Factor VII Gene Defects: Review of Functional Studies and Their Clinical Implications

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ABSTRACT

Coagulation factors belong to a family of plasma glycosylated proteins that should be activated for appropriate blood coagulation. Congenital deficiencies of these factors cause inheritable hemorrhagic diseases. Factor VII (FVII) deficiency is a rare bleeding disorder with variable clinical symptoms. Various mutations have been identified throughout the F7 gene and can affect all the protein domains. The results of previous experiments have partly revealed the correlation between genotype and phenotype in patients with FVII deficiency. Nevertheless, each particular variant may affect the coagulative function of FVII, mainly via altering its expression level, extra-cellular secretion, tissue factor binding affinity, or proteolytic activity. The pathogenicity of the variants and molecular mechanisms responsible for clinical symptoms in patients with FVII deficiency should be characterized via in silico and in vitro, as well as in vivo functional studies. This review has highlighted the most important functional studies reported on F7 gene variants, including relevant reports regarding Iranian FVII deficiency patients. DOI: 10.29252/irb.23.3.165

Keywords: Factor VII deficiency, in vitro techniques, Mutation

INTRODUCTION

Thrombosis and coagulation factors

In the coagulation process, which ultimately prevents bleeding, various mechanisms such as vascular contraction, platelet aggregation, and clot formation are activated¹². The coagulation cascade (Fig. 1) is triggered by the tearing of vessel and progress through complex sets of biochemical reactions that are carried out by blood coagulation factors¹³. Coagulation factors belong to a family of plasma glycosylated proteins that should be activated for appropriate blood coagulation⁴. In general, coagulation factors are present in plasma at very low levels and are dependent on vitamin K for their activity⁵. Congenital deficiencies of these factors cause inheritable hemorrhagic diseases, which are often rare⁶. Defective function of coagulation factors can be quantitative or qualitative. In qualitative type, although functional tests may indicate coagulation factor deficiency, antigen detection assays show that their plasma level is normal or increased⁷. The main consequence of coagulation cascade is the formation of active substances that are called prothrombin activators⁸. The prothrombin activators catalyze the conversion of prothrombin to thrombin, which converts fibrinogen into fibrin fibers. Eventually, fibrin fibers trap the platelets and form the clot. The prothrombin activators are formed in two ways that interact with each other: (1) the extrinsic pathway that starts with damage to the vascular walls and their surrounding tissues and (2) the intrinsic pathway that starts inside the blood. Factor VII (FVII) plays a pivotal role in the commencement of blood coagulation through the extrinsic pathway⁹.
Coagulation factor VII

FVII is a serine protease produced in the liver and presents in plasma as a zymogen at a concentration of 10 nM (0.5 µg/ml)\(^{10-12}\). This vitamin K-dependent glycoprotein is circulating in plasma in two forms, mainly as inactive single-chain zymogen and partly as active form (FVIIa) consisted of heavy and light chains. Following vascular injury, FVII is converted into its active form and binds to the tissue factor (TF) to form the TF/FVIIa complex. The conversion of FVII to FVIIa occurs by breaking the peptide linkage between amino acids Ile\(^{153}\) and Arg\(^{152}\)\(^{13}\). TF consists of phospholipids derived from tissue membranes plus lipoprotein complexes of damaged tissue. The TF/FVIIa complex acts as an enzyme on Factor X (FX) and converts it into its active form (FXa) in the presence of calcium ion. FXa is rapidly combined with tissue phospholipids, a part of TF, or released from the platelets\(^{14}\). Together with FV, they form the prothrombin-activating complex. Then this complex converts prothrombin into thrombin in the presence of calcium ion, and the coagulation process proceeds\(^{3,15}\).

