Review Article

Congenital Prothrombin Deficiency

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Abstract

Congenital prothrombin deficiency is an extremely rare hemorrhagic disorder with estimated prevalence of 1 per 2,000,000 in the general population. Since the disorder is an autosomal recessive disorder, the disorder is more frequent in areas with high rate of consanguinity. Clinical manifestations of the disorder are highly variable ranging from mild bleeding episodes to severe life-threatening hemorrhages. The disorder can be diagnosed based on routine and specific tests. Deficiency in concentration of specific factor II (FII) is available, but patients can receive fresh frozen plasma (FFP) and prothrombin complex concentrate (PCC). Traditionally patients with prothrombin deficiency receive on-demand therapy, but secondary prophylaxis can be used for those patients with high risk of severe life-threatening bleeding. With timely diagnosis and appropriate management of disorder quality of life in these patients can significantly improve.

Keywords: Prothrombin deficiency, Clinical manifestations, Diagnosis, Treatment

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Introduction

Prothrombin deficiency or factor II (FII) deficiency is an extremely rare hemorrhagic disorder with estimated incidence of one per 2 million in the general population. The disorder is more common in areas with high rate of consanguinity. Patients with FII deficiency (FIID) present various clinical presentation ranges from asymptomatic conditions to severe life-threatening bleeding. Patients may present mild bleeding episodes such as epistaxis, gum bleeding and bruising to severe hemorrhages such as intracranial hemorrhage and gastrointestinal bleeding. Patients with FIID usually require on-demand replacement therapy, but prophylaxis may be used for those patients at high risk of severe life-threatening hemorrhages. Diagnosis of disorder is made based on simultaneous prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT) and decreased FII activity. FII antigen assay can be used to determine the type of disorder (type I and type II).

Different therapeutic choices are available for these patients including fresh frozen plasma (FFP) and prothrombin complex concentrate (PCC), but no specific concentrate is available. With appropriate management of disorder the rate of morbidity and mortality can significantly decreased and the quality of life of patients can increased.

Prothrombin structure and activation

Prothrombin, or coagulation FII, circulates in the blood at a concentration of 0.1 mg/ml, as an inactive precursor of α-thrombin which synthesized in liver and released into the blood stream (1, 2). The

prothrombin is a 70-kD glycoprotein (579-residues), composed of a N-terminal Gla domain (10 γ-carboxyglutamic residues), k1 domain (Kringle-1), k2 domain (Kringle-2), and a serine protease catalytic domain with A and B chains (figure 1) (2-4).

Prothrombin to thrombin conversion requires two cleavages at Arg273-Thr274 and Arg322-Ile323 sites (1, 5, 6). Since cleavage at different sites is necessary for prothrombin activation and conversion to thrombin, there are three possible pathways for a thrombin formation (figure 2) (6-8). In coagulation cascade for prothrombin to α-thrombin conversion, this protein must be cleaved by prothrombinase complex (consists of activated FXa, cofactor Va, phosphatidylserine and calcium) (4, 9). In the first pathway (reaction A in figure 2), FXa initially cleaves prothrombin at position Arg273-Thr274 and yields the fragment 1.2 (F1.2), as an activation peptide and a catalytically inactive intermediate species known as prethrombin 2 (Pre2). F1.2 comprised of Gla and kringle domains, and Pre2 intermediate molecule comprised of A-chain and B-chain with band at Arg322–Ile323 intact, which is necessary for making Pre2 catalytically inactive. Further proteolysis of this band (reaction B) converts Pre2 to α-thrombin with 35.5 kDa molecular weight, which composed of A-chain (36-residues) and a catalytic B-chain (259-residues) linked by a disulfide bridge C1-C122 (6-8). α-thrombin contains four disulfide bands, therefore A and B chains can covalently link to one of them (C1-C122) and three bonds, C48-C52, C168-C182 and C191-C220 are present as intra-chain disulfide bridges in B-chain (10, 11).

