

Genetic Analysis of Hereditary Coagulation Factor V Deficiency in Two Chinese Families Caused by Compound Heterozygous Mutations

Yuan Chen¹ Ke Zhang¹ Yanhui Jin¹ Manlin Zeng¹ Kaiqi Jia¹ Lihong Yang¹ Mingshan Wang¹

¹Department of Clinical Laboratory, Key Laboratory of Clinical Laboratory Diagnosis and Translational Research of Zhejiang Province, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

Address for correspondence Mingshan Wang, MD, Department of Clinical Laboratory, Key Laboratory of Clinical Laboratory Diagnosis and Translational Research of Zhejiang Province, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China (e-mail: wywms@126.com).

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Abstract

Objective This study aims to provide a preliminary discussion of the molecular basis of FV deficiency caused by compound heterozygous mutations in two Chinese families.

Methods Relative coagulation index was measured by the one-stage clotting method and the FV:Ag was measured by ELISA. All exons and flanking regions of the *F5* gene were amplified by PCR and directly sequenced. ClustalX-2.1-win was used to analyze the conservation of mutations. The online software was used to predict the pathogenicity of mutations. PyMOL was used to analyze the variation in the spatial structure of the FV protein before and after mutations. Calibrated automated thrombogram was used to analyze the function of the mutant protein.

Results Phenotyping suggested that both probands had a simultaneous decrease in FV:C and FV:Ag. Their genetic tests showed that proband A had a missense mutation p.Ser111Ile in exon 3 and a polymorphism p.Arg222Gly in exon 25. At the same time, the proband B had a missense mutation p.Asp96His in exon 3 and a frame-shift mutation p.Pro798Leufs*13 in exon 13. Meanwhile, the p.Ser111Ile is conserved among homologous species. The bioinformatics and protein model analysis revealed that p.Ser111Ile and p.Pro798Leufs*13 were pathogenic and could affect the structure of the FV protein. The thrombin generation test revealed that the clotting function of proband A and B had been affected.

Conclusion These four mutations may be responsible for the reduction of FV levels in two Chinese families. Moreover, the p.Ser111Ile mutation is a novel pathogenic variant that has not been reported.

Keywords

- coagulation factor V deficiency
- *F5* gene
- bioinformatics
- thrombin generation

Introduction

Coagulation factor V (FV) is a single-chain glycoprotein with a molecular weight of 330 kb produced by hepatocytes and megakaryocytes. It can be cleaved by thrombin and activated coagulation factor X (FXa) within the B domain at positions Arg709, Arg1018, and Arg1545 to generate activated FV (FVa).¹ It is well-known that FVa acts as a membrane receptor for FXa

in the prothrombinase complex and can enhance the catalytic activity of FXa to convert prothrombin into thrombin and facilitate the interaction between prothrombin and the prothrombinase complex. Moreover, the FV has an anticoagulating function as a cofactor of APC in inactivating activated coagulation factor VIII (FVIIIa). But this anticoagulant function disappears at the time of procoagulant activation of the FV.²

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Hereditary FV deficiency is a kind of rare autosomal recessive bleeding disorder caused by *F5* gene defects, with an incidence of one case per million in the general population.³ Patients with hereditary FV deficiency have a wide variety of clinical manifestations, ranging from asymptomatic to epistaxis, oral mucosal bleeding, menorrhagia, postpartum hemorrhage, and abnormal bleeding after trauma or surgery. And there is no evidence to prove that the clinical manifestations have relationships with the FV level.¹ According to the decrease of FV activity (FV:C) and FV antigen (FV:Ag), the hereditary FV deficiency is divided into two types: type I is a quantitative defect (FV:C and FV:Ag are both decreased) and type II is a qualitative defect (decreased FV:C but normal FV:Ag).⁴ The hereditary FV deficiency is considered to have connected with the *F5* gene defects. Until now more than 160 mutations that caused FV deficiency have been collected in the Human Gene Mutation Database (HGMD; <http://www.hgmd.org>), and most of them were characterized by type I.

In this article, we described two Chinese families with hereditary FV deficiency. And we preliminarily discussed the molecular pathogenesis of FV deficiency in these two families.

