Identification of two novel mutations in three children with congenital factor VII deficiency

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Congenital factor VII deficiency (FVIID) is a rare F7 gene mutation causing bleeding disorder inherited in an autosomal recessive manner. In this study, we aimed to identify genetic defects and analyze their relationships with phenotype in three Chinese FVIID patients. The diagnosis of FVIID was made based on FVII coagulant activity (FVII:C) levels assessed through prothrombin time assay. Direct sequencing and protein modeling were performed to detect genetic mutations and the resulting protein expression. Patient 1, a 2-year-old girl, presented with mild bleeding and was found to have a FVII:C of 0.2% and a compound heterozygous F7 Cys389Gly/Cys115Arg mutation. Patient 2, a 7-year-old boy, consulted for moderate bleeding and was found to have a FVII:C of 0.8% and a compound heterozygous F7 Thr241Asn/Pro324Leu mutation. Patient 3, a 5-year-old boy who developed a mild bleeding after trauma was found to have a FVII:C of 1.8% and a compound heterozygous F7 Thr241Asn/IVS5-2A>G mutation. We hereby report three congenital FVIID patients with FVII:C less than 2% and their respective F7 mutations, two of which (F7 Cys115Arg, Pro324Leu) are novel. The molecular model analysis of the two novel mutations F7 Cys115Arg and Pro324Leu respectively indicated impairment of the proper folding of epidermal growth factor 1 domain situated on F7 gene and impairment of the procoagulant function of FVII both leading to the congenital deficiency of FVII. Blood Coagul Fibrolysis 32:340–343 Copyright © 2021 The Author(s).

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Introduction

Inherited factor VII (FVII) deficiency, first reported by Alexander et al. [1], is a rare bleeding disorder caused by mutations in the FVII (F7) gene. It is more likely to be found in countries where consanguineous marriages are more frequent [2] with an estimated incidence of 1/500000 in the general population. Low levels of FVII coagulant activity (FVII:C), prolonged prothrombin time (PT), and bleeding tendency are the main characteristics. However, FVII:C levels share a poor correlation with hemorrhagic manifestations [3], making it difficult to precisely determine the predictors of bleeding risk. Bleeding features are quite heterogeneous, ranging from asymptomatic to life-threatening including gastrointestinal bleeding and intracranial hemorrhage. Bleeding presentations are usually found in homozygous or compound heterozygous states, with FVII:C levels less than 2% usually manifesting as severe cases [4], whereas heterozygous states appear to be asymptomatic most of the time.

In the current study, we describe the phenotype and genotype of three congenital FVII deficiency (FVIID) patients with FVII:C less than 2%, and report two novel F7 gene missense mutations.

Material and methods

Patients

Patient 1, a 2-year-old girl, presented with recurrent gum bleeding and easy bruising. Clinical investigations revealed a mild bleeding presentation. Patient 2, a 7-year-old boy consulted for easy bruising, recurrent epistaxis and gum bleeding. He was found to have a moderate bleeding manifestation. Patient 3, a 5-year-old boy developed a scalp hematoma after trauma. He was classified as mild bleeding according to the International Registry Factor Seven Study Group (Table 1).

Methods

Plasma factor VII coagulant activity

PT and activated partial thromboplastin time (APTT) were detected by a clotting assay. The determination of FVII:C was based on the PT assay using FVII deficient plasma kit (Coagulation assay, FVII deficient plasma, Brea, California, USA).

DNA sequencing

The DNA of the patients and their parents was extracted from peripheral blood leukocytes using a FlexiGene DNA kit (product number 51206; Qiagen, Hilden, Germany), according to the manufacturer’s instructions. All exons and the flanking sequences of the F7 gene were amplified by PCR using the specific primers designed by the Primer Z software (http://genepipe.ncgm.sinica.edu.tw/primerz/primerz4.do). F7 gene sequencing was performed on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster, California, USA). The sequencing
results were compared with the $F7$ sequence published by the NCBI Genbank to find the mutations. We searched the Pubmed database, Human Gene Mutation Database and FVII Gene Variant Database to confirm the novel mutations and consulted the 1000Genomes database and database of Single Nucleotide Polymorphism database to eliminate common polymorphisms.

**Protein molecular modeling**

We analyzed the molecular structure of the mutant protein by using PyMOL software (http://pymol.org) based on the known three-dimensional structure of FVII.

**Results**

Patient 1 had markedly prolonged PT of 149.2 s, normal APTT, and FVII:C of 0.2%. Patient 2 was found to have a prolonged PT of 82.1 s, normal APTT, and FVII:C of 0.8%. Patient 3 was found to have a prolonged PT of 85.5 s, normal APTT, and FVII:C of 1.8% (Table 1).