In the second pathway (figure 2, reaction C), FXa cleaves prothrombin at Arg322-Ile323 site and generates an intermediate molecular protein known as meizothrombin (mIIa) which is a catalytically active molecule that then (reaction D) is converted to F1.2 and α-thrombin followed by further proteolysis of meizothrombin by FXa at Arg273-Thr274 site (2, 6, 7). In the third pathway (reaction E), prothrombin molecule produces α-thrombin without releasing intermediate molecules from the enzyme. This reaction is performed by prothrombinase complex and, since it occurs simultaneously and quickly, prothrombin is converted directly to thrombin (6-8).

Due to catalytic domain, α-thrombin is very similar to trypsin and other similar proteases (all of them cleave proteins at ARG residues) (9, 12). Alpha-thrombin, which composed of two β-barrels and helix, has a special structure due to its active site, exosite, and electrostatic fields with positive and negative charges (figure 3). The active site is located in the central part of α-thrombin. Three residues, Ser195, His57, and Asp102, which are located between both β-barrels, comprised the active site groove extending across both β-barrels perpendicular to the junction (9, 12).

Due to 27 additional residues in thrombin compared to chymotrypsin, large loops are formed on thrombin surface, especially around the active site. Two interesting loops that make active site groove deep and narrow are 60-loop and 149-loop (also known as autolysis loop, 147-loop and γ-loop) (9, 13-15). Sixty-loop is more hydrophobic and can interact with hydrophobic residues, but 149-loop is hydrophilic (14). The function of these loops is to block the access of macromolecular proteins, substrates or inhibitors to the active site and the specificity of thrombin (9, 13).

From electrostatic fields, three patches are more important, one with negative charge and two with positive charge. The negative patch is located around the active site in the packet S1 with D819 and E192 (9, 13). The first positive patch is away from the active site and located on the outer side and is called anion binding exosite I (ABE-I) (8, 16). This patch can directly or indirectly interacts with many important proteins, including thrombomodulin (TM), protease activated receptors 1 (PAR1), PAR3, FV, FVIII, FXIII, and especially fibrinogen that is also known as fibrinogen binding site due to binding to fibrinogen (8, 15). In contrast to the second electrostatic patch with positive charge is called anion binding exosite (ABEII) (8, 16), which is the main site for the binding of poly-anion ligands such as heparin and that is why it is also known as heparin binding site (8, 15).

An important point is that fibrinogen and PAR1 binds to thrombin at active site and exosite I. Protein C (pro-C) interacts with binding site around fibrinogen or PAR1, but thrombomodulin binds to exosite 1 (8, 15). Due to the overlaps of the binding sites of TM, Fibrinogen and PAR1, TM can completely interrupt the procoagulant effect of thrombin and induce cleavage of protein C and enhance thrombin anticoagulant property (8, 15).

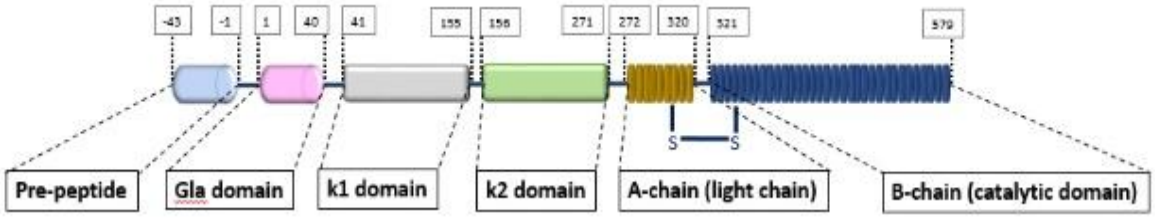

Figure 1. Schematic structure of human prothrombin. Prothrombin consists of two parts, leader peptide (43 residues) and mature protein (579 residues). Mature protein includes Gla domain, k1 domain (kringle1 domain), k2 domain (kringle2 domain), A-chain (α-thrombin light chain) and B-chain (α-thrombin heavy chain; catalytic domain; serine protease domain).

