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CASE REPORT

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Hereditary coagulation factor VII deficiency caused by novel compound heterozygous mutations in a Chinese pedigree: A case report

Ruimin Cai | Yi Li | Wei Xu | Xue Gao | Qiang Feng

Department of Clinical Laboratory, The Affiliated Taian City Central Hospital of Qingdao University, Taian, China

Correspondence
Qiang Feng, Department of Clinical Laboratory, The Affiliated Taian City Central Hospital of Qingdao University, No. 29, Longtan Road, Taishan District, Taian 271000, Shandong Province, China.
Email: fengqiang915@163.com

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Abstract

Background: Congenital coagulation factor VII (FVII) deficiency is a rare, autosomal-recessive haemorrhagic disorder with an estimated incidence of 1:500,000. This disorder is caused by mutations in the F7 gene.

Case description: Here, we report a pedigree of congenital FVII deficiency. The proband was a 30-year-old female with severely low FVII activity and a history of menorrhagia and epistaxis since her childhood who was subsequently diagnosed with congenital compound heterozygous FVII deficiency. A genetic study revealed a novel combination of compound heterozygous mutations (c.64G 〉 A, p.Gly22Ser and c.1027G 〉 A, p.Gly343Ser). Her father and older son had the c.64G 〉 A, p.Gly22Ser (heterozygous) mutation. Her mother and younger son had the c.1027G 〉 A, p.Gly343Ser (heterozygous) mutation. The predicted results of PolyPhen-2 and MutationTaster indicated that these mutations were probably damaging and disease-causing, respectively.

Conclusion: In this study, we identified a novel combination of genetic mutations that could expand the mutant library and help in elucidating the pathogenesis of hereditary human coagulation FVII deficiency. A novel combination of compound heterozygous mutations was reported for the first time in Chinese individuals.

KEYWORDS
factor VII deficiency, gene mutation, hemorrhagic disorder, pedigree analysis, protein structure

1 | INTRODUCTION

Coagulation factor VII (FVII) is a vitamin K-dependent serine protease that plays a key role in the initiation of the endogenous coagulation pathway. FVII is synthesized in the liver and circulates in the blood at a concentration of approximately 0.5 μg/ml.[1,2] The factor 7 (F7) gene is located at chromosome 13q34 and consists of nine exons.[3] The mature secreted FVII protein consists of 406 amino acid residues, with an amino terminal γ-carboxy glutamic acid domain, two epidermal growth factor-like (EGF) domains and a C-terminal serine proteinase domain. When a blood vessel is injured, FVII interacts with the cell surface receptor tissue factor (TF) and is then converted to the active form after the cleavage of Arg212 and Ile213.[4] The TF-FVIIa complex is an important initiator of the endogenous coagulation pathway.

Congenital FVII deficiency is a rare, autosomal-recessive hemorrhagic disorder with an estimated incidence of 1:500,000; this disorder is caused by mutations in the F7 gene.[1] Affected patients show considerable heterogeneity in clinical manifestations, from mild to life-threatening; for example, heterozygous carriers usually show no bleeding symptoms, but the bleeding events of homozygous or compound heterozygous carriers are characterized by menorrhagia,

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epistaxis, gum bleeding, easy bruising, gastrointestinal bleeding, hematoma, and intracranial hemorrhage, among others.[5] According to research, the factor VII activity levels in individuals heterozygous for mutations did not lead to the occurrence of bleeding symptoms,[6] and congenital FVII deficiency is widespread among female adolescents who experience heavy menstrual bleeding.[7] Furthermore, recent studies have reported certain new mutations in congenital FVII deficiency. A homozygous mutation of IVS7+1G>T in the FVII gene splice site led to severe gastrointestinal tract and intracranial hemorrhage.[8] Cys115Arg and Pro324Leu, situated on the F7 gene, were responsible for the impairment of the proper folding of the EGF 1 domain, which led to congenital deficiency of FVII.[9] Phe84Ser and p.Gly156Cys affected the Gla and EGF domains of FVII, respectively, causing hereditary coagulation factor VII deficiency.[10]

Here, we report a pedigree of congenital FVII deficiency, the proband was a 30-year-old female with severely low FVII activity who was subsequently diagnosed with congenital compound heterozygous FVII deficiency. The clinical manifestations and laboratory results of the proband and her family members are presented, the genetic analysis of the proband was performed by second-generation sequencing, and the bioinformatics and amino acid homology analyses were conducted. Our findings showed that the proband with severe FVII deficiency was compound heterozygous for c.64G>A and c.1027G>A mutations in the F7 gene, resulting in p.Gly22Ser and p.Gly343Ser.

