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# Clinical Phenotype and Genetic Analysis of Twins With Congenital Coagulation Factor V Deficiency

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**Objective:** The aim was to investigate the clinical characteristics and molecular pathogenic mechanism of twins with congenital factor V (FV) deficiency.

**Methods:** We comprehensively analyzed the clinical manifestations and laboratory test results of a set of twins and their parents and performed point mutation analysis with direct high-throughput exon sequencing.

**Results:** The prothrombin time and activated partial thromboplastin time were prolonged for both probands, and the FV activity levels were 13.0% and 9.8%. Next-generation sequencing showed that the affected individuals harbored a paternal c.5113A > C (p.S1705R) and a maternal c.4949C > T (p.A1650V) heterozygous variants in the *FV* gene, which conformed to an autosomal recessive inheritance pattern. This is the first report of these point mutations. The older boy also had a congenital patent foramen ovale.

**Conclusion:** In this set of twins, missense mutations of the *FV* gene were related to congenital FV deficiency but unrelated to the patent foramen ovale observed in the older boy.

**Key Words:** coagulation factor V deficiency, *FV* gene, gene mutation

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Factor V deficiency (FVD) (OMIM 227 400), an autosomal recessive disorder, is a rare hereditary coagulation disorder related to factor V (FV) mutations with a prevalence of one per one million individuals.[1–3] The *FV* gene is located on human chromosome 1q23 and contains 25 exons and 24 introns, and its messenger RNA (mRNA) has 6914 base pairs that encode FV, an inactive protein of 2224 amino acids (OMIM 612309). FV, a high-molecular-weight glycoprotein (330 kDa), consists of 6 domains (A1, A2, B, A3, C1, and C2) and is involved in coagulation by regulating the formation of thrombin.[4–6] Clinical manifestations of FVD vary among patients, ranging from mild epistaxis, bruises, and increased menstrual flow to severe spontaneous bleeding of the digestive tract, skin, and mucous membranes, as well as bleeding problems after injury or surgery.[5] To date, few cases of *FV* genetic variation have been reported domestically or abroad. Mutations are classified according to the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines with reference to the Human Gene Mutation Database (HGMD) and database of single nucleotide polymorphisms (dbSNP) public databases,[7] and 159 mutation sites have been found to be related to FVD as of this manuscript's submission. In this study, we used targeted capture and high-throughput next-generation sequencing (NGS) to analyze the genetic variations in a set of twins and their parents with FVD to provide a basis for genetic counseling of the family.

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Y.W. participated in the entire process of research, data collection, and operation log recording. Y.H. analyzed and evaluated the treatment and curative effects. The supervisor, Professor X.G., guided the entire process in terms of theory and practice and revised the manuscript. Y.W. wrote the first draft of the paper, and the remaining authors jointly revised the paper.

This study was approved by the Ethics Committee of Xinxiang Medical University affiliated with Puyang Oilfield General Hospital (review number: 2020-08-0006-E05) and was performed with the consent of the patients and their family members. The authors obtained consent from the participants to publish individual patient data.

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## CASE DESCRIPTION

### Patients

Ten-year-old male twins visited the Department of Hematology of our hospital because of recurrent, multiple, scattered bruises on the skin of the limbs and intermittent nasal bleeding. The maximum range of ecchymosis was 94×43 mm, and the smallest was 23×16 mm. The parents were unaffected. Laboratory tests showed that the activated partial thromboplastin time (APTT) and prothrombin time (PT) were significantly prolonged; fibrinogen, thrombin time, and the international normalized ratio were normal; and the activities of factors V, VII, and XII were low. A physical examination showed that the twins were conscious, without additional signs of bleeding, such as vomiting blood or blood in stools. All other tests were normal. Color ultrasound B examination of the heart, liver, gallbladder, spleen, pancreas, and kidneys showed congenital patent foramen ovale in the older boy but no abnormal findings in the younger boy. The parents of the probands were not in a consanguineous marriage and had normal laboratory test results and no history of bleeding or thrombosis.

### Methods

#### Sample Collection

After obtaining informed consent and signatures from the probands' guardian and their parents, 2 peripheral blood samples (3 to 5 mL for each sample) were collected in ethylenediaminetetraacetic acid-treated tubes from each proband and parent; one sample was centrifuged to collect the supernatant for routine coagulation tests, and the other sample was used to extract DNA for polymerase chain reaction (PCR) analysis.

#### Genetic Analysis

A blood sample (3 to 5 mL) from each proband and their parents was sent to Guangzhou KingMed Diagnostics (Guangzhou, China) for targeted genetic testing for hematology with high-throughput sequencing (Illumina, USA), with coverage of 99% or higher for targeted sequences. Candidate gene variation sites were screened and

**TABLE 1.** Laboratory Tests in the Probands and Pedigree Members With Hereditary FVD

| | APTT (s) | PT (s) | V:C (%) | VIII:C (%) | XII:C (%) | vWF:Ag (%) |
|---|---|---|---|---|---|---|
| Older boy | 55.40 | 16.00 | 9.80 | 35.60 | 46.00 | 89.20 |
| Younger boy | 49.60 | 15.30 | 13.00 | 38.20 | 47.60 | 92.90 |
| Mother | 31.50 | 12.30 | 63.20 | 53.50 | 80.20 | 108.20 |
| Father | 32.20 | 11.70 | 64.60 | 63.20 | 91.30 | 103.20 |
| Reference range | 27-45 | 11-14 | 62-139 | 50-150 | 50-150 | 94.10 ± 32.50 |

APTT indicates activated partial thromboplastin time; FVD, factor V deficiency; PT, prothrombin time.

verified by Sanger sequencing. The primers were designed based on the following variant sites: *FV* exon 14: forward primer TGTAAAACGACGGCCAGTCAGGGAAACAGATATTGAAGACTC and reverse primer CAGGAAACAGCTATGACCACCTGCACCTTTACAACCC; and *FV* exon 15: forward primer TGTAAAACGACGGCCAGTGATAAATGCAGACTGTTAACCACAC and reverse primer CAGGAAACAGCTATGACCAGCATCCAGACCGTATTCTCTAC. The primers were synthesized by Guangzhou KingMed Diagnostics. The PCR (ABI 9700 PCR Instrument, USA) conditions were as follows: denaturing at 95°C for 10 minutes; 35 cycles of denaturing at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute; final extension at 72°C for 7 minutes; and then incubation at 25°C until use. The amplified products were directly sequenced with an ABI 3500 sequencer (USA).

### Changes in the Spatial Structure of the Protein

On the basis of the crystal structure model from PubMed (pdb: 1FV4), the three-dimensional structure of the FV protein with c.4949C > T (p.A1650V) and c.5113A > C (p.S1705R) genetic variants was predicted with SWISS-MODEL, a protein structure homology modeling server.

## RESULTS

### Laboratory Tests and Clinical Manifestations

Laboratory tests for routine blood coagulation and coagulation factor activities of all pedigree members showed that the PT and APTT were prolonged in the twins (16.00 and 55.40 s for the older twin; 15.30 and 49.60 s for the younger twin) and were normal in both parents. For the set of twins, the relative activity of FV (V:C) was decreased to 9.8% and 13.00%; for factor VIII, (VIII:C) activity was decreased to 35.60% and 38.20%; and for factor XII, (XII:C) activity was decreased to 46.00% and 47.60%. All other coagulation factors were normal. The parents had normal results (Table 1).

### Sanger Sequencing

Sanger sequencing revealed that the twins harbored a c.4949C > T (p.A1650V) missense mutation in *FV* exon 14 and a c.5113A > C (p.S1705R) missense mutation in *FV* exon 15, which is consistent with the NGS results (Fig. 1). Pedigree verification revealed maternal exon 14 mutation and paternal exon 15 mutations, indicating autosomal recessive inheritance. A review of several databases revealed that c.4949C > T (p.A1650V) was included only in the dbSNP database with "unknown" pathogenicity and had not been reported in any other database, such as HGMD, the exome variant server ESP-6500, and the 1000 Genomes Project; c.5113A > C (p.1705R) had not been reported in any of these databases.

### Analysis of the Biological Characteristics of the Protein

Both twins harbored both mutation sites, 1 maternal and 1 paternal. The paternal mutation, c.5113A > C, alters FV residue 1750. We then used SWISS-MODEL to predict the structure of the FV variant S1705R. Figure 2 shows that residue 1750 was changed from serine (Ser) to arginine (Arg), with a significant impact on the conformation of surrounding amino acid residues mainly because of the increased volume of the Arg side chain. Moreover, Arg has a different charge and can form more hydrogen bonds and electrostatic interactions with surrounding residues. Figure 2 shows that the change from Ser1705 to Arg1705 enhances hydrogen bonding between Ser1626 and Arg1659, with certain effects on the conformation of surrounding residues. However, the maternal mutation, c.4949C > T, alters FV residue 1650. Figure 3 shows that residue 1650 was changed from alanine (Ala) to valine (Val), with minimal effects on the conformation of surrounding amino acid residues. However, the larger side chain allows Val1650 to form hydrophobic interactions with more surrounding amino acid residues. The combination of the 2 mutations further aggravated the change in protein conformation.

## DISCUSSION

FV was discovered by Paul Owren in 1943.[8] FV is encoded by the *FV* gene, located on chromosome 1q23, and consists of 25 exons and 24 introns.[9] The *FV* gene is related to the multicopper oxidase family and is highly homologous to factor VIII and ceruloplasmin. FV is primarily produced in the liver as a proprotein with 2240 amino acids, which is then preprocessed and secreted as a single-stranded glycoprotein with 2196 amino acids (molecular weight ≈33,000). FV has domains A1, A2, B, A3, C1, and C2 and is circulated in the blood as a large single-stranded cofactor.[10] In FV domain B, proteolysis at Arg709, Arg1018, and Arg1545 simultaneously removes the inhibitory B domain and forms the heterodimer cofactor Va.[11] Factor Va consists of a heavy chain containing domain A1-A2 (molecular

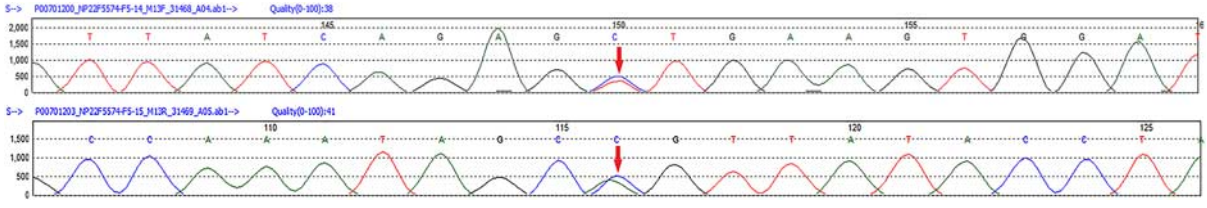

**FIGURE 1.** Schematic diagram of the patient mutation site in Fasta format as shown by next-generation sequencing.

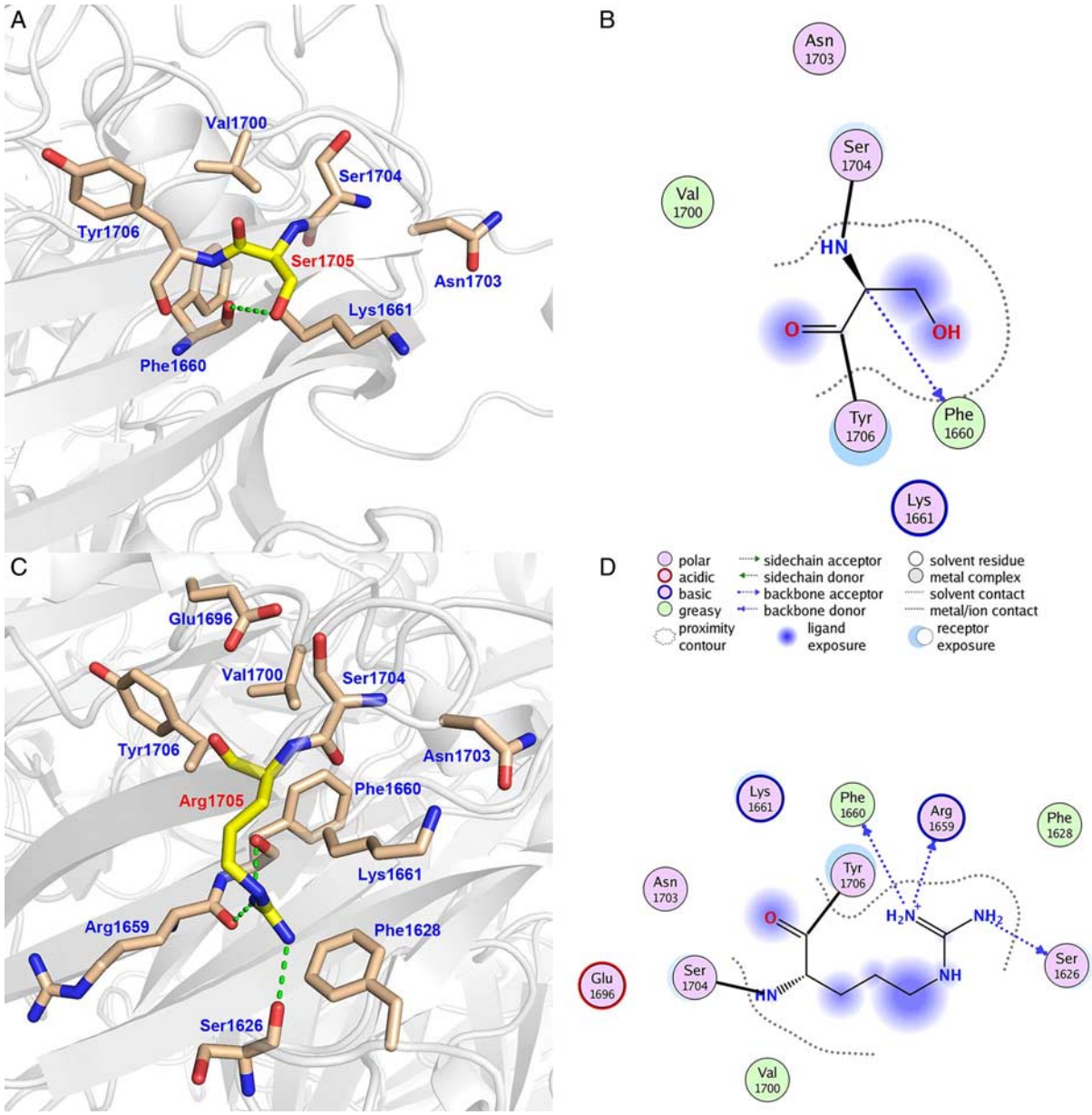


**FIGURE 2.** Interaction between residue 1705 and surrounding residues in wild-type FV (A and B) and the S1705R variant (C and D).

weight ≈105,000) noncovalently bound to a light chain containing domain A3-C1-C2 (molecular weight ≈74,000) through calcium ions.[12] The amino acid sequence of the light chain is partially homologous to the carboxyl terminal sequence of human factor VIII. The factor Va level is ~20 nM.[13–15] Factor Va acts as a cofactor during coagulation to substantially improve the catalytic efficiency of large enzyme complexes, thereby playing an important role in the rapid formation of thrombin. Congenital FVD is usually related to *FV* gene defects that lead to loss of function of FV. The condition is characterized by autosomal recessive inheritance and was first discovered in 1954 by Kingsley[16] in a consanguineous pedigree. Clinically relevant nonsense, frameshift, missense, and splice site mutations of the *FV* gene have been described. The serum FV level is usually less than 10% to 20% in affected individuals with homozygous or compound heterozygous mutations and less than 50% in heterozygous individuals with mild or moderate FVD.[17] FV activity is also abnormal. Researchers are still debating the relationship between FV activity and the severity of bleeding.[4] Studies have shown that FV activity > 15% is generally observed in asymptomatic individuals or individuals with mild bleeding,[17] while FV activity <10% is associated with varying clinical manifestations and varying severity of bleeding.[18] Kingsley[16] examined 2 pedigrees and found that heterozygous individuals were asymptomatic when FV activity was 24% to 68%. Other studies note that the severity of bleeding varies in patients with the same mutation or FV activity, including those with the same genotype and low FV activity (< 1%).[19] In addition, studies show that in most cases, FVD manifests as skin and mucous membrane bleeding rather than joint bleeding, as observed