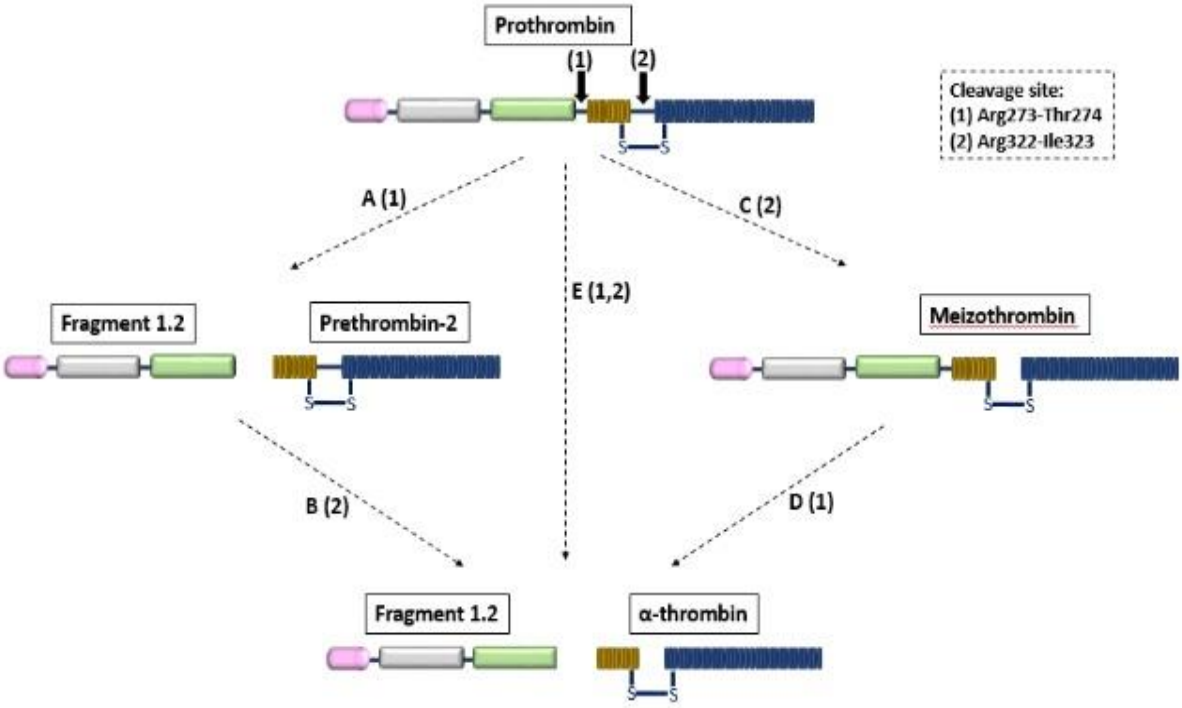

Figure 2. Schematic activation of human prothrombin. there are three pathways for prothrombin thrombin conversion. First pathway: A. activated factor X (factor Xa)a cleaves prothrombin after Arg273 amino acid and yields fragment 1.2 (F1.2) and prothrombin 2 (pre2). B. Further proteolysis of pre2 after Arg322 amino acid generates α-thrombin consists of A and B chains. Second pathway: C. cleavage of prothrombin at Arg322 position yields catalytically active intermediate Meizothrombin (mIIa). D. factor Xa cleaves mIIa after Arg273 amino acid and generate α-thrombin and F1.2. Third pathway: E. Simultaneously cleavage of prothrombin at Arg273 amino acid and Arg322 amino acid, produces F1.2 and α-thrombin.

Sodium binding site is another thrombin surface patch, where NA^+ binding to thrombin is considered as one of the thrombin regulator mechanisms (8, 13). As thrombin is an allosteric enzyme, this interaction affects thrombin function making it "slow" or "fast". These thrombin forms are characterized by the absence ("slow" form) or presence ("fast" form) of NA^+ ion. Converting slow thrombin to fast form increased its binding to substrates such as fibrinogen and its catalytic and procoagulant functions (8, 13).