2 | CASE PRESENTATION

The proband (III) was a 30-year-old female from Shandong Province, China, who was admitted to the hospital because of her pregnancy. She had been pregnant for 38 weeks and had a history of menorrhagia and epistaxis since her childhood. A complete blood count showed a white cell count of 2.9×10^9/L, a hemoglobin level of 83 g/L and a platelet count of 212×10^9/L. Routine coagulation tests showed that the prothrombin time (PT) was markedly elevated (35.0 s, normal range 10–14 s); the international normalized ratio (INR) was 2.92 (normal range 10–14); the FVII activity level was extremely low at 2.1% of normal on a Siemens automatic blood coagulation analyzer (Siemens 5100); and the activated partial thromboplastin time (APTT), thrombin time (TT) and D-dimer were within their respective reference ranges. The FVII:Ag was checked with an enzyme-linked immunosorbent assay (ELISA) kit (Changfeng), the phenotypes of this family are shown in Table 1, and the pedigree is shown in Figure 1. Informed consent was obtained from all family members.

Genomic DNA of all the family members was extracted from fresh blood samples using a DNA extraction kit (TIANGEN Biotech Co., Ltd.). Second-generation sequencing of the proband and Sanger sequencing of the family members were performed by Yinfeng Gene Technology Company. The sequences of all the primers and PCR (polymerase chain reaction) reaction conditions are described

TABLE 1 Results of routine coagulation tests of the family members

Family members	PT(s)	INR	FVII:C (%)	FVII:Ag (%)
I	12.8	1.07	70.5	89.0
II	13.9	1.16	66.2	82.0
III	35	2.92	2.1	25.6
IV	13.7	1.14	39.9	56.0
V	13.5	1.13	49.6	96.5
Normal range	10–14	0.8–1.2	50–150	80–120

Abbreviations: INR, International standardized ratio of prothrombin; PT, prothrombin time.

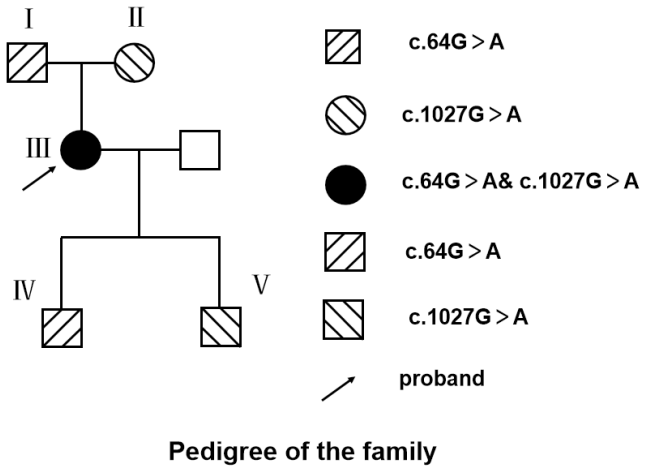

FIGURE 1 Pedigree of the family

in the literature.[11] The sequenced PCR products and corresponding GenBank sequence (NM_000131.2) were analyzed by Megalign software to identify mutation sites. The result showed that the sequence of the proband had a missense mutation in exon 1 (c.64G>A, p.Gly22Ser) and a missense mutation in exon 8 encoding the serine protease domain of FVII (c.1027G>A, p.Gly343Ser). Her father and older son had the c.64G>A, p.Gly22Ser (heterozygous) mutation. Her mother and younger son had the c.1027G>A, p.Gly343Ser (heterozygous) mutation (Figures 2 and 3). No other mutations were found in the exons of the F7 gene.