Gene analysis showed patient 1 harbored a compound heterozygous mutation of a known paternal missense mutation $c.1165T>G$ (Cys389Gly) in exon 9 and a novel maternal missense mutation $c.343T>C$ (Cys115Arg) in exon 5 of the $F7$ gene. In patient 2, compound heterozygosity of a known paternal missense mutation $c.722C>A$ (Thr241Asn) in exon 8 and a novel maternal missense mutation $c.971C>T$ (Pro324Leu) in exon 9 of $F7$ gene was identified. Patient 3 was identified with a compound heterozygosity of a known maternal missense mutation $c.722C>A$ (Thr241Asn) in exon 8 and a known paternal mutation $c.572-2A>G$ (IVS5-2A>G) in intron 6 of $F7$ gene (Fig. 1).

| Patient ID | Sex | Age (years) | Clinical severity | FVII:C (%) | PT (s) | APTT (s) |
|------------|-----|-------------|------------------|------------|--------|----------|
| 1          | Female | 2 | Mild | 0.2 | 149.2 | 36 |
| 2          | Male  | 7 | Moderate | 0.8 | 82.1 | 31.3 |
| 3          | Male  | 5 | Mild | 1.8 | 85.5 | 32.6 |

APTT, activated partial thromboplastin time; FVII:C, factor VII coagulant activity; PT, prothrombin time.

Table 1 Clinical characteristics for the patients

![Fig. 2](image-url)

Crystal structure model of the $F7$ Cys115Arg mutation. The yellow stick represents the disulfide bond, the rose red and cyan stick represents the residue Cys115 and Cys130, respectively. The Cys115 forms a disulfide bond with Cys130 within the epidermal growth factor 1 domain, the amino acid substitution at Cys115Arg disrupts the disulfide bond.

![Fig. 1](image-url)

Representative chromatograms from direct sequencing of the $F7$ gene. The red arrow indicates the mutation position. (a) Represents the mutation $F7$ Cys115Arg in patient 1. (b) Represents the mutation $F7$ Thr241Asn in patients 2 and 3. (c) Represents the mutation $F7$ Pro324Leu in patient 2. (d) Represents the mutation $F7$ Cys389Gly in patient 1. (e) Represents the mutation $F7$ IVS5-2A>G in patient 3.
Crystal structure model showed that the Cys115 forms a disulfide bond with Cys130 within the epidermal growth factor (EGF)1 domain. The amino acid substitution at Cys115Arg eliminated Cys residue which is important for disulfide bond formation, resulting in the impairment of the proper folding of EGF1 domain (Fig. 2). Model analysis suggested that the residue Pro324 constitutes a flexible loop within the catalytic domain of FVII. Although the replacement of Proline by Leucine does not implicate a significant structural change of the FVII molecule, the mutation Pro324Leu may well hinder the secretion of the expressed protein or impair the procoagulant function of FVII, leading to the congenital deficiency of FVII in individuals harboring the mutation (Fig. 3).

**Discussion**

FVII is a vitamin K-dependent serine protease synthesized by the liver playing a vital role in the activation of the exogenous coagulation pathway. FVII has a molecular weight of 50 kD and consists of 406 amino acids, including a gamma-carboxy glutamic acid domain (Gla domain), two EGF-like domains and a serine protease domain [5]. F7 gene is located on chromosome 13 (13q34), spans about 12 kb, and is comprised of nine exons and eight introns. Their respective encoding domains are as follow:
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Exon 1a and exon 1b encode prepro leader sequence, exon 2 encodes Gla domain, exon 3 and exon 4 encode EGF1 domain, exon 5 encodes EGF2 domain, exons 6, 7, and 8 encode serine protease domain [6,7]. There are more than 200 mutations responsible for FVIIID, of these mutations the missense mutations are the most common variants (http://www.factorvii.org/index.php).

In this study, we identified four F7 missense mutations (Cys389Gly, Pro324Leu, Thr241Asn, Cys115Arg) and one F7 splicing mutation (IVS5-2A>G) in three unrelated Chinese children. Three mutations (Cys389Gly, Thr241Asn, IVS5-2A>G) had been previously reported [8,9], whereas F7 Cys115Arg and Pro324Leu are novel.

Previous studies have shown that EGF1 domain and Gla domain are related to the binding of FVII and tissue factor [10–12] and in this study patient 1 who harbored a compound heterozygous mutation of a mutation Cys115Arg in the EGF1 domain and a mutation Cys389Gly in the serine protease domain of the F7 gene presented with mild bleeding. Model analysis showed that the amino acid substitution at Cys115Arg broke disulfide bond formation, resulting in the impairment of the proper folding of EGF1 domain. It has been also reported in the literature [13,14] that the amino acid residues (Leu323 and Glu325) next to the Pro324 play an important role in maintaining the stability of the FVII molecular structure. Patient 2 was found to have a novel mutation F7 Pro324Leu and the model analysis suggested that the mutation Pro324Leu may well hinder the secretion of the expressed protein or impair the procoagulant function of FVII, leading to the congenital deficiency of FVII in individuals harboring the mutation. Since a study reported that the F7 Thr241Asn mutation has no obvious effect on the secretion/stability of FVII molecule [9], the novel F7 Pro324Leu mutation may be the main molecular mechanism of patient 2 who harbored a compound heterozygous mutation F7 Thr241Asn/Pro324Leu and had a moderate bleeding manifestation.

In conclusion, we investigated three patients diagnosed with severe (FVII:C < 2%) hereditary FVII deficiency. Genetic analysis identified five F7 mutations, two of which were novel. This study enriches the FVII Gene Variant Database and gives a better understanding of phenotype and genotype.

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Conflicts of interest
The authors declare no conflicts of interest to disclose.

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