Materials and Methods

Patients

Family A

Proband A is a 52-year-old Chinese man who was admitted to the hospital due to a “pulmonary nodule” for 4 months. The routine blood coagulation test revealed that his prothrombin time (PT) and activated partial thromboplastin time (APTT) were prolonged. In addition, the coagulation factor detection found that his FV:C and FV:Ag were reduced. His other coagulation indices were within the normal range. The pedigree investigation, of a total of 10 persons in three generations (→ Fig. 1), revealed that none of them had a history of spontaneous bleeding or thrombosis except his little sister who experienced abnormal bleeding during one delivery. No abnormal function of the liver and kidney was found in this family.

Family B

Proband B is a 47-year-old Chinese woman who suffered from “chest heart palpitations” for 1 year. Four months ago, she had a routine test at a primary hospital and found that PT and APTT were prolonged. In our hospital, routine blood coagulation tests revealed that her FV:C and FV:Ag were significantly reduced. In total, six members of her family, spanning three generations, were examined (→ Fig. 2); abnormal liver and kidney function, thrombosis, and bleeding were not discovered.

One hundred healthy individuals were engaged in this study as healthy controls to set the reference range for the coagulation index, including 58 males and 42 females with an average age of 35 years (range: 19–63 years). None of them had a history of abnormal bleeding or a thrombosis tendency, and none had liver or kidney disease. This study was approved by the Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University.

Family A

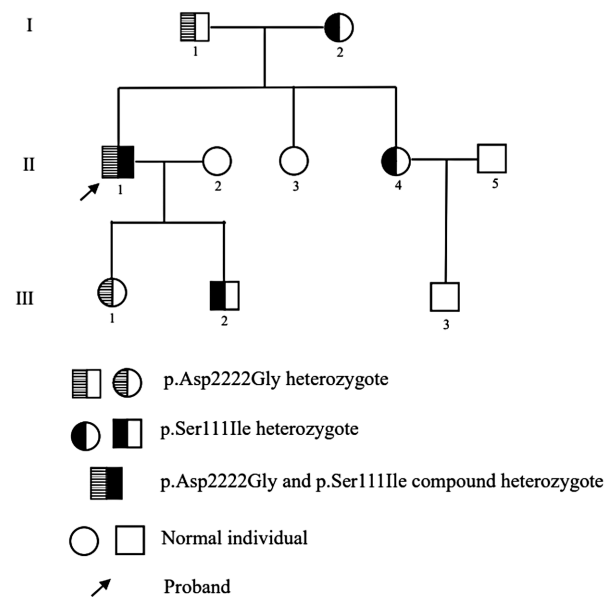


Fig. 1 The pedigree chart of the FV deficiency: family A.

Family B

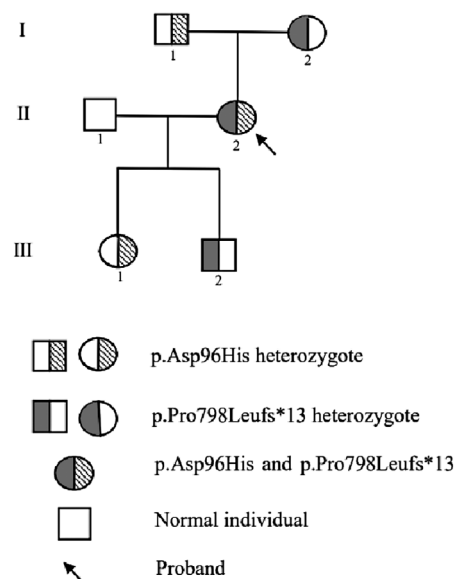


Fig. 2 The pedigree chart of FV deficiency: family B.

Methods

Coagulation Index Detection

Peripheral blood was collected from two probands and their family members into sodium citrate anticoagulant tubes and centrifuged at 2,100g for 10 minutes to prepare the upper platelet-poor plasma and lower blood cells. The plasma was used to detect the coagulation indices and the blood cell was

used to extract DNA. The PT, APTT, FV:C, factor II activity (FII:C), factor VII activity (FVII:C), and factor X activity (FX:C) were tested on the STA-R-Max analyzer (Stago, Asnières-sur-Seine, France) through the one-stage clotting method. The reagents used to detect the coagulation indices in this study were all companion reagents to the STA-R-Max analyzer. The FV:Ag was measured by an ELISA kit (Cedarlane Labs, North Carolina, United States).