Based on the p.Gly22Ser and p.Gly343Ser mutations in the F7 gene, the mutation score was 0.968 and 1.0 by PolyPhen-2, respectively, which indicates that the mutations are probably damaging. The predicted results for both of these mutations by MutationTaster were both disease-causing, and the score for both mutations was 56 (Table 2). Conservative analysis of the amino acid sequences of the F7 gene between different species was performed by using ClusterX software, which was used to compare the sequence of F7 in *Homo sapiens* (NP_062562.1) with the species *P. troglodytes* (XP_001149885.2) and *M. mulatta* (NP_001073605.1). The results showed that Gly22 and Gly343 in F7 are conserved among species. A structural analysis of the F7 molecule produced

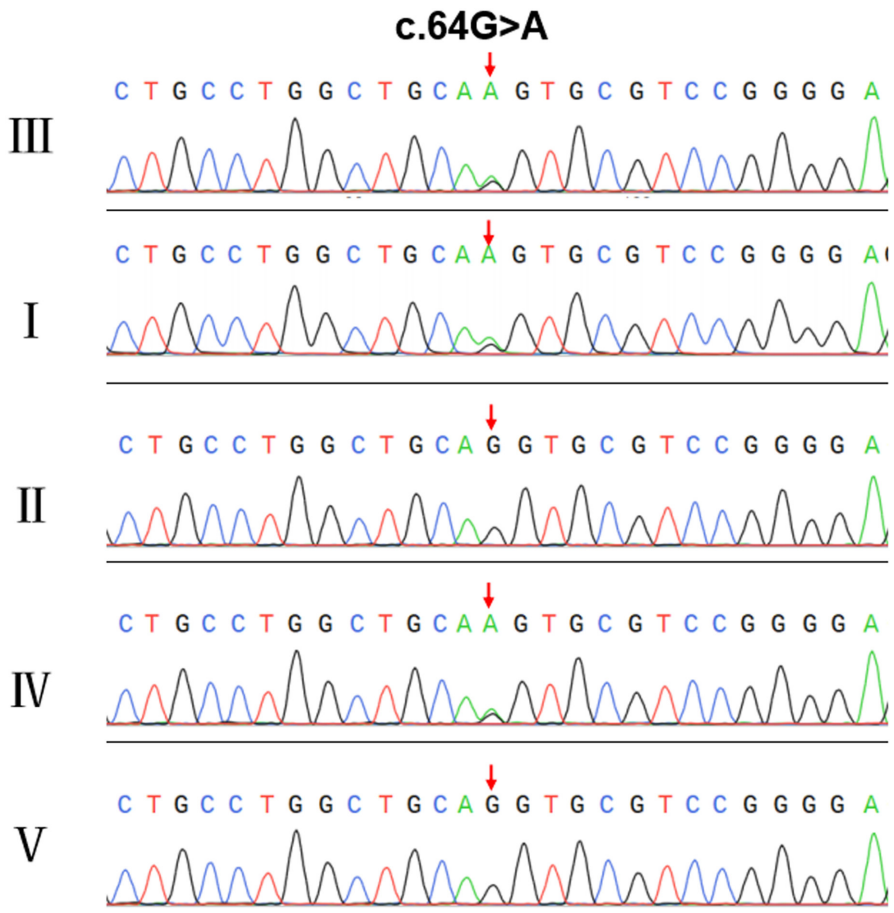

FIGURE 2 Fragment of chromatogram showing the sequence of exon 1 of F7 gene in the pedigree. The genetic variant c.64G>A is marked with red arrow, III: the proband. I: father of the proband. II: mother of the proband. IV: elder son of the proband. V: younger son of the proband.

