul A. Factor VII Padua: a congenital coagulation disorder due to an abnormal factor VII with a peculiar activation pattern. J Lab Clin Med. 1978;91(3):387–95.

60. Girolami A, Treleani M, Scarparo P, Bonamigo E, Lombardi AM. Considerations on a tentative classification of FVII deficiency suited for practical clinical purposes. Clin Appl Thromb Hemost. 2012;18(6):654–7.
61. Kirkel D, Lin T-W, Fu SW, Dlott JS, Sahud MA, McCaffrey T, et al. Asymptomatic factor VII deficiency: gene analysis and structure–function relationships. Blood Coagul Fibrinolysis. 2010;21(1):91–4.
62. Gallardo CA, Wong LJL, Sum CLL, Goh LL, Ong KH. Compound heterozygous factor VII deficiency c.1025G>A p.(Arg342Gln) with novel missense variant c.194C>G p.(Ala65Gly). J Hematol. 2022;11(1):29.
63. Simpson H, Meade T, Stirling Y, Mann J, Chakrabarti R, Woolf L. Hypertriglyceridaemia and hypercoagulability. Lancet. 1983;321(8328):786–90.
64. Shams M, Dorgalaleh A, Safarian N, Emami AH, Zaker F, Tabibian S, et al. Inhibitor development in patients with congenital factor VII deficiency, a study on 50 Iranian patients. Blood Coagul Fibrinolysis. 2019;30(1):24–8.
65. Cid A, Lorenzo J, Haya S, Montoro J, Casana P, Aznar J. A comparison of FVII: C and FVIIa assays for the monitoring of recombinant factor VIIa treatment. Haemophilia. 2001;7(1):39–41.
66. Friederich PW, Henny CP, Messelink EJ, Geerdink MG, Keller T, Kurth K-H, et al. Effect of recombinant activated factor VII on perioperative blood loss in patients undergoing retropubic prostatectomy: a double-blind placebo-controlled randomised trial. Lancet. 2003;361(9353):201–5.
67. Gruppo R, Malan D, Kapocsi J, Nemes L, Hay C, Boggio L, et al. Phase 1, single-dose escalating study of marzeptacog alfa (activated), a recombinant factor VII a variant, in patients with severe hemophilia. J Thromb Haemost. 2018;16(10):1984–93.
68. Neuenschwander PF, Morrissey JH. Deletion of the membrane anchoring region of tissue factor abolishes autoactivation of factor VII but not cofactor function. Analysis of a mutant with a selective deficiency in activity. J Biol Chem. 1992;267(20):14477–82.
69. Morrissey JH, Macik BG, Neuenschwander PF. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. Blood. 1993;81(3):734–44.
70. Philippou H, Adami A, Amersey RA, Stubbs PJ, Lane DA. A novel specific immunoassay for plasma two-chain factor VIIa: investigation of FVIIa levels in normal individuals and in patients with acute coronary syndromes. Blood. 1997;89(3):767–75.
71. Tabibian S, Shams M, Naderi M, Dorgalaleh A. Prenatal diagnosis in rare bleeding disorders—an unresolved issue? Int J Lab Hematol. 2018;40(3):241–50.
72. Colman RW. Hemostasis and thrombosis: basic principles and clinical practice. Lippincott Williams & Wilkins; 2006.
73. Peyvandi F, Garagiola I, Palla R, Marziliano N, Mannucci P. Role of the 2 adenine (g. 11293_11294insAA) insertion polymorphism in the 3′ untranslated region of the factor VII (FVII) gene: molecular characterization of a patient with severe FVII deficiency. Hum Mutat. 2005;26(5):455–61.
74. van't Hooft FM, Silveira A, Tornvall P, Iliadou A, Ehrenborg E, Eriksson P, et al. Two common functional polymorphisms in the promoter region of the coagulation factor VII gene determining plasma factor VII activity and mass concentration. Blood. 1999;93(10):3432–41.