Analysis of F5 Gene

The genomic DNA of all participants was extracted from peripheral blood using a DNA blood extraction kit (Tiangen Biotech, Beijing, China). All exons and flanking regions of the *F5* gene were amplified by polymerase chain reaction (PCR). The primer sequences and PCR reaction conditions are listed in the literature.³ The PCR products were sent to Sunny Biotechnology Corporation (Shanghai, China) for purification and direct sequencing. After identifying the mutation site, all exons and flanking regions of the *F5* gene of family members were amplified to confirm if they carried the same mutation.

Conservation Analysis

The multiple sequence alignment software ClustalX-2.1-win was used to analyze the conservation of Ser111 in the FV protein in multiple alignment modes. A total of nine homologous species were investigated: *Homo sapiens* (NP_000121.2), *Mus musculus* (NP_032002.1), *Rattus norvegicus* (NP_001041343.1), *Pan troglodytes* (XP_513984.4), *Macaca mulatta* (XP_001093072.2), *Canis lupus familiaris* (XP_005622605.1), *Bos taurus* (NP_776304.1), *Gallus gallus* (XP_001231901.3), and *Danio rerio* (NP_001007209.2). Conservation analysis was performed after the alignment of the homologous sequences was completed.

Bioinformatic Analysis

Four online bioinformatic softwares—PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/index.shtml>), PROVEAN (<https://www.jcvi.org/research/provean>), Mutation Taster (<https://www.mutationtaster.org/>), and SIFT (<http://sift.jcvi.org>)—were used to predict the pathogenicity of p.Ser111Ile mutation in protein function through the scoring results. According to the known FV protein structure (AF-P12259-F1) in UniProt (<https://www.uniprot.org/>), the difference in the spatial structure of FV protein before and after mutation was analyzed by PyMOL software. The entire protein model was shown in cartoon format. Amino acids at the mutation sites and those that interact with them were displayed by sticks with different colors.

Thrombin Generation Experiment

According to the literature,⁵ the calibrated automated thrombin (CAT) generation assay was used to examine thrombin generation. Following the protocol of the Thrombinoscope BV company (Rijswijk, the Netherlands), we measured the lag time, peak height, time to peak (ttPeak), and endogenous thrombin potential (ETP) to evaluate the pro-coagulant activity of FV on a Fluoroskan Ascent FL reading meter (Thermo Electron and Fisher Scientific Company,

Waltham, Massachusetts, United States). The FluoCa kit and PPP-Reagent (Stago) were used to perform the CAT analysis. Moreover, the PPP-Reagent used for the CAT assay was of medium Tissue Factor (TF) concentration.

Results

Laboratory Analysis

The APTT and PT of proband A prolonged to 47.7 seconds (reference range: 29.0~43.0 s) and 17.1 seconds (reference range: 11.5~14.8 s), respectively. Meanwhile, the APTT and PT of proband B prolonged to 73.6 and 28.2 seconds, respectively. The FV:C and FV:Ag of proband A had reduced to 13 and 20% (reference range: 82~118%), respectively. Meanwhile, the FV:C and FV:Ag of his father, mother, younger sister, son, and daughter were all reduced. There are no obvious abnormalities in FV:C and FV:Ag of other members of his family. The FV:C and FV:Ag of proband B were significantly reduced to 3 and 5%, respectively. At the same time, the FV:C and FV:Ag of her father, mother, son, and daughter were all reduced. However, her husband's FV:C and FV:Ag were within the normal range (▶Table 1).

Genetic Analysis

Family A

Genetic analysis revealed that the proband A carried a heterozygous missense mutation c.332G > T in exon 3 which resulted in a p.Ser111Ile mutation. Meanwhile, he also carried a polymorphism c.6665A > G in exon 25 which resulted in a p.Asp2222Gly mutation. The pedigree study revealed that his mother, younger sister, and son were p.Ser111Ile heterozygotes. However, his father and daughter were p.Asp2222Gly heterozygotes. Other members of this family were found to carry the wild-type *F5* gene (▶Fig 3).

The p.Ser111Ile missense mutation is not found in gnomAD, meaning that this mutation is not a polymorphism. Furthermore, this missense mutation has not been found in HGMD and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>).