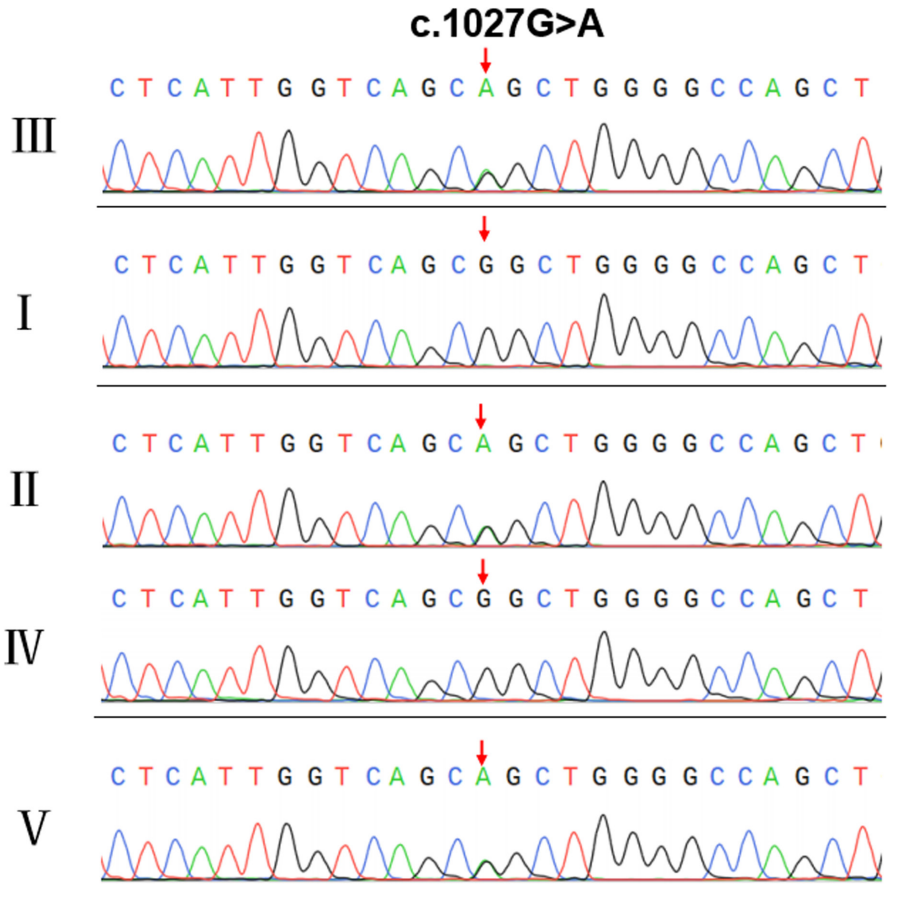

FIGURE 3 Fragment of chromatogram showing the sequence of exon 8 of F7 gene in the pedigree. The genetic variant c.1027G>A is marked with red arrow, III: the proband. I: father of the proband. II: mother of the proband. IV: elder son of the proband. V: younger son of the proband.

TABLE 2 F7 mutation sites and corresponding nucleotide and amino acid changes in the pedigree

Family members	Exon	Nucleotide change	Amino acid change	Genotype	MutationTaster prediction	Polyphen2 prediction
I	1	c.64G>A	p.Gly22Ser	Heterozygote	Disease-causing score: 56	Probably damaging score: 0.968
II	8	c.1027G>A	p.Gly343Ser	Heterozygote	Disease-causing score: 56	Probably damaging score: 1.0
III	1 8	c.64G>A c.1027G>A	p.Gly22Ser p.Gly343Ser	Compound heterozygote	Disease-causing score: 56	Probably damaging score: 0.968 score: 1.0
IV	1	c.64G>A	p.Gly22Ser	Heterozygote	Disease-causing score: 56	Probably damaging score: 0.968
V	8	c.1027G>A	p.Gly343Ser	Heterozygote	Disease-causing score: 56	Probably damaging score: 1.0

Note: The scores are provided by the MutationTaster and Polyphen2 online software.