Factor VII biosynthesis and functions

The coagulation factors (FVII, FIX, and FX) and prothrombin have almost the same protein structure characteristics. All of these proteins have a signal peptide that is necessary for their transmission to the endoplasmic reticulum. They also contain a pro-peptide sequence that carries out vitamin K-dependent \(\gamma\)-carboxylation in mature protein and is cleaved after transferring to Golgi's system\(^{16,17}\). The FVII protein also contains two epidermal growth factor-like (EGF-like) domains and an activation peptide with a glycosylated asparagine that provides a proteolytic cleavage site. The catalytic regions exhibit the serine protease activity, which leads to various functions of the protein\(^{18}\). The role of FVII in the pathogenesis of various cancers has extensively been studied\(^{19-24}\). Though the molecular pathogenesis of the increased expression of FVII by cancer cells has not precisely been described, the ectopic expression of FVII may promote the division, migration, and invasion of cancer cells. This process seems to be mainly mediated through the TF/FVIIa/PAR2 complex\(^{25}\). Recently, it has been shown that FVII is an important target of androgen receptor in breast cancer cells. That study indicated that the androgen receptor binds to the \(F7\) promoter near the ATG translation start codon, which suggests that the androgen receptor directly activates \(F7\) gene expression in cancer cells\(^{19}\).

Factor VII deficiency

The deficiency of FVII was first described in 1951. The disease is known as a hereditary bleeding disorder with prevalence of 1 in every 300,000-500,000 individuals\(^{17,26}\). Children with congenital
FVII deficiency may be diagnosed following a gastrointestinal tract or central nervous system bleeding in the first six months of their life. Patients with severe FVII deficiency may experience joint and muscle bleeding, easy bruising, and postoperative hemorrhage. Bleeding can also occur spontaneously in the mouth, the nose, the genitals, and the urinary tract. Furthermore, the affected women often suffer from severe menorrhagia. In sum, FVII deficiency is a rare bleeding disorder with variable clinical symptoms. However, in many cases, there is no direct correlation between the factor plasma levels and the severity of the disease symptoms. In some people with very low levels of FVII may demonstrate mild symptoms. In cases of very low factor levels, the clinical manifestation of the disease may be similar to hemophilia symptoms. However, the patients are generally treated with the administration of recombinant FVII.

**Factor VII gene (F7)**

The F7 gene is located on chromosome 13 (13q34). This gene has nine exons and eight introns, which, besides the gene promoter region, composes a 12-kb gene locus near the telomeric region of the chromosome. Two other genes (i.e. FX and PROZ) which encode vitamin K-associated proteins, are also located close to the F7 gene locus. The complete sequence of this gene was reported in 1987 by O'Hara et al. The length of the introns in this gene varies between 68 nucleotides and 2.6 kb, while the gene exons are between 25 nucleotides and 1.6 kb. The exons 1a, 1b, and a part of the exon 2 join together and encode the pre-pro leader sequence. The presence or absence of exon 1b assigns the pre-pro leader with a size of 60 or 38 amino acids, respectively. Both variants are naturally occurring in humans, although the lack of exon 1b is more common. The rest of the exon 2 plus the remaining exons encode the mature protein. Regardless of the transcribed alternate exons, the mature FVII protein in the plasma is a single chain protein with a molecular weight of 50 kDa that contains 406 amino acids. In contrast, FVIIa has a light chain (gamma carboxyl-glutamic acid domain and two other EGF-like domains) and a heavy chain with catalytic activity (Fig. 2). The promoter and regulatory regions of this gene have extensively been studied. The main transcription initiation region is located at [-57CCCGTCAGTCCC-46] upstream of the transcription starting point. The binding region for the transcription factor HNF4, which affects the expression of other genes in the liver, spans bases from -63 to -58 (Fig. 3). There is also a gene locus on chromosome 8 that may play a role in regulating FVII levels. The presence of this locus was suggested by observing FVII deficiency in patients with trisomy of chromosome 8. Eventually, Fagan et al. have
assigned this regulatory locus on 8p23.2-p23.1 chromosomal region.