Family B

According to the result of genetic analysis, proband B carried a heterozygous mutation c.286G > C in exon 3 that led to missense mutation p.Asp96His. She also carried a heterozygous mutation c.2393-2393delC in exon 13 which led to a frameshift mutation p.Pro798Leufs*13. The pedigree study revealed that her father and daughter were p.Asp96His heterozygotes. At the same time, her mother and son carried p.Pro798Leufs*13 heterozygotes. Her husband carried a wild-type *F5* gene (▶Fig. 3).

Conservation Analysis

After completing the alignment, the sequence near Ser111 was discovered. A comparison of the homologous sequences of the nine species using the ClustalX-2.1-win software revealed that the Ser111 was conserved among those homologous species (▶Fig. 4). The conservation analysis of Asp2222 was listed in the literature.⁵

Table 1 Phenotypes and genotypes of two hereditary FV deficiency families

Patient	PT (s)	APTT (s)	TT (s)	FV:C (%)	FV:Ag (%)	FII:C (%)	FVII:C (%)	FX:C (%)	Mutation
Family A									
Proband A	17.1	47.7	18.2	13	20	110	113	104	Asp2222Gly Ser111Ile
A-I ₁	15.5	45.9	14.4	60	58	97	95	95	Asp2222Gly
A-I ₂	15.1	44.3	18.0	55	53	89	98	107	Ser111Ile
A-II ₂	12.8	31.2	17.8	94	109	94	110	99	Wild-type
A-II ₃	13.5	41.2	17.0	112	97	92	98	100	Wild-type
A-II ₄	15.2	43.5	15.3	52	56	102	111	106	Ser111Ile
A-II ₅	13.1	32.0	14.9	107	111	95	109	103	Wild-type
A-III ₁	15.0	44.4	16.0	58	57	103	103	94	Asp2222Gly
A-III ₂	15.7	45.1	19.6	51	55	90	105	91	Ser111Ile
A-III ₃	12.9	38.1	20.1	110	108	101	89	98	Wild type
Family B									
Proband B	28.2	73.6	15.7	3	5	103	98	100	Asp96His Pro798Leufs*13
B-I ₁	16.3	47.5	18.3	57	58	105	94	102	Asp96His
B-I ₂	15.5	48.3	17.0	56	53	107	92	99	Pro798Leufs*13
B-II ₁	13.0	31.0	15.8	107	110	96	99	94	Wild-type
B-III ₁	15.8	49.9	16.2	51	49	92	91	101	Asp96His
B-III ₂	15.6	48.1	18.8	55	52	102	100	96	Pro798Leufs*13
Reference range	11.5~14.8	29.0~43.0	14.0~20.0	82~118	82~118	86~112	86~114	90~110	

Bioinformatics Analysis

The p.Ser111Ile missense mutation was predicted to be “damaging,” “probably damaging,” “disease-causing,” and “damaging” by online software SIFT, PolyPhen-2, mutation taster, and PROVEAN, respectively. The pathogenicity analyses of p.Pro798Leufs*13 and p.Asp2222Gly were listed in the literature.⁵

The protein model analysis of FV by PyMOL software revealed that Ser111 in wild type located in A1 domain formed a hydrogen bond with Tyr66, Gly125, Ser174, and His175, respectively. When Ser111 was mutated to Ile111, the hydrogen bond formed between Ile111 and Gly125 disappeared along with the hydrogen bond between Ile111 and His175. The other hydrogen bonds and amino acids were unchanged (→Fig. 5).

As shown in →Fig. 6, the Pro798 located in the B domain of FV protein; when p.Pro798Leufs*13 happened right here, it resulted in a frame-shift of 13 amino acids in FV protein and predicted a downstream premature termination at amino acid Leu1010, then part of the B domain and entire A3, C1, C2 domains had disappeared.

Thrombin Generation Experiment

Compared with the healthy control, the quantity and peak height of thrombin generation in probands A and B were

significantly reduced, while the ttPeak and lag time had prolonged. Moreover, according to the thrombin generation curve, we found that the p.Arg2222Gly polymorphism had higher quantities and the peak height of thrombin generation than the p.Ser111Ile, p.Asp96His, and p.Pro798Leufs*13 mutations heterozygotes (→Fig. 7).