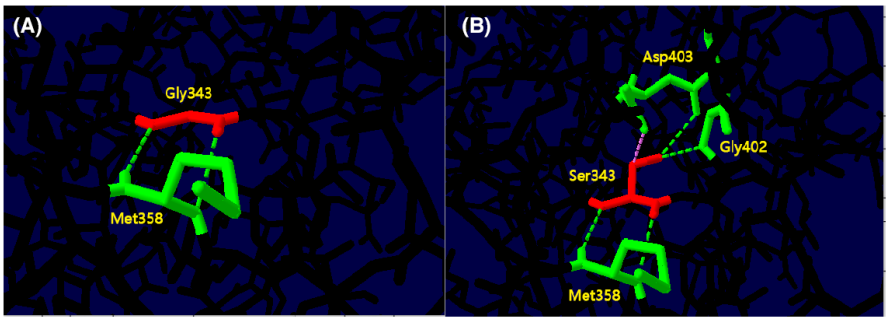

FIGURE 4 Predicted structure of FVII protein. (A) The wild type; (B) the mutant (p.Gly343Ser). p.Gly343Ser is shown in the protein three-dimensional model in red. It shows that p.Gly343Ser would cause a steric collision with Asp403, the collision is depicted as the pink line, and the hydrogen bonds are depicted as green lines.

by Swiss-PdbViewer is shown in Figure 4, which shows that the Gly343Ser mutation causes steric collision with Asp403 and generates new hydrogen bonds with Asp403 and Gly402. The structure of F7 resulting from the Gly22Ser mutation could not be predicted by Swiss-PdbViewer.

3 | DISCUSSION

Congenital FVII deficiency is a rare hemorrhagic disorder with a prevalence of 1:500,000; in this disorder, the majority of FVII mutations are single amino acid substitutions according to the literature. Approximately 70 different mutations that could cause symptoms of FVII deficiency have been included in the EAHAD FVII variant database (https://f7-db.eahad.org/). The hemorrhagic tendency might not be related to the plasma FVII activity levels, as the proband in our study, who had a history of menorrhagia and epistaxis only in her childhood, had no hemorrhagic symptoms when she gave birth to her sons, while her plasma FVII activity levels were extremely low, at 2.1% of normal. Unsurprisingly, her sons were asymptomatic and exhibited only slight reductions (39.9% and 49.6%) in the plasma level of FVII.

The calcium-binding site on which most of the missense mutations were located might be related to the formation of a complex of FVII and tissue factor. In our study, we found that the Gly343Ser mutation causes a severe collision with the side chain of Asp403; it would also generate new hydrogen bonds with Asp403 and Gly402, which could enhance the polarity. The steric collision and the new hydrogen bonds would lead to a reduction in the stability of the protease domain. According to research,[11] the location of the 64G>A mutation at the 3′ end of exon 1a may make splicing inefficient and reduce the mRNA levels. Both mutations have been previously reported,[5,11] but this combination of mutation sites (c.64G>A and c.1027G>A) in a Chinese pedigree was described here first. The mutations occurring in other domains of the FVII protein would also lead to the plasma antigen levels and function of FVII protein, as described. Asn57Ile and Asn57Asp mutations, which lead to the loss of an important intramolecular hydrogen bond between Asn57 and Cys81, alter the folding of the first EGF domain.[12] The 6070+1G>A substitution occurs at the invariable dinucleotide splice site of intron 4,[13] and the frameshift 11125delC mutation,[14] and the 5886+5G>A transition[15] could generate a truncated or elongated FVII protein. Tang et al.[16] reported a summary of the novel missense mutations in the FVII gene reported in recent years.

According to the clinical symptoms and routine laboratory test and genetic sequencing results, we reported here that a novel combination of compound heterozygous mutations, the heterozygous missense mutations of c.64G>A and c.1027G>A in exon 1 and exon 8 of the F7 gene (p.Gly22Ser and p.Gly343Ser), respectively, probably underlies the congenital FVII deficiency in this pedigree, suggesting that these missense mutations changed the molecular spatial conformation of the FVII domain. A novel combination of compound heterozygous mutants was reported for the first time in Chinese individuals.

AUTHOR CONTRIBUTION

All authors have approved the final article.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest in relation to this work.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available in the [https://pan.baidu.com/] repository, https://pan.baidu.com/s/1n-oyAdaBDF4KME4rAX7Z4A%C2%A0, access code: mshq.

ORCID

Ruimin Cai https://orcid.org/0000-0002-4529-9283

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