Prothrombin deficiency (Factor II Deficiency)

Prothrombin deficiency (FIID) is a rare coagulation disorder, with a prevalence of about 1:2000000 (38). Prothrombin deficiency can be diagnosed and classified by routine laboratory tests such as PT and aPTT, FII activity and antigen assays (39). According to the measurement of functional and antigenic plasma levels, FIID has been classified into two types: hypoprothrombinemia (type I) which is also known as true deficiency that is associated with low

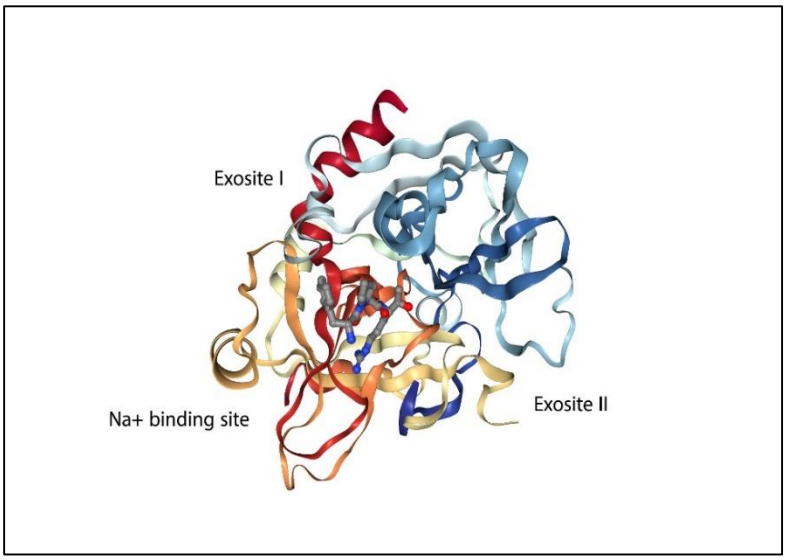

Figure 3. X-ray structure of α-thrombin with PPACK. In the central part of the structure, the active site is located with PPACK inhibitor (stick model). PPACK (D-Phe-Pro-Arg-methyl-ketone) is the small covalent inhibitor. Exosite I (ABE-I; fibrinogen binding site; positively charge patch) is located in the northwest of active site and Exosite II is located in the southeast of active site. Na^+ binding site shown in the southwest of active site. PDB of prothrombin crystal structure downloaded from Research Co laboratory for Structural Bioinformatics (RCSB) protein data bank (www.pdb.org).

antigen level and reduced plasma activity of FII. Dysprothrombinemia (type II) is associated with low activity and normal or near normal antigen level (19, 38, 39). Based on the FII level, FIID can be classified into three forms, severe (less than 5%), moderate (5-10%) and mild (more than 10%) (39). Clinical manifestations of patients with FIID are highly variable (38). Bleeding complications include spontaneous hematomas/bruising, gastrointestinal hemorrhages, menorrhagia, intracerebral hemorrhage, Post-dental extraction bleeding and post-surgical bleeding (38, 39).

Clinical manifestations

Complete absence of prothrombin has not been reported due to its necessity for being alive. Investigations revealed that mice with knockout prothrombin gene show embryonic lethally and neonatal death (40). There is a wide spectrum of bleeding episodes among patients with FIID ranges from mild episodes to severe life-threatening presentations. These episodes may be vary from patient to patient even in those who are affected by same mutation (42). However, due to rarity of FIID, there is little information about patients suffering from this disorder (43).