**F7 gene mutations**

According to the reported data (http://www.factorvii.org), the F7 gene harbors more than 200 different variants. These variants include missense, nonsense, small insertion/deletion, and splice site mutations, which may affect every region of the gene (Fig. 4A), and the mutations identified throughout the F7 gene can affect all the protein domains (Fig. 4B). Although F7 mutations are very heterogeneous, some are common in particular populations\(^{[51]}\). On the other hand, many patients with a specific mutation in the F7 gene may have no significant clinical manifestation. Currently, comprehensive information regarding these mutations is available at the FVII gene variants database (http://www.factorvii.org)\(^{[26]}\). Point mutations are the main causes of FVII inherited defects, where missense mutations are the most frequent variants. Exon 8 is the largest exon of the gene and harbors a large number of mutations. Like other hereditary coagulation defects, such as FIX deficiency (hemophilia B), many mutations occur in CpG hot spot regions. To date, frequent examples of such mutations have been described \((R79Q/W, 6071G>A, A244V, R304Q, and T359M)\(^{[22]}\). In a comprehensive study on 717 patients in Latin America and Europe, 131 mutations were observed in 73 homozygotes, 145 heterozygote compounds, and 499 heterozygotes patients, of which 71% of homozygous and 50% of compound heterozygotes cases were symptomatic. Interestingly, despite the observation of FVII deficiency symptoms in some patients, almost 10% of the patients had no mutations in the screening analysis\(^{[20]}\). Whether the FVII deficiency is due to the defects in genes other than F7 has yet to be described. It is also believed that the plasma levels of the factor are regulated by F7 gene polymorphisms. However, their effect on the severity of patients’ clinical manifestation is not clear. In general, the most severe cases are either homozygous or compound heterozygous with FVII: C levels less than 2.0% of normal, but occasionally, heterozygous carriers display hemorrhagic symptoms that can be severe in rare cases. For instance, a heterozygous 19-year-old patient with severe spontaneous intracranial bleeding was reported; the patient had no previously recorded hemorrhagic symptoms\(^{[53]}\).

**Functional studies on F7 gene variants**

Up to now, various functional studies\(^{[46,54,55]}\) have been conducted to show the effects of F7 gene variants, mainly on the secretion rate, ligand binding, and coagulation activity of the protein (Table 1). These studies are often based on the *in vitro* expression of mutant recombinant FVII in mammalian cells in culture. Their results partly revealed the correlation between the genotype and phenotype in the patients with FVII deficiency. Nevertheless, each particular variant may affect the coagulative function of FVII, chiefly via altering its expression level, extracellular secretion, TF-binding affinity, or proteolytic activity. Functional analysis of the F7 mutations has demonstrated that the binding of FVII to TF occurs through a large interface between the two proteins, which comprise all four FVIIa domains and two TF extracellular domains\(^{[54,56]}\). The Gla domain binds to the C-terminal membrane domain, while EGF1 interacts with both domains of TF. EGF2 and the FVII protease domains form a merged surface interacting with the N-terminal of the TF. Although the mechanism by which TF increases the catalytic activity
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<td>Reporter gene expression assay/ electrophoretic mobility shift assay</td>
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SPCD, serine protease catalytic domain; PA, pathogenic; LP, likely pathogenic; LS, low secretion; LCA, low coagulative activity; LTB, low TF binding; IPF, impaired protein folding; HE, high expression; LE, low expression; CM, confocal microscopy; FM, fluorescence microscopy; CA, coagulation assay; Ref. reference
of FVIIa is not well known, previous studies have indicated that different F7 gene variations can change this interaction and decrease the coagulation activity of the protein\(^{[54]}\). For instance, the R79Q mutation has no effect on the expression of the FVII protein but decreases its TF binding affinity\(^{[57,58]}\). Protein structure analysis by X-ray crystallography has displayed that the region that contains this residue plays an important role in the interaction of EGF1 with TF\(^{[54]}\). In the same way, the Q100R mutation may affect the protein expression and cause defective FVIIa/TF complex formation\(^{[59]}\). Peyvandi et al.\(^{[60]}\) have studied Pro303Thr variant in an Iranian patient with relatively severe hemorrhage. The functional study of this variant was performed using in vitro expression of the defective FVII protein, followed by biochemical coagulation tests. The mutation was induced by site-directed mutagenesis in exon 8 of F7 gene, and the mutated protein was expressed in mammalian cells. Quantitative tests have suggested that the expression and the secretion of the mutated protein were normal. However, further experiments have revealed that impaired binding of FVII to the TF diminishes its proteolytic activity\(^{[60]}\). It has been reported that mutant FVII protein with R152Q mutation has no detectable activity. This mutation occurs at the proteolytic cleavage site required for the conversion of FVII into FVIIa. Thus, the mutation affects the protein activity by inhibiting the activation of FVII serine proteases\(^{[58]}\). In another functional analysis, although the F328S variant led to partially diminished TF binding, the protein was not able to activate FX, possibly due to a defective substrate binding site\(^{[61]}\).

