OPEN

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

Kairong Liang[a,*], Lauriane Nikuze[a,*], Fuyong Zhang[b], Zhengjing Lu[a], Manlv Wei[a] and Hongying Wei[a]

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 Fibrinolysis* 32:340–343 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

Blood Coagulation and Fibrinolysis 2021, **32**:340–343

Keywords: congenital factor VII deficiency, genotype, phenotype

[a]Department of Pediatrics and [b]Department of Clinical Laboratory, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

Correspondence to Hongying Wei, Department of Pediatrics, The First Affiliated Hospital of Guangxi Medical University, No. 6, Shuangyong Road, Qingxiu District, Nanning 530021, Guangxi, China
Tel: +86 15977767103; e-mail: whylhr@qq.com

Received 3 October 2020 **Revised** 11 January 2021
Accepted 25 January 2021

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 1/500 000 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

* Kairong Liang and Lauriane Nikuze contributed equally to the article.

 DOI:10.1097/MBC.0000000000001022

Table 1 **Clinical characteristics for the patients**

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.

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

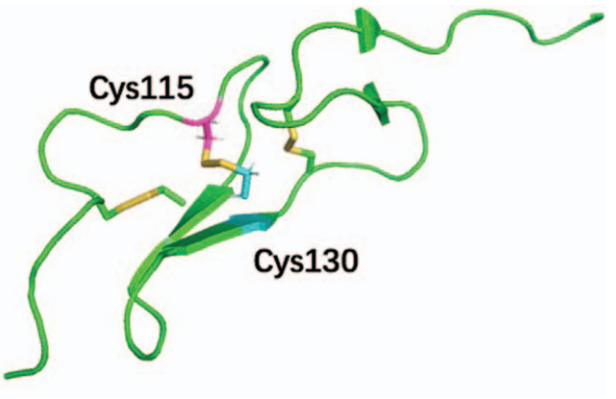
We analyzed the molecular structure of the mutant protein by using PyMOL software (http://pymol.org) based on the known three-dimensional structure of FVII.

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).

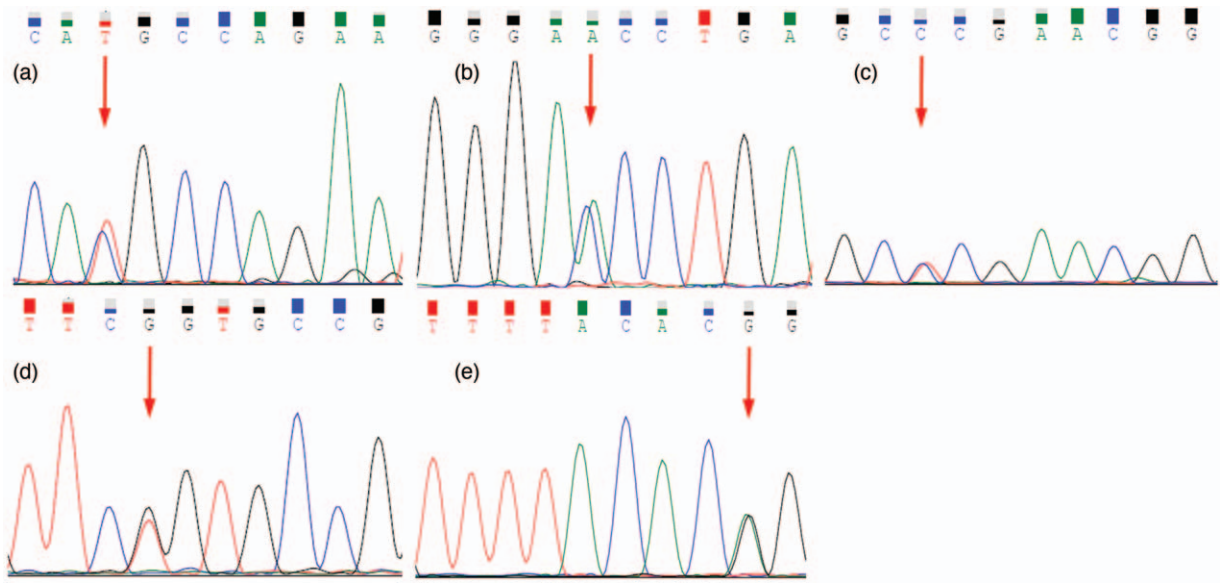
Gene analysis showed patient 1 harbored a compound heterozygous mutation of a known paternal missense mutation c.1165T>G (Cys389Gly) in exon 9 and a novel maternal missense mutation c.343T>C (Cys115Arg) in exon 5 of the *F7* gene. In patient 2, compound heterozygosity of a known paternal missense mutation 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).