Discussion

The human F5 gene is located on chromosome 1q23 and consists of 25 exons encoding the A1–A2–B–A3–C1–C2 domains. Each domain has different functions in FV protein. The A1 domain is considered to contribute to the FVa/FXa interaction and the binding between heavy and light chains of FVa, the A2 domain has several sites of proteolytic processing by thrombin and APC, and the C1 and C2 domains provide a platform for interaction with phospholipid membranes.^{6,7} However, the B domain is poorly conserved among homologous species and is removed when FV protein is converted to FVa, which consists of a heavy chain (A1–A2 domains) and a light chain (A3–C1–C2 domains) linked through a noncovalent interaction by two Ca²⁺ ions and a Cu²⁺ ion.⁸ This arrangement prevents the destruction of the domain structure and stabilizes the interaction at A1/A3 interface.

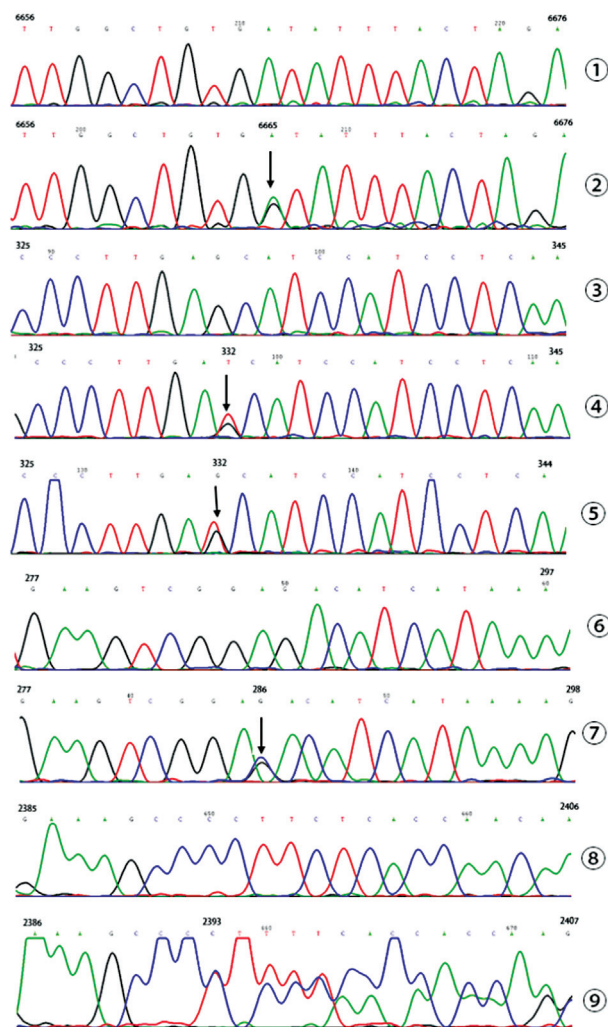


Fig. 3 The direct sequence of two FV deficiency families. The site of the variant is indicated with an arrow. ① is wild-type of c.6665A > G (p.Asp2222Gly) and ② is forward sequencing of heterozygous c.6665A > G (p.Asp2222Gly). ③ is wild-type of c.332G > T (p.Ser111Ile), ④ is forward sequencing of heterozygous c.332G > T (p.Ser111Ile), ⑤ is reverse sequencing of heterozygous c.332G > T (p.Ser111Ile). ⑥ is wild-type of c.286G > C (p.Asp96His) and ⑦ is forward sequencing of heterozygous c.286G > C (p.Asp96His). ⑧ is wild-type of c.2393-2393delC (p.Pro798Leufs*13) and ⑨ is forward sequencing of heterozygous c.2393-2393delC (p.Pro798Leufs*13).

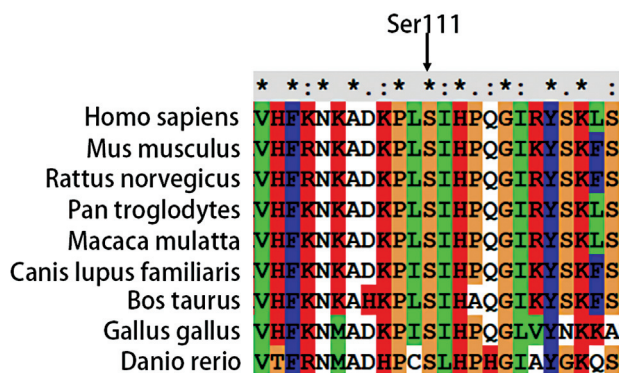


Fig. 4 The conservative analysis of Ser111. (“*” means conserved; “:” means highly conserved; “.” means lowly conserved.)