75. Giansily-Blaizot M, Rallapalli PM, Perkins SJ, Kemball-Cook G, Hampshire DJ, Gomez K, Ludlam CA, McVey JH. The EAHAD blood coagulation factor VII variant database. Human Mutation. 2020;41(7):1209–19.
76. Giansily-Blaizot M, Thorel D, Khau Van Kien P, Behar C, Romey MC, Mugneret F, et al. Characterisation of a large complex intragenic re-arrangement in the FVII gene (F7) avoiding misdiagnosis in inherited factor VII deficiency. Br J Haematol. 2007;138(3):359–65.
77. Hewitt J, Ballard JN, Nelson TN, Smith VC, Griffiths TA, Pritchard S, et al. Severe FVII deficiency caused by a new point mutation combined with a previously undetected gene deletion. Br J Haematol. 2005;128(3):380–5.

78. Dorgalaleh A, Bahraini M, Shams M, Parhizkari F, Dabbagh A, Naderi T, et al. Molecular basis of rare congenital bleeding disorders. Blood Rev. 2022:101029.
79. Farah R, Al Danaf J, Braiteh N, Costa JM, Farhat H, Mariani G, et al. Life-threatening bleeding in factor VII deficiency: the role of prenatal diagnosis and primary prophylaxis. Br J Haematol. 2015;168(3):452–5.
80. Loddo A, Cornacchia S, Cane FL, Barcellona D, Marongiu F, Melis GB, et al. Prophylaxis of peripartum haemorrhage using recombinant factor VIIa (rfVIIa) in pregnant women with congenital factor VII deficiency: a case report and literature review. Eur J Obstet Gynecol Reprod Biol. 2019;235:77–80.
81. Lee EJ, Burey L, Abramovitz S, Desancho MT. Management of pregnancy in women with factor VII deficiency: a case series. Haemophilia. 2020;26(4):652–6.
82. Şenol BK, Zülfikar B. Clinical problems and surgical interventions in inherited factor VII deficiency. Turk Arch Pediatr. 2020;55(2):184.
83. Peyvandi F, Menegatti M. Treatment of rare factor deficiencies in 2016. ASH Educ Program Book. 2016;2016(1):663–9.
84. Asif M, Siddiqui A, Scott LJ. Recombinant factor VIIa (eptacog alfa): a review of its use in congenital or acquired haemophilia and other congenital bleeding disorders. Drugs. 2005;65(8):1161–78.
85. Croom KF, McCormack PL. Recombinant factor VIIa (eptacog alfa): a review of its use in congenital hemophilia with inhibitors, acquired hemophilia, and other congenital bleeding disorders. BioDrugs. 2008;22(2):121–36.
86. Hedner U, Glazer S, Pingel K, Alberts KA, Blonback M, Schulman S, et al. Successful use of recombinant factor VIIa in patient with severe haemophilia a during synovectomy. Lancet. 1988;332:1193.
87. Sadeghi N, Kahn D, Syed D, Iqbal O, Abro S, Eshraghi R, et al. Comparative biochemical and functional studies on a branded human recombinant factor VIIa and a biosimilar equivalent product. Clin Appl Thromb Hemost. 2014;20(6):565–72.
88. Faranoush M, Abolghasemi H, Toogeh G, Karimi M, Eshghi P, Managhchi M, et al. A comparison between recombinant activated factor VII (Aryoseven) and Novoseven in patients with congenital factor VII deficiency. Clin Appl Thromb Hemost. 2015;21(8):724–8.
89. Faranoush M, Abolghasemi H, Mahboudi F, Toogeh G, Karimi M, Eshghi P, et al. A comparison of efficacy between recombinant activated factor VII (Aryoseven) and Novoseven in patients with hereditary FVIII deficiency with inhibitor. Clin Appl Thromb Hemost. 2016;22(2):184–90.
90. Miller RD, Eriksson LI, Fleisher LA, Wiener-Kronish JP, Cohen NH, Young WL. Miller's anesthesia E-book. Elsevier Health Sciences; 2014.
