Clinical Characterization and Molecular Analysis of Fourteen Chinese Patients with Factor V Deficiency

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Abstract

Introduction Coagulation factor V (FV) functions as a vital cofactor that performs procoagulant roles in the coagulation system. We investigated 14 unrelated patients whose plasma FV levels were all below the reference range.

Methods FV activity (FV:C) and FV antigen were detected by one-stage clotting and ELISA, respectively. All 25 exons of the *F5* gene in patients were amplified by the PCR, and they were sequenced directly. Haplotype analysis was performed with different polymorphisms on *F5*. Protein modeling was applied to analyze the potential molecular mechanisms.

Results Of five patients with higher FV levels (FV:C > 10%), only one had minor bleeding symptoms. In contrast, of the remaining eight patients with lower FV levels (FV:C < 10%), six showed various bleeding manifestations. A total of 10 mutations were detected from 14 patients (6 were novel mutations). Interestingly, the homozygous p.Phe190Ser was found in five pedigrees, and haplotype analysis showed that they shared almost the same haplotype, indicating the common origin rather than a hotspot mutation. *In silico* analysis preliminarily investigated the potential pathogenic mechanism of the mutation. Modeling analysis showed that all six missense mutations would lead to conformational alterations in the FV protein. Among them, three (p.Gly1715Ser, p.Ser1753Arg, and p.Asp68His) would decrease hydrogen bonds.

Conclusion This is the largest genetic analysis of a single cohort of FV deficiency in Chinese. The study demonstrated that FV levels tended to be correlated with the probability of hemorrhage. The identification of a large number of unique FV-deficient pedigrees highlighted the screening for mutations in *F5*.

Keywords
- factor V deficiency
- sequencing
- mutation profile
- haplotype
- hemorrhage

Introduction

Coagulation factor V (FV) is a large single-chain glycoprotein (330 kDa) synthesized principally by the liver. FV is present in two places, 80% circulating in plasma and the other 20% stocking within the platelet α granules.[1] Plasma FV acts as a cofactor of activated protein C (APC) together with protein S (PS), exerting its anticoagulant effect of degrading activated coagulation factor VIII (FVIIIa).[2,3] In the presence of thrombin and activated coagulation factor X (FXa), FV is cleaved at

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arginine residues Arg709, Arg1018, and Arg1545 and transformed into activated FV (FVa), which performs as a cofactor for FXa.[4] Then, under the mediating effect of Ca^{2+}, FVa combines with FXa on negatively charged phospholipids to generate the prothrombinase complex, which is pivotal in accelerating prothrombin activation and thrombin generation.[5,6] The form of FVa is thoroughly stable, but APC could exert its proteolytic activity to inactivate FVa completely at three arginine residues (Arg506, Arg306, and Arg679) in sequence. In recent years, it has been demonstrated that a physiological subtype of FV (FV short) functions as a carrier and cofactor for the tissue factor pathway inhibitor (TFPI) in the inhibition of FXa.[1,7]

The matching gene coding for human *F5* gene maps to the long arm of chromosome 1 (1q24.2) and spans 74.5 kb. It consists of 25 exons and transcribed into a 7-kb mRNA that translates into FV zymogen (2,224 residues). The mature FV protein composed of 2,196 amino acids is created after modifications and processing in the endoplasmic reticulum and Golgi apparatus.[8] The single-chain protein comprises six domains arranged sequentially from the N-terminal to the C-terminal in the form of A1–A2–B–A3–C1–C2. The B domain is excised during activation of FV, making FVa yield a heavy chain (A1 and A2) and a light chain (A3, C1, and C2). Moreover, the B domain is not highly conserved in all researched species. This seems to explain the low sequence identity of the B domain of FV with that of FVIII.[9]