In our study, we found two mutations of the *F5* gene, including p.Ser111Ile and a polymorphism p.Asp2222Gly in family A, and two mutations including p.Pro798Leufs*13 and p.Asp96His in family B. In these two families, the four mutations in the heterozygous state all showed simultaneously decreased in FV:C and FV:Ag, which could be classified as type I FV deficiency. The thrombin production curve suggested that the coagulation function of patients with *F5* gene defects was affected, and the effects on probands A and B were the most severe. Therefore, we held an opinion that the decrease in FV level in the two families was related to the *F5* gene defects. In addition, it is worth mentioning that the mutation p.Ser111Ile is discovered for the first time in the world.

The Ser111 site is located in the A1 domain, which contains ligands entirely coordinated with Ca^{2+9} . Moreover, Adams et al⁹ suggested that there may be a ring composed of Lys121-Asp140 in the A1 domain, whose conformation leads to several fundamental interactions between the A1 and A3 domains and facilitates the binding between heavy and light chains. And the destruction of this structure may lead to the dissociation of the light and heavy chains of FVa. Conservation analysis showed that the Ser111 is conserved in biological evolution, suggesting that it is an essential functional site for the FV protein. Then by constructing the structure model of the FV protein, we found that Gly125 is located at the loop of Lys121-Asp140 and is spatially close to the Lys121. The alteration of the hydrogen bond between Ile111 and Gly125 and the extended side chain might destroy the spatial conformation of the Lys121 and affect the stable structure of the loop. That might result in the dissociation of the heavy and light chains of FVa. Furthermore, Liu et al¹⁰ reported a p.His175Arg variant, which had affected the normal conformation of His1845 and led to the reduction of FV:C. The His1845 is a residue of Cu^{2+} coordination between the A1 and A3 domains and is spatially close to His175 in the FV protein.¹¹ Therefore, we suggested that the change in the hydrogen bond between Ile111 and His175 may have affected the stability of the spatial structure at His175, and subsequently also affected His1845. Consequently, these hydrogen bond changes might lead to the deterioration of FVa stability and result in the degradation of FVa, which in turn leads to a decrease in FV level.

The polymorphism p.Asp2222Gly was involved in the R2 haplotype.¹² As we know, this variant was first reported by Yamazaki et al¹³; they suggested that p.Asp2222Gly caused the misfolding of FV protein and subsequently led to the retention of protein in endoplasmic reticulum (ER) and the intracellular degradation by the cell's quality control mechanism. It is worth mentioning that p.Arg96His discovered in family B had a similar pathogenic mechanism to p.Asp2222Gly polymorphism. The p.Arg96His was reported by Liu et al¹⁴; they suggested that it could result in misfolding of FV protein after changing in hydrogen bond between Arg96 and Arg4 and increased protein retention in the ER, subsequently, leading to intracellular degradation. As a result, both the p.Asp2222Gly polymorphism and the p.Arg96His mutation exhibit unstable expression and low