Severe deficiency of prothrombin is characterized by less than 5% plasma level in homozygote or compound heterozygote patients and manifests with severe bleeding including post-surgical bleeding, mucosal bleeding and subcutaneous and muscle hematoma. Gastrointestinal bleeding have been reported in only a few cases (44). Hematoma and spontaneous bruising are the most common manifestations of the disorder (42, 45, 46). Hemarthrosis and post-dental extraction bleeding are less common. Menorrhagia is a common symptom that can be observed among ~20% of homozygote women (47). Generally, the most common clinical manifestations in patients with FIID are epistaxis, prolonged and heavy menstruation, bleeding in joints and muscles and bleeding from mouth and gums. Gastrointestinal bleeding, umbilical cord bleeding and bleeding after major or minor surgeries are occasionally reported in these patients (48), while severe life-threatening bleeding such as intracranial hemorrhage are reported in less than 10% of patients (49).

In an investigation conducted in Iranian patients with prothrombin disorders, 11 out of 14 patients had hypothrombinemia with 4-10% prothrombin level and 3 were diagnosed with dysprothrombinemia. Hemarthrosis and muscle hematoma were the most common severe manifestations among these patients. Intracranial hemorrhage was reported in one case and

umbilical cord bleeding had observed in two neonates (50). In a study on 26 Italian patients with hypothrombinemia similar clinical presentations were observed and mucosal bleeding, soft tissue hematoma and hemarthrosis were reported as relatively common presentations (51).

Heterozygote patients with 30% to 60% prothrombin plasma level are usually asymptomatic. However, excessive bleeding after trauma, tooth extraction and surgical procedures may occur. Patients with dysprothrombinemia show different bleeding tendency that is usually less severe than hypoprothrombinemia (47).

Molecular basis

Prothrombin (coagulation FII) is a single chain 72 kDa protein expressed primarily in liver encoded by a 21 kb gene located on chromosome number 11 and has 14 exons (52). Thirty two different prothrombin causing mutations were identified until 2000, but the number is increased to more than 40, so far (47, 43-45). The majority of these mutations is occur in catalytic region, while some of them disrupt regulatory domains including anion-binding exosite-I, Na^+ binding loop and light A chain. Most of the mutations that resulting in hypoprothrombinemia are missenses, but a number of nonsense mutations causing stop codon and a single nucleotide deletion are also identified (47). More than 17 mutations have been described in dysprothrombinemia; all of them are missense mutations. These mutations result in amino acid replacement inside the prothrombin cleavage site that is activated FX substrate; therefore, mutations interfere with serine protease region of thrombin. On the other hand, hypothrombinemia-causing mutations are usually occur near to Gla and kringle domains and A chain (56).

Homozygote mutations in F2 gene are not always lead to severe bleeding; for example Arg76His mutation decreases FII activity to less than 20%, but causes little bleeding tendency (57). Similarly some other mutations including homozygote Glu466Ala and compound heterozygote Met337Thr-Arg388His do not cause bleeding manifestations (58, 59). In addition, mutations like Arg596Leu (called prothrombin Yukuhashi) that cause prothrombin with disturbed Na^+ binding loop, not only do not identify with hemorrhagic phenotypes, but also can lead to hereditary thrombosis (60).

Laboratory diagnosis

PT and aPTT are usually sensitive enough to detect coagulation factors abnormalities, but their results may be normal in some coagulation factors deficiency including fibrinogen, prothrombin and FXIII. Therefore, diagnosis of FIID may require specific assessments (43, 56). In case of suspicion to coagulation factors abnormality based on clinical manifestations and family history, an immunological assessment for indenting dysprothrombinemia is required. In these patients antigenic levels of FII is relatively normal without appropriate function. After that factor assay or one-stage standard clotting assay using specific factor depleted plasma and thromboplastin should be conducted (43). There are many assessments using different snake venoms for prothrombin to thrombin conversion. These assessments are including *Echis carinatus* venom test that does not require phospholipids. Taipan venom and Textarin venom tests are phospholipid dependent. Results of PT and some venom tests are significantly lower than antigenic assays in dysprothrombinemia (56). Immunological assays are fairly related to functional assays in hypoprothrombinemia and both are decreased, while dysprothrombinemia is characterized by decreased functional assays with normal or near normal immunological tests (51, 61).