FV deficiency is an autosomal recessive hemorrhagic disorder. The first FV-deficient patient was identified in 1947,[10] whereas the first FV deficiency caused by a genetic mutation was not discovered until more than 50 years later.[11] One of the reasons for this is its extremely low prevalence, about 1:1 million. Patients with FV deficiency exhibit a variety of clinical features, ranging from asymptomatic cases to severe bleeding. Studies of patients with bleeding manifestations have found that the most common clinical sign is mucosal bleeding (in particular epistaxis and oral cavity bleeding), followed by abnormal postoperative bleeding and posttraumatic bleeding. In addition, central nervous system (CNS) bleeding, gastrointestinal (GI) bleeding, and recurrent miscarriages are quite rare.[12]

Hereditary FV deficiency has been well reported in Chinese. In this study, we recorded 10 mutations (6 novel mutations) from 14 unrelated probands with FV deficiency. This work aims to investigate the clinical characterization and molecular basis of this genetic disease.

Materials and Methods

Patients

A total of 14 unrelated index patients were under investigation. To set up a laboratory reference range and exclude the polymorphism, 100 healthy individuals were randomly selected as controls, 54 males and 46 females, with no liver or kidney disease and history of hemorrhage or thrombosis. Bleeding scores of 14 patients were calculated according to ISTH-BAT (https://practical-haemostasis.com), a bleeding assessment tool which was introduced by Rodeghiero et al.[13] For adult men, adult women, and children (<18 years old), the normal ranges for bleeding score are 0 to 3, 0 to 5, and 0 to 2, respectively. The FV levels were measured before transfusion. Other platelet or coagulation defects were excluded. Patients 1, 3, 4, 8, 9, and 14 and their families did not have any bleeding symptoms and all were detected with FV defects during routine preoperative coagulation screening. Patient 6 had no bleeding symptoms in general, but her sister had a hemorrhage during a cesarean section more than 10 years ago, and after resuscitation and blood transfusion, the pregnancy proceeded successfully. His parents were close relatives of aunt and uncle.

Patient 2 was a 35-year-old woman who was admitted to our hospital with recurrent epistaxis for several years, which worsened for 3 months. There was no history of bleeding in her family members.

Patient 5 was a 59-year-old male who presented with incessant bleeding after injuries during childhood. None of the family members had a bleeding tendency, except for bruising in the proband's sister. The parents of the proband were consanguineously married.

Patient 7, a 64-year-old male with a traumatic injury that resulted in a left temporal scalp abrasion and the formation of a hematoma, was diagnosed with FV deficiency after coagulation testing and genetic testing; he was treated with fresh frozen plasma (FFP) and his symptoms improved. The parents of the proband were consanguineous.

Patient 10 was a 49-year-old male who was admitted to our hospital with the chief complaint of recurrent gingival bleeding for more than 3 months. He was diagnosed with FV deficiency after performing coagulation tests and was treated with FFP. The parents of the proband were consanguineous.

Patient 11 was a 47-year-old woman who was found to have abnormal coagulation and a severe deficiency in FV levels on routine coagulation testing prior to thyroid surgery. The patient underwent thyroidectomy after prophylactic transfusion of FFP. After surgery, active bleeding developed in the inferior thyroid vein. No bleeding tendency was reported for the proband's relatives.

Patient 12, a 16-year-old girl, presented with mild bleeding incessantly after the surgery for intraocular nodules, and the diagnosis of hereditary FV deficiency was confirmed after performing coagulation tests and genetic analysis. None of the family members had a history of hemorrhage.

Patient 13 was a 34-year-old woman who was admitted to the hospital with menopause for more than 3 months with lower abdominal constriction and was diagnosed with preterm miscarriage and treated with fetal preservation. Due to abnormal postpartum vaginal bleeding, the patient received an FFP transfusion, after which the patient's bleeding decreased. She was diagnosed with FV deficiency due to decreased FV activity (FV:C) in coagulation test. No other members of the family had a bleeding tendency.

Informed consent was received from all participants and our studies were approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).

Peripheral Blood Collection and DNA Extraction

Peripheral venous blood samples from 14 probands and 100 healthy controls were collected in the sodium citrate anticoagulant tubes. Upper plasma for coagulation tests was obtained by centrifugation at 1,500*g* for 15 minutes, and human genomic DNA was extracted from lower blood cells by using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) according to the instruction of manufacturer.