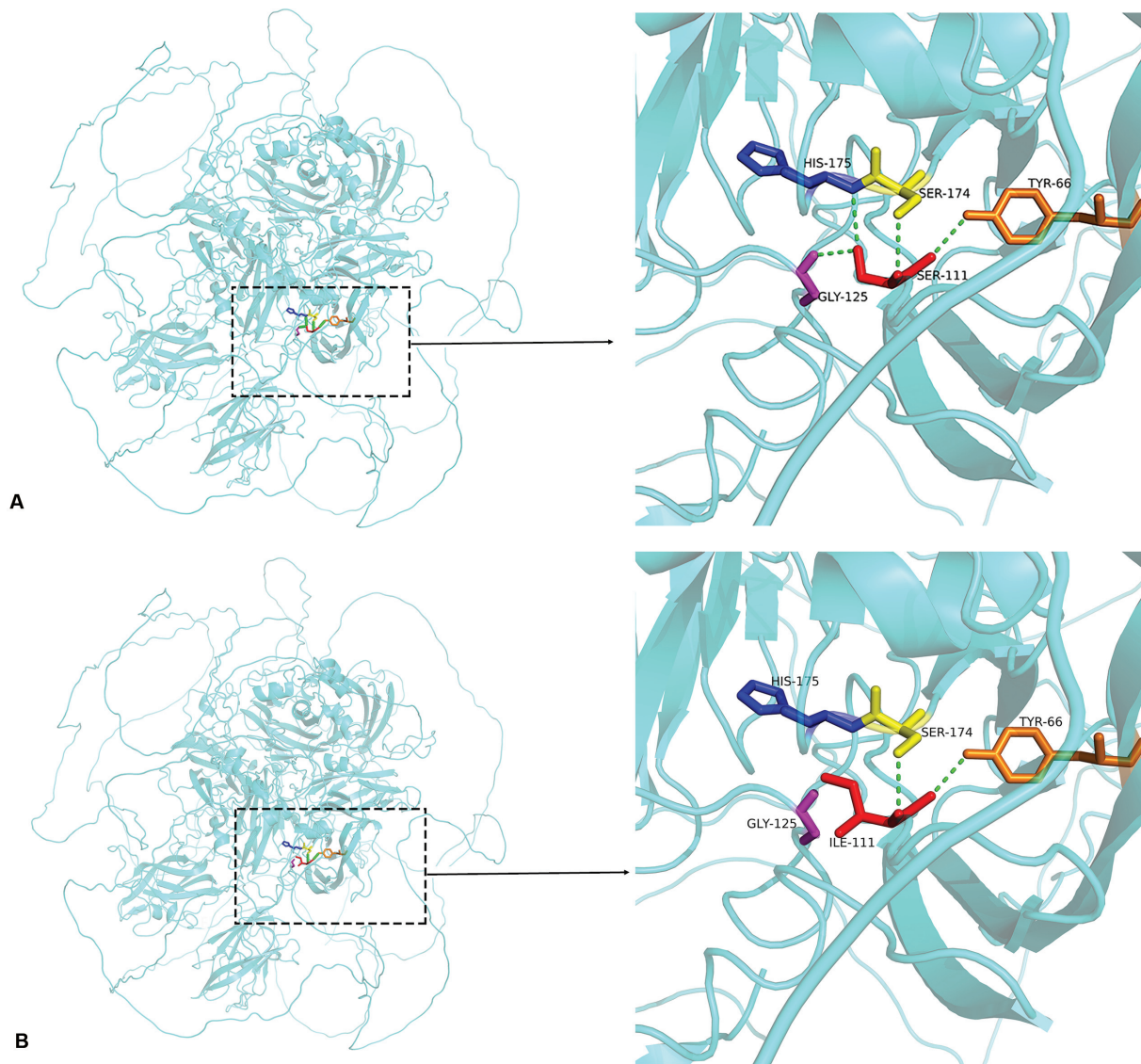


Fig. 5 The model analysis of p.Ser111Ile. (A) Wild type; (B) mutant type. Hydrogen bonds are indicated by green dotted lines.

secretion rates of FV proteins. This may be the reason for the decrease of FV:Ag in p.Asp222Gly and p.Arg96His heterozygotes. The p.Pro798Leufs*13 was first identified by Deng et al⁵, which suggested that it was a disease-causing mutation and might give rise to damage to the function and structure of the FV protein. According to our protein structure model, the p.Pro798Leufs*13 mutation caused the generation of premature termination of FV protein, subsequently, resulting in the formation of truncated protein. The production of truncated proteins in the cell is detrimental to the organism and is easily degraded by the cell's protection mechanism, which protects the cell from the dominant negative effects of nonfunctioning polypeptides.¹⁵ As a result, the secretion of FV protein is reduced, which subsequently leads to a reduction in FV levels.

In conclusion, it was reasonable for us to speculate that the compound heterozygous mutations of p.Ser111Ile and p.Asp222Gly, p.Arg96His and p.Pro798Leufs*13 connected to two hereditary FV deficiency Chinese families. Moreover, we

have preliminarily discussed the potential pathogenesis of p.Ser111Ile and p.Pro798Leufs*13 mutations, which would help us better understand the FV deficiency. The specific pathogenic mechanism, however, still needs to be demonstrated by expression study in vitro.

Ethical Approval

Our study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).

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Conflict of interest

The authors declare that they have no conflict of interest.