91. Bauer KA. Treatment of factor VII deficiency with recombinant factor VIIa. Pathophysiol Haemost Thromb. 1996;26(Suppl. 1):155–8.
92. Chrisentery-Singleton T, Amos LE, Bonzo D, Escobar M, Giermasz A, Lagrue E, et al. A preliminary analysis of Athn 16: safety of coagulation factor VIIa (recombinant)-Jncw for the treatment of bleeding events in patients with congenital hemophilia a or B with inhibitors. Blood. 2022;140(Supplement 1):11360–1.
93. Meeks SL, Leissinger CA. The evolution of factor VIIa in the treatment of bleeding in haemophilia with inhibitors. Haemophilia. 2019;25(6):911–8.
94. Awad NI, Cocchio C. Activated prothrombin complex concentrates for the reversal of anticoagulant-associated coagulopathy. Pharm Ther. 2013;38(11):696.
95. Sørensen B, Spahn DR, Innerhofer P, Spannagl M, Rossaint R. Clinical review: prothrombin complex concentrates-evaluation of safety and thrombogenicity. Crit Care. 2011;15(1):201.
96. Dentali F, Marchesi C, Pierfranceschi MG, Crowther M, Garcia D, Hylek E, et al. Safety of prothrombin complex concentrates for rapid anticoagulation reversal of vitamin K antagonists. Thromb Haemost. 2011;106(3):429–38.
97. Grottke O, Rossaint R, Henskens Y, van Oerle R, ten Cate H, Spronk HM. Thrombin generation capacity of prothrombin complex concentrate in an in vitro dilutional model. PLoS One. 2013;8(5):e64100.

98. Pabinger I, Brenner B, Kalina U, Knaub S, Nagy A, Ostermann H. Prothrombin complex concentrate (Beriplex® P/N) for emergency anticoagulation reversal: a prospective multinational clinical trial. J Thromb Haemost. 2008;6(4):622–31.
99. Schulman S, Bijsterveld NR. Anticoagulants and their reversal. Transfus Med Rev. 2007;21(1):37–48.
100. Strengers P, Drenth J. PPSB as first choice treatment in the reversal of oral anticoagulant therapy. Acta Anaesthesiol Belg. 2001;53(3):183–6.
101. Lusher J, editor. Thrombogenicity associated with factor IX complex concentrates. Seminars in hematology. 1991.
102. Schulman S, Johnsson H, Lindmarker P. Thrombotic complications after substitution with a FVII concentrate. Thromb Haemost. 1991;66(5):619.
103. Escoffre M, Zini J, Schuamser L, Mazoyer E, Soria C, Tobelem G, et al. Severe arterial thrombosis in a congenitally factor VII deficient patient. Br J Haematol. 1995;91(3):739–41.
104. Ferster A, Capouet V, Deville A, Fondu P, Corazza F. Cardiac surgery with extracorporeal circulation in severe factor VII deficiency. Pathophysiol Haemost Thromb. 1993;23(1):65–8.
105. Behring CSL. Beriplex® P/N 250, 500 and 1000 IU [package leaflet]. Marburg, Germany: CSL Behring GmbH; 2014.
106. Behring CSL. KCENTRA (prothrombin complex concentrate (human)), [package insert]. Marburg Germany: CSL Behring GmbH; 2013.
107. Sanquin. Cofact 250, 500 IU [information for use]. Amsterdam, Netherlands: Sanquin Plasma Products B.V.; 2016.
108. LFB-BIOMEDICAMENTS. KASLADIL (human coagulation factor II) [Patient information leaflet]. FRANCE. 2010.
109. Octapharma. Octaplex 500, 1000. [package leaflet]. Vienna, Austria: Octapharma Pharmazeutika Produktionsges.m.b.H.; 2015.
110. Baxalta. Prothromplex TOTAL 600 IU [package leaflet]. Vienna, Austria: Baxalta Innovations GmbH; 2015.
111. Baxter. Proplex-T (factor IX complex). Glendale: Baxter Healthcare Corporation; 2000.