Genotyping and molecular assays of FIID are conducted only on a few patients. These molecular analyses of confirmation of FIID generally only are available in research centers (62, 63).

Diagnosis of prothrombin deficiency may be difficult especially in immature neonates due to low level of vitamin K. Reassessment after vitamin K replacement may be required in these patients (56).

In many countries, efficient diagnosis of bleeding disorders using laboratory tests is very difficult due to unavailability and unstandardized laboratory assays (64). Due to small amount of coagulation factors required for normal results in traditional laboratory tests, the factors deficiency could be undetectable using these assays. Therefore laboratory assessments evaluating general hemostatic capacity had been developed (65). These assessments including constant thrombin generation, thrombin generation test (TGT), clot formation,

thromboelastography (TEG) and simultaneous generation of thrombin and plasmin, are used to hemostasis evaluation (66-70). TGT and TEG are used for monitoring of treatment in different bleeding episodes in RBD (71). It is crucial to continue to undertake attempts for standardize and quality assessment of laboratory technologies like TEG and TGT; since these strategies can be helpful in coagulation deficiencies with high risk of thrombotic events during therapeutic periods (43).

Treatment

Treatment of FIID should be individualized. The purpose is to maintain FII levels as enough as required level for normal hemostasis. Traditional treatment for patients with FIID is on-demand therapy that means beginning of treatment as soon as possible afterbleeding, but secondary prophylaxis can be considered for those with high risk of life threatening bleeding. The dosage and frequency of therapeutic agents administration are depend on the minimal level of deficient factor, its plasma half-life and type of bleeding episodes (43, 62). The main treatment of prothrombin deficiency is replacement therapy using fresh frozen plasma (FFP) andprothrombin complex concentrate (PCC). Moreover, administration of antifibrinolytic agents, with or without replacement therapy, can be useful in the management of mild bleeding episodes. Administration of antifibrinolytic agents accompanied by PCC is contraindicated because thrombotic complications are potential risk of PCC injection and fibrinolysis is the only body defending mechanism against undesirable clot formation (72).

Hormonal interference might help to decrease menorrhagia by estrogen-progesterone consumption (43). For patients with severe bleeding manifestations prophylactic treatment should be considered. It includes primary and secondary prophylaxes that are different in administration timing (before bleeding onset to prevent or after bleeding to recurrence debarment). In general, due to inadequate information about patients with FIID, regular replacement therapy should be monitored to prevent thrombotic complications (43).

There is no pure prothrombin concentrate available; so PCC is therapeutic choice. Most of PCCs are containing therapeutic amounts of three coagulation factors: FII, FIX and FX. There are also PCCs containing four coagulation factors that have FVII in addition to above (73, 74). In absence of a proper PCC, viral inactivated FFP can be administered as source of prothrombin. Although viral inactivation can eliminate blood born infections, other complications of blood products injection are remain including inhibitor formation, thrombosis and rash (43).

Injection of every single unit of prothrombin increases plasma prothrombin level for about 1 IU/dl. It seems to 20-30 IU/dl level of prothrombin is adequate for normal hemostasis. Administration of 20-30 IU/kg dose seems effective; however, higher doses may be required for life-threatening bleedings management. Prothrombin half-life is about 72 hours; so the frequency of injections lowered to every 2 or 3 days (56, 75).

Severe life-threatening bleeding episodes are not observed widely in infancy; however umbilical cord bleeding is reported occasionally. Thus primary prophylactic replacement therapy does not conduct for patients in early infancy (56). Patients with cardiovascular diseases are at risk for circulatory overload; so it is better to inject PCC instead of FFP in these patients (75).

Conclusion

This study provides a general overview about prothrombin, associated disorders and how to manage them. Given the rarity of congenital prothrombin deficiency, the information about the disorder is limited. We hope this survey could provide an insight for better understanding, identification and management of FIID.

Acknowledgment

None.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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