Recently, we have reported the FVII functional defects consequent to C91S mutation in a homozygote patient with mild bleeding symptoms\(^{[62]}\). We expressed the mutant protein in CHO-K1 cells in vitro and assessed its properties using coagulation assays and immunocytochemistry. In spite of increased secretion of FVII in the culture medium of the cells expressing the mutant FVII, C91S substitution severely affected the coagulant activity of FVII. The C91S substitution was first reported in a British patient with FVII deficiency\(^{[63]}\). The mutation occurs in the exon 5 of F7 gene and alters residue 91 in EGF2 (EGF-like 2) domain of the protein. The EGF-like and the serine protease domains are necessary for FVII and TF interaction\(^{[64]}\). Previous studies have also shown that EGF2 mutations dramatically impair FVII coagulant activity by affecting protein-protein interactions\(^{[18,54]}\). The review by Peyvandi et al.\(^{[54]}\), which included 21 families with FVII deficiency, has identified nine new missense mutations in the Gla, EGF-2, or serine protease domains (Table 1). They analyzed the protein crystal structure to describe the functional effects of these variants on FVIIa and FVIIa/TF complex. In a similar study, Millar et al.\(^{[63]}\) have evaluated 23 new mutations in 38 British patients with FVII deficiency. They also used crystal structure analysis and molecular modeling of the FVIIa/TF complex to determine the variants pathogenicity. In a study on Italian patients, D’Andrea et al.\(^{[18]}\) have reported a 6-year-old female with FVII deficiency who was identified as compound heterozygote for Asp123Tyr and Arg110Cys mutations, both of which in the EGF-2 domain. In order to evaluate the importance of the EGF-2 motif and the pathogenicity status of the variants, a functional study was performed on the both mutations. When the recombinant variants were expressed in mammalian cells, FVII:C and FVII:Ag were assessed in the cell lysate and culture medium of the host cells. They observed that these mutations decreased the intracellular accumulation and the secretion rate of FVII protein. They concluded that the mutations in EGF-2 domain could affect FVII processing, stability, or secretion\(^{[18]}\). Also, the effect of Gly97Cys and Gln100Arg mutations on FVII secretion and function was studied. These mutations that occur in EGF-2 may alter the intracellular localization and the secretion of the protein. To evaluate the pathogenic outcome of these variants, COS-1 and CHO cells were transfected with expression vectors containing wild type and mutated alleles. The host cells were examined by immunostaining to reveal intracellular localization of FVII protein. The results showed that the mutations in EGF-2 domain can alter the localization pattern as well as the secretion rate of FVII protein\(^{[18]}\).