Coagulation Assays

Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, factor II activity, factor VII activity, FVIII activity (FVIII:C), FX activity, and FV:C were detected on the STAGO STA-R-Max automatic blood analyzer (Diagnostica Stago, Asnieres sur Seine, France) using a commercial kit by the one-stage clotting method. The FV antigen (FV:Ag) was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Changfeng, Wenzhou, China).

Polymerase Chain Reaction Amplifications and Gene Analysis

F5 gene, including all 25 exons and exon–intron boundaries, was amplified on a thermal cycler (ABI Thermal Cycler 2720; ABI, Foster City, California, United States). The procedure and materials of PCR were performed as previously described.[14] The products of amplification were identified and sequenced by Sunsoon Bio-Technology Corporation (Shanghai, China). Primers for PCR were designed following the *F5* sequence (accession number: NM_000130.5).

In Silico Analysis

Two online bioinformatics softwares, including Mutation Taster (http://www.muta tiontaster.org/) and PROVEAN (http://provean.jcvi.org/index.php), were used as an electronic tool for predicting the potential impacts of amino acid substitutions on the function and structure of human FV protein. I-Mutant 3.0 analysis was performed to evaluate the stability change upon single site mutations starting from the protein structure at temperature 25 °C and pH 7.0. The PyMOL software was applied to analyze and predict changes in the three-dimensional spatial structure of the FV protein caused by amino acid variations. The changes in the internal structure of the protein could be clearly observed with this tool. The FV protein crystal structure data could be obtained from Protein Data Bank (PDB; https://www.rcsb.org/, PDB ID: 1FV 4).

Results

Phenotype of 14 Patients

In general, all 14 Chinese patients had considerably lower plasma FV levels (►Table 1). According to FV:C,[15] there were five patients with mild deficiency (>10%), eight patients with moderate deficiency (1%-10%), and only one patient with severe deficiency (<1%). The FVIII:C was within the normal range in all patients, hence excluding the presence of combined FV + VIII deficiency. Eight of the patients presented with various bleeding symptoms, including bruising, bleeding after surgery, postpartum hemorrhage, posttraumatic bleeding, hematoma, epistaxis, oral cavity bleeding, GI bleeding, and CNS bleeding. The bleeding scores varied from 1 to 5. The bleeding symptoms of four patients (patients 6, 10, 11, and 13) improved after the treatment with the FFP. Except for the eight patients mentioned earlier, the remaining six patients did not show any evidence of bleeding.

Genotype of 14 Patients

In total, ten disease-causing genetic variants of which six mutations were identified for the first time. The genetic mutations were dispersed in domains of the protein encoded by the *F5* gene (►Fig. 1). All six missense mutations identified were distributed in the A domain (three in A1, two in A3, and one in A2), two of the three frame shift mutations were in the B domain and one in the A3 domain, and the only nonsense mutation was located in the C1 domain. Among the ten mutants, half were recurrent mutations, of which Phe190Ser was identified in five unrelated pedigrees, and four variations (p.Gly276Glu, p.Gly392Cys, p.Pro770-LeufsX13, and p.Glu1572LysfsX19) were also found twice. Amino acid positions were displayed in the absence of the signal peptide.

Haplotype Analysis

In addition to the mutations described in this report, all patients exhibited a variety of single nucleotide polymorphisms (SNPs). A total of 20 SNPs were identified through the analysis of patients with recurrent mutations, of which a number were clustered in the B domain encoded by exon 13 (►Table 2). The c.3809C > A in exon 13 was a newly identified polymorphism that resulted in a mutation from alanine to aspartic acid at the 1242 site of the mature FV protein. Except for c.3809C > A, all other SNPs could be matched in ExAC (https://ngdc.cncb.ac.cn/) and 1,000 genomes (https://www.internationalgenome.org/). As shown in ►Table 2, the three recurrent mutations (p.Phe190Ser, p.Gly276Glu, and p.Pro770LeufsX13) shared almost the same haplotype, indicating a common origin of the mutations rather than a hotspot mutation. The data of proband 12 and 13 were not shown.