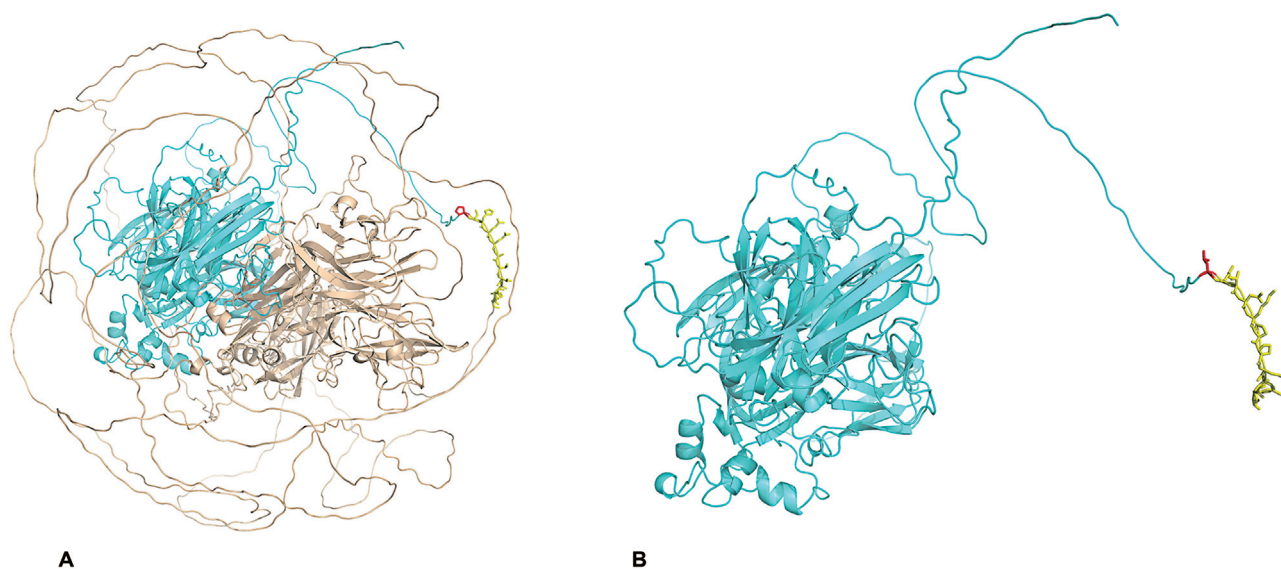


Fig. 6 The model analysis of p.Pro798Leufs*13. (A) Wild type; (B) mutant type. The frame-shifted amino acids were shown in yellow and the disappeared areas were shown in wheat.

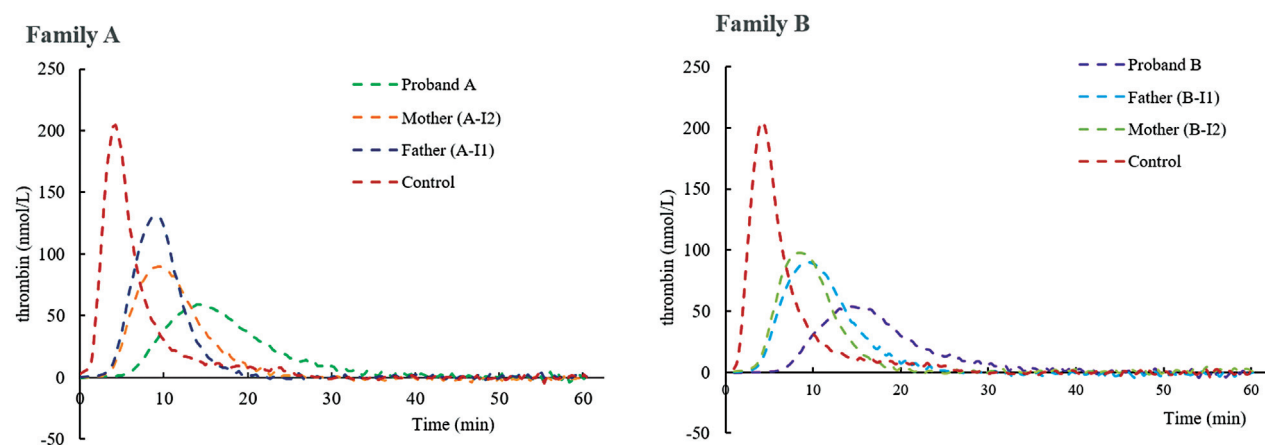


Fig. 7 The graph of thrombin generation of families A and B.

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