Cysteine residues play an important role in FVII function, in particular, Cys329 that is strongly preserved in the serine proteases is critical for TF binding and, thus, the catalytic function of FVIIa. Disruption of disulfide bond between Cys329 and Cys310 dramatically affects the structure and the function of the protein\(^{[65]}\). So far, numerous patients with Cys329Gly mutation and a patient with Cys329Arg have been reported\(^{[60,66]}\). The molecular mechanisms involved in the pathogenesis of FVII deficiency consequent to the mutations in the serine protease catalytic domain have widely been studied in vitro. In a study by Chollet et al.\(^{[55]}\), CHO-K1 cells were transiently transfected to describe the mechanisms by which these three different mutations reduce the levels of FVII. They revealed impaired secretion of the defective FVII protein in the culture medium. These results were consistent to the low FVII levels measured in patients carrying these mutations. In another study, we performed a functional study on H348R and S282R mutations detected in compound
heterozygous status in a FVII-deficient patients\textsuperscript{[43,67]}. The both variants could lead to lowered secretion of the mutant proteins and undetectable coagulation activity in vitro.

The expression of chimeric FVII/GFP proteins has been analyzed to identify the effects of nonsense mutations on the biosynthesis and secretion of FVII. Further studies have been conducted to investigate the expression features of F7 promoter variants. The mutations in the promoter consensus sequence of F7 gene (\textasciitilde94C\textgt T; \textasciitilde61T\textgt G; \textasciitilde55C\textgt T) affect the binding of transcription factors that are important for the expression of FVII. These three mutations have been studied with the help of reporter genes in transfected cells. The transcripts containing the reporter gene along with the mutated upstream sequences of the F7 gene showed decreased expression rate compared to the wild-type gene. It has also been shown that 94C\textgt T mutation occurs at SP1 binding site and \textasciitilde61T\textgt G mutation at HNF4 binding sequence. The mutation at \textasciitilde55C\textgt T also caused a significant reduction in the binding affinity of HNF4 to this sequence. The severe clinical phenotype observed in the patients carrying these mutations can be explained by reduced binding efficacy of the transcription factors for the F7 promoter\textsuperscript{[68]}.

By developing advanced \textit{in silico} analysis methods and genotype-phenotype association studies, more comprehensive data on the effects of F7 gene variants on the function of FVII protein are being provided\textsuperscript{[69,70]}. Tiscia \textit{et al.}\textsuperscript{[70]} have described molecular consequences related to novel variants detected in FVII deficiency patients by using the bioinformatics software, including PROMO, SIFT, and PolyPhen-2. Structural characteristics of the mutant FVII proteins have also been evaluated by \textit{in silico} functional analysis on SPDB viewer software. The data of an \textit{in silico} study predicted a possible damaging effect of the Cys400Ser missense mutation on the conformation of FVIIa via disrupting the Cys400-Cys428 disulfide bond. Very recently, the association of FVIIa levels with the incidence of coronary heart disease and the mortality rate of ischemic stroke has been assessed by Olson \textit{et al.}\textsuperscript{[69]}. They performed a genome-wide single nucleotide polymorphisms association analysis for FVIIa in European-Americans (\textit{n} = 2410) patients and reported that rs1755685 in the F7 promoter region on chromosome 13 was the most significantly relevant single nucleotide polymorphism to FVIIa levels. Interestingly, a functional \textit{in vitro} site-directed mutagenesis study has previously demonstrated that allelic variants rs1755685 may increase F7 gene expression\textsuperscript{[68]}.

Overall, various functional analysis methods may be implemented for the evaluation of each variant in F7 gene. However, the best choice depends on the nature of the variant, the genotype-phenotype correlation in the patients, as well as previous studies on the population of interest.

Though the mutational spectrum of F7 gene has been substantially described, the genotype-phenotype correlation in patients with FVII deficiency and the functional defects of the mutant FVII protein have yet to be precisely elucidated. This attempt may be more complicated in symptomatic patients with heterozygote variants. The pathogenicity and clinical severity of each particular F7 gene variant should be evaluated considering overall data provided by \textit{in vitro} and \textit{in silico} functional analyses, as well as the presence of other interfering variants throughout the patients’ genome.

\textbf{CONFLICT OF INTEREST.} None declared.

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