In Silico Analysis of Missense Mutations

Six missense mutations were identified, including p.Asp68His, p.Phe190Ser, p.Gly276Glu, p.Gly392Cys, p.Gly1715Ser, and p.Ser1753Arg, which were all predicted to "disease causing" and "damaging" according to Mutation Taster and PROVEAN (►Table 3). I-Mutant 3.0 predicted that all mutations resulted in "large decrease" in FV protein stability, except the p.Ser1753Arg (neutral). ►Fig. 2 portrayed the modification of the local spatial structure of FV protein before and after the mutation. Three missense mutations (p.Gly1715Ser, p.Ser1753Arg, and p.Asp68His) would weaken the local structural stability by reducing the hydrogen bonds formed with other amino acid residues. The remaining three (p.Phe190Ser, p.Gly276Glu, and p.Gly392Cys) would affect the function of FV by altering the local protein conformation.

Table 1 Phenotype and genotype of 14 unrelated Chinese patients with factor V deficiency

Patient	Gender/ Age	PT (s)	APTT (s)	FV:C (%)	FV:Ag (%)	Bleeding manifestation	Bleeding score	Nucleotide change	Mature protein alteration	Exon/ Domain	Genotype
1	F/36	15.2	41.8	55	62	N	0	c.911G > A	Gly276Glu	E6/A1	Heterozygote
2	F/35	17.9	46.9	24	28	E	2	c.2393delC	Pro770LeufsX13[a]	E13/B	Heterozygote
3	M/44	20.3	59.2	13	17	N	0	c.2851delT	Ser923LeufsX8[a]	E13/B	Heterozygote
4	M/56	18.3	44.9	26	20.3	N	0	c.6175C > T	Gln2031Ter[a]	E22/C1	Heterozygote
5	M/59	22.0	60.6	7	3.4	PTB	2	c.653T > C	Phe190Ser	E5/A1	Homozygote
6	M/50	23.5	50.5	8	<1	N	0	c.653T > C	Phe190Ser	E5/A1	Homozygote
7	M/64	25.7	99.8	8	7	H	3	c.653T > C	Phe190Ser	E5/A1	Homozygote
8	F/66	29.6	79.2	4	5	N	0	c.653T > C	Phe190Ser	E5/A1	Homozygote
9	F/29	20.5	50.7	11	12	N	0	c.653T > C	Phe190Ser	E5/A1	Homozygote
10	M/49	26.3	73.5	3	7	OCB	1	c.5227G > A	Gly1715Ser[a]	E16/A3	Homozygote
11	F/47	28.2	78.6	3	8.7	BS	3	c.286G > C c.2393delC	Asp68His Pro770LeufsX13	E3/A1 E13/B	Compound heterozygote
12	F/16	42.8	126.6	<1	1.3	BS	3	c.1258G > T c.4797delG	Gly392Cys[a] Glu1572LysfsX19[a]	E8/A2 E14/A3	Compound heterozygote
13	F/34	34.6	103.9	3	5	PPH	3	c.1258G > T c.4797delG	Gly392Cys Glu1572LysfsX19	E8/A2 E14/A3	Compound heterozygote
14	F/74	34.2	119.3	3	6	N	0	c.911G > A c.5343C > G	Gly276Glu Ser1753Arg[a]	E6/A1 E16/A3	Compound heterozygote
Reference range		11.3–14.5 s	27.0–41.0 s	72–133%	70–140%						

Abbreviations: APTT, activated partial thromboplastin time; BS, bleeding after surgery; E, epistaxis; FV:Ag, factor V antigen; FV:C, factor V activity; H, hematoma; N, no bleeding; OCB, oral cavity bleeding; PPH, postpartum hemorrhage; PT, prothrombin time; PTB, posttraumatic bleeding.
[a]Novel mutation.

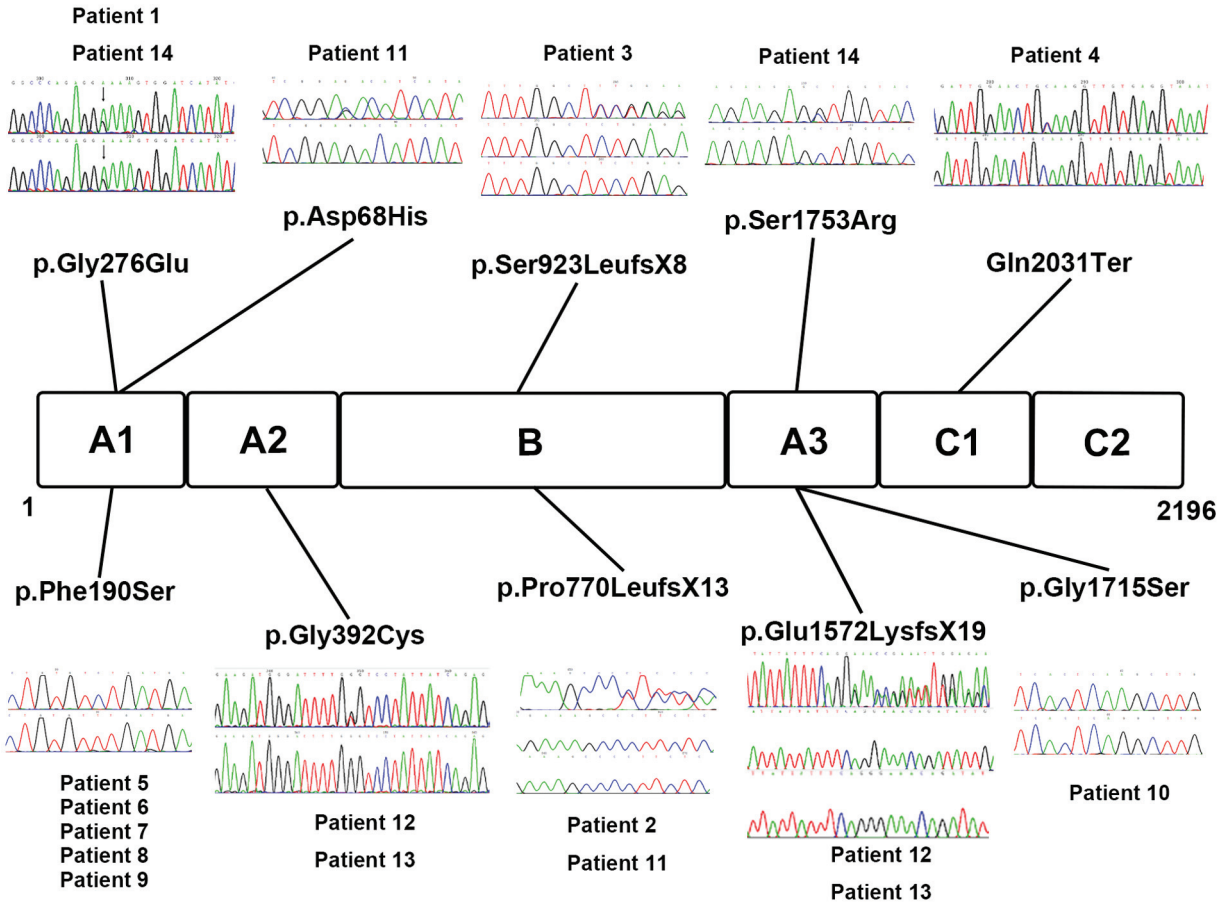

Fig. 1 Ten mutations identified in 14 unrelated patients. The sequencing results and mutation distribution were shown. Six missense mutations and one nonsense mutation were demonstrated in sequencing chromatograms (upper panels) and corresponding wild-type chromatograms (lower panels). Clone sequencing was introduced in three frame shift mutations (middle panels). Domains (approximately to scale) were indicated by boxes.

PTC Mutation

The three frame shift mutations (p.Glu1572LysfsX19, p. Pro770LeufsX13, and p.Ser923LeufsX8) and one nonsense mutation (p.Gln2031Ter) were further defined as premature termination codons (PTC) mutations, which would lead to the generation of a termination signal preceding the natural termination codon, resulting in the degradation of corresponding mRNA transcript by nonsense-mediated mRNA decay (NMD) and/or the production of truncated proteins. All PTC mutations (PTCs) were predicted to "disease causing" according to Mutation Taster.

Discussion

As a rare hemorrhagic genetic disorder, FV deficiency can be classified into two types.[16] Type I disease is characterized by consistently low levels of FV:C and FV:Ag. Differently, type II presents reduced FV:C and normal FV:Ag. In this study, the means of FV:C and FV:Ag were 12.07 and 13.12%, respectively, in 14 patients with type I defects. The 14 patients all had normal bleeding scores, even though FV:C was not detectable, and bleeding scores were only 3. These are indicative of the fact that clinical symptoms in patients with FV defects tend to be mild. FV is a variable factor and FV deficiency is often considered to have a poor correlation between the level of coagulation activity and the severity of bleeding. Explicitly, one reason seems to be associated with the existence of platelet FV and low levels of TFPI.[17] In our cohort data, of the five patients with mild FV deficiency (FV:C > 10%), only one had minor bleeding symptoms. In contrast, of the remaining eight patients with moderate FV deficiency and one with severe FV deficiency, six showed various bleeding manifestations. This phenomenon demonstrated that FV levels tended to be correlated with the probability of hemorrhage, although it may be limited by the size of the model. Therefore, those patients with severe FV deficiency deserve more attention and management, such as preventive treatment of FFP.

FV deficiency has been described throughout the world, but is more prevalent in countries where parental consanguinity is a common occurrence.[18] The proportion of homozygotes (43%) in our cohort also illustrated the high prevalence of FV deficiency in China. Mutations in the heterozygous state will tend to reduce plasma FV levels by about half. The lower FV:C of patients 2 to 4 (13–26%) can be explained by the known pathogenic SNPs such as the p.Asp2194Gly (rs6027) and the p.Arg485Lys (rs6020). Four patients were compound heterozygotes, which demonstrates high levels of allelic heterogeneity of the disease.

Table 2 Haplotype of probands in *F5* sequence

cDNA	Exon	Probands																	
		P1		P14		P5		P6		P7		P8		P9		P2		P11	
237A > G	E2	A	A	A	A	G	G	G	G	G	G	G	G	G	G	A	A	A	A
405G > A	E4	G	G	G	G	G	G	G	G	G	G	G	G	G	G	A	A	A	A
552G > T	E4	G	T	G	T	G	G	G	G	G	G	G	G	G	G	T	T	T	T
1238T > C	E8	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	C	T	C
1380C > T	E9	C	T	C	T	C	C	C	C	C	C	C	C	C	C	C	T	C	T
1538G > A	E10	G	G	G	G	A	A	A	A	A	A	A	A	A	A	G	G	G	G
1716G > A	E11	G	A	G	A	G	G	G	G	G	G	G	G	G	G	G	A	G	A
1926C > A	E12	C	A	C	A	C	C	C	C	C	C	C	C	C	C	C	A	C	A
2208C > T	E13	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	C	T
2235T > C	E13	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	C	T	C
2289A > G	E13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	G
2301A > G	E13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	G
2773A > G	E13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	G
2938C > T	E13	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	C	T
3804T > C	E13	T	T	T	T	T	T	T	T	T	T	T	T	T	T	C	C	C	C
3809C > A	E13	C	C	C	C	C	A	C	A	C	A	C	A	C	A	C	C	C	C
4189C > T	E13	C	C	C	C	T	T	T	T	T	C	T	T	T	T	C	C	C	C
5022A > G	E15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	G
5290A > G	E16	A	G	A	G	A	A	A	A	A	A	A	A	A	A	G	G	G	G
6665A > G	E25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	G

Note: The grey squares indicate the mutated bases.

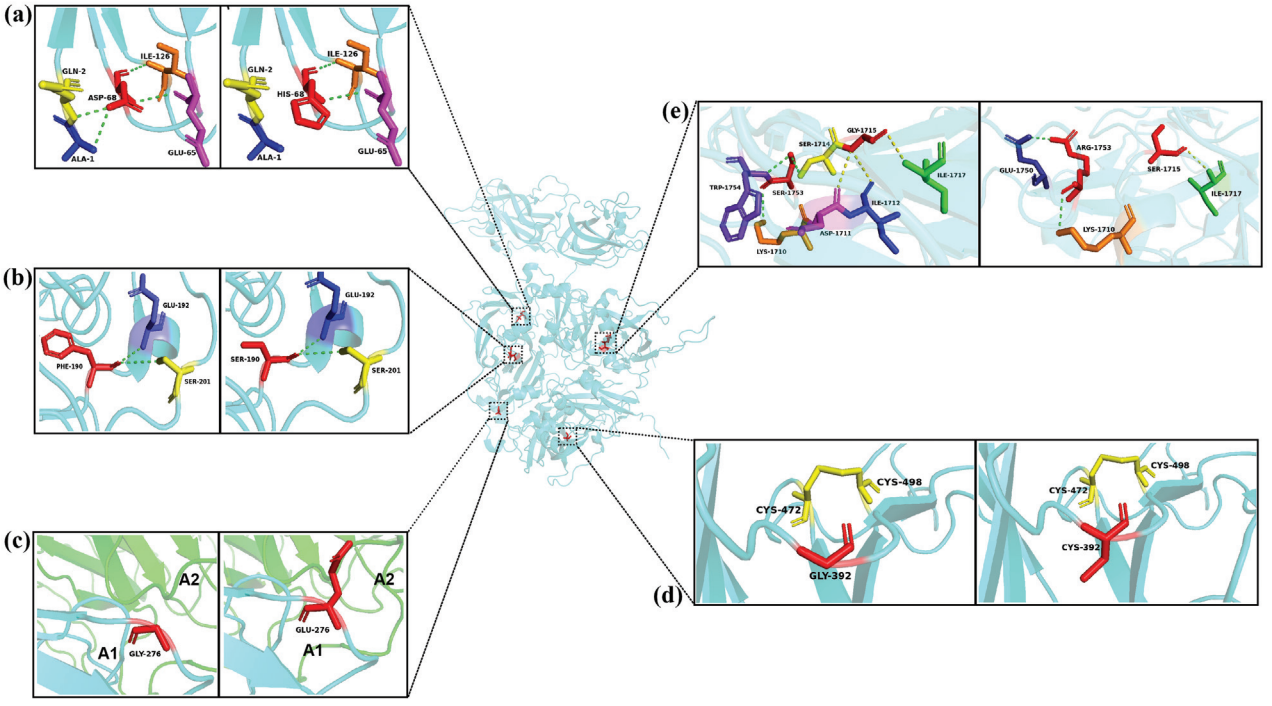

Fig. 2 Comparison of structural differences between FV wild-type and mutation-type model with the PyMOL software. Asp68His variant (**a**); Phe190Ser variant (**b**); Gly276Glu variant (**c**); Gly392Cys variant (**d**); Gly1715Ser; and Ser1753Arg variant (**e**). The wild-type model was presented first in each pairing. Each of the amino acid residues studied were shown with stick (red) and other residues were shown with cartoon (cyan). The hydrogen bonds between amino acid residues were shown as green or yellow dashed lines.

Table 3 Predictive results of the harmfulness of six missense mutations

	Mutation Taster	PROVEAN	Stability
Asp68His	Disease causing	Damaging	Large decrease
Phe190Ser	Disease causing	Damaging	Large decrease
Gly276Glu	Disease causing	Damaging	Large decrease
Gly392Cys	Disease causing	Damaging	Large decrease
Gly1715Ser	Disease causing	Damaging	Large decrease
Ser1753Arg	Disease causing	Damaging	Neutral

Our data for all the detected mutants were relatively consistent with the mutation profiles in the *F5* gene mutation database, especially the missense mutations, which both accounted for approximately 60%. Interestingly, all six missense mutations were clustered in the A domain, only two frame shift mutations located in the B domain. It is not hard to find that the frequency of mutations in B domain does not match its size. Moreover, all genetic alterations in the B domain were found to be alleles coding for null. According to the Human Gene Mutation Database (HGMD; https://www.hgmd.cf.ac.uk/docs/login.html), there are only seven missense mutations in the large B domain. Its tolerance to amino acid variants is confirmed by the high polymorphism and low interspecific conservation. In our opinion, the missense mutation in the B domain of the *F5* gene should be interpreted with caution to properly distinguish benign polymorphisms from pathogenic missense mutations.

The majority of mutants in this study were missense mutations. In general, these mutations cause a defect in secretion, which can lower plasma FV levels. For the six missense mutations we reported, online bioinformatics software revealed that they were all deleterious variants. The consistency of the results reflected the reliability of the online analysis, but other more definitive analyses were warranted before associating mutants with FV defects. The PyMOL software was utilized to simulate the impact of mutations on the FV protein and visualize any local changes. Glycine is highly hydrophilic but is a nonpolar amino acid. Half of these six missense mutations involved the small glycine residue, which would generate remarkable conformational variations. The mutation of glycine to glutamate, cysteine, or serine resulted in a longer side chain, which may disrupt the normal protein folding. Apart from this, the glycine392 residue in the A2 domain is conserved in FV and its homologous counterparts, FVIII and ceruloplasmin.[8] The cysteine introduced at position 392 may bridge these two cysteines or interfere with the formation of original disulfide bridge (Cys472–Cys498), thus interfering with the spatial folding of the FV protein (►**Fig. 2d**). Undoubtedly, the effects of such mutations were severe, as in patients 12 and 13 who were compound heterozygous with a PTC and both had bleeding manifestations. Protein helical arrangement enhances structural stability due to the presence of hydrogen bonds. Three missense mutations (p.Gly1715Ser, p.Ser1753-Arg, and p.Asp68His) would weaken the local structural stability by reducing the hydrogen bonds formed with other amino acid residues. However, I-Mutant 3.0 predicted the p.Ser1753Arg would not result in decreased spatial structural stability of the FV, which may explain the asymptomatic state of patient 14.

Among the 10 identified mutations, the p.Phe190Ser exhibited the highest mutant frequency. The p.Phe190Ser was detected in five patients, who all presented in the homozygous state. The average FV:C was 7.6%, which was moderate FV deficiency. Even though their FV defects were caused by the same pathogenic mutation, only two patients presented bleeding symptoms, suggesting a poor association between clinical symptoms of FV deficiency and the location of the mutation. Kanaji et al[19] revealed that the two probands with p.Phe190Ser did not share the same haplotype, and thus inferred that p.Phe190Ser may be a mutational hotspot. In our cohort, however, haplotype analysis revealed the founder effects. Due to the large size and high polymorphism ratio of the *F5* gene, most of the mutations causing FV deficiency are restricted to specific families and lack mutational hotspots.

In conclusion, we described the molecular basis of 14 unrelated FV-defect Chinese patients. To the best of our knowledge, this is the largest single cohort genetic analysis of FV deficiency in the Chinese population. The clinical manifestations and identified mutations were generally consistent with the previously identified bleeding symptoms and mutation spectrum of the disease. There is a strong necessity for molecular genetic analysis of individuals with FV levels below the normal range and for measures to prevent abnormal bleeding after injury or surgery. The molecular mechanisms of pathogenesis of the newly identified mutations still require further investigation.

Statement of Ethics

Our study was approved by the Ethics Committee in Clinical Research (ECCR) of the First Affiliated Hospital of Wenzhou Medical University (number: KY2022-R193). All participants signed the written informed consent.

Data Availability Statement

The data that support the conclusions of this study are available from the corresponding author upon request. Further inquiries can be directed to the corresponding author.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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