Fig. 2



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



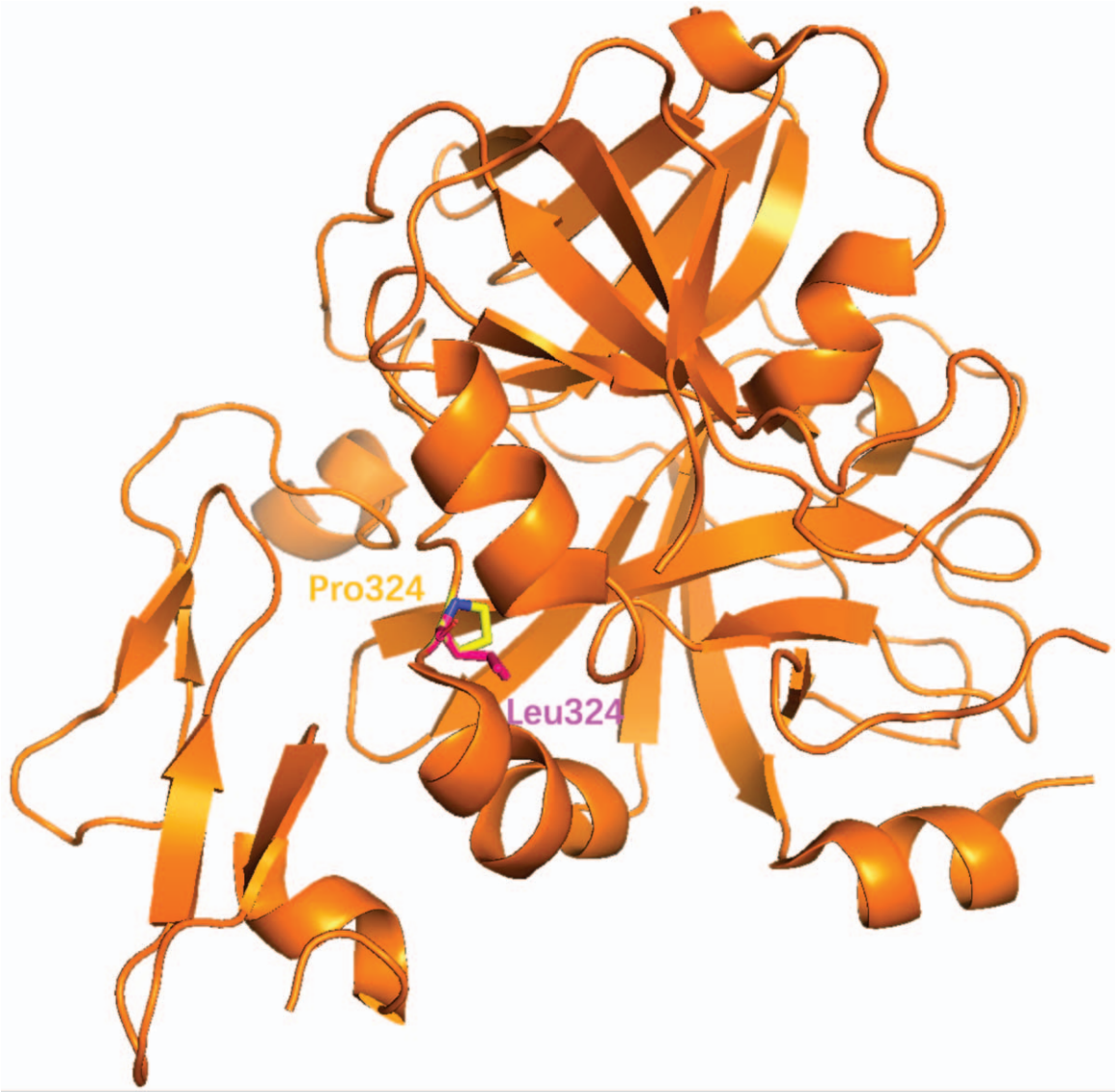
Representative chromatograms from direct sequencing of the *F7* gene. The red arrow indicates the mutation position. (a) Represents the mutation *F7* Cys115Arg in patient 1. (b) Represents the mutation *F7* Thr241Asn in patients 2 and 3. (c) Represents the mutation *F7* Pro324Leu in patient 2. (d) Represents 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:

Fig. 3



Crystal structure model of the *F7* Pro324Leu mutation. The residue Pro324 constitutes a flexible loop within the catalytic domain of factor VII. Replacement of Proline by Leucine appear to not implicate significant structural change of the factor VII molecule.

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 FVIID, 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.

Acknowledgements

We express our gratitude to the patients for their participation in this study. We are grateful to Prof Wenman Wu (Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China) for helpful technical support.

Conflicts of interest

The authors declare no conflicts of interest to disclose.

References

1 Alexander B, Goldstein R, Landwehr G, Cook CD. Congenital SPCA deficiency: a hitherto unrecognized coagulation defect with hemorrhage rectified by serum and serum fractions. *J Clin Invest* 1951; **30**:596–608.
2 Perry DJ. Factor VII deficiency. *Br J Haematol* 2002; **118**:689–700.
3 Traivaree C, Monsereenusorn C, Meekaewkunchorn A, Laoyookhong P, Suwansingh S, Boonyawat B. Genotype and phenotype correlation in intracranial hemorrhage in neonatal factor VII deficiency among Thai children. *Appl Clin Genet* 2017; **10**:37–41.
4 Sevenet PO, Kaczor DA, Depasse F. Factor VII deficiency: from basics to clinical laboratory diagnosis and patient management. *Clin Appl Thromb Hemost* 2017; **23**:703–710.
5 Mashayekhi A, Shahbazi S, Omrani M. Functional and molecular characterization of C91S mutation in the second epidermal growth factor-like domain of factor VII. *Iran J Biotechnol* 2018; **16**:e1813.
6 Herrmann FH, Wulff K, Auberger K, Aumann V, Bergmann F, Bergmann K, *et al.* Molecular biology and clinical manifestation of hereditary factor VII deficiency. *Semin Thromb Hemost* 2000; **26**:393–400.
7 McVey JH, Boswell E, Mumford AD, Kemball-Cook G, Tuddenham EG. Factor VII deficiency and the FVII mutation database. *Hum Mutat* 2001; **17**:3–17.
8 Yu T, Wang X, Ding Q, Fu Q, Dai J, Lu Y, *et al.* Using a minigene approach to characterize a novel splice site mutation in human *F7* gene causing inherited factor VII deficiency in a Chinese pedigree. *Haemophilia* 2009; **15**:1262–1266.
9 Millar DS, Kemball-Cook G, McVey JH, Tuddenham EG, Mumford AD, Attock GB, *et al.* Molecular analysis of the genotype–phenotype relationship in factor VII deficiency. *Hum Genet* 2000; **107**:327–342.
10 Clarke BJ, Ofosu FA, Sridhara S, Bona RD, Rickles FR, Blajchman MA. The first epidermal growth factor domain of human coagulation factor VII is essential for binding with tissue factor. *FEBS Lett* 1992; **298**:206–210.
11 Giansily-Blaizot M, Aguilar-Martinez P, Biron-Andreani C, Jeanjean P, Igual H, Schved JF. Analysis of the genotypes and phenotypes of 37 unrelated patients with inherited factor VII deficiency. *Eur J Hum Genet* 2001; **9**:105–112.
12 Leonard BJ, Chen Q, Blajchman MA, Ofosu FA, Sridhara S, Yang D, *et al.* Factor VII deficiency caused by a structural variant N57D of the first epidermal growth factor domain. *Blood* 1998; **91**:142–148.
13 Mota L, Shetty S, Idicula-Thomas S, Ghosh K. Phenotypic and genotypic characterization of factor VII deficiency patients from Western India. *Clin Chim Acta* 2009; **409**:106–111.
14 Bernardi F, Castaman G, Pinotti M, Ferraresi P, Di Iasio MG, Lunghi B, *et al.* Mutation pattern in clinically asymptomatic coagulation factor VII deficiency. *Hum Mutat* 1996; **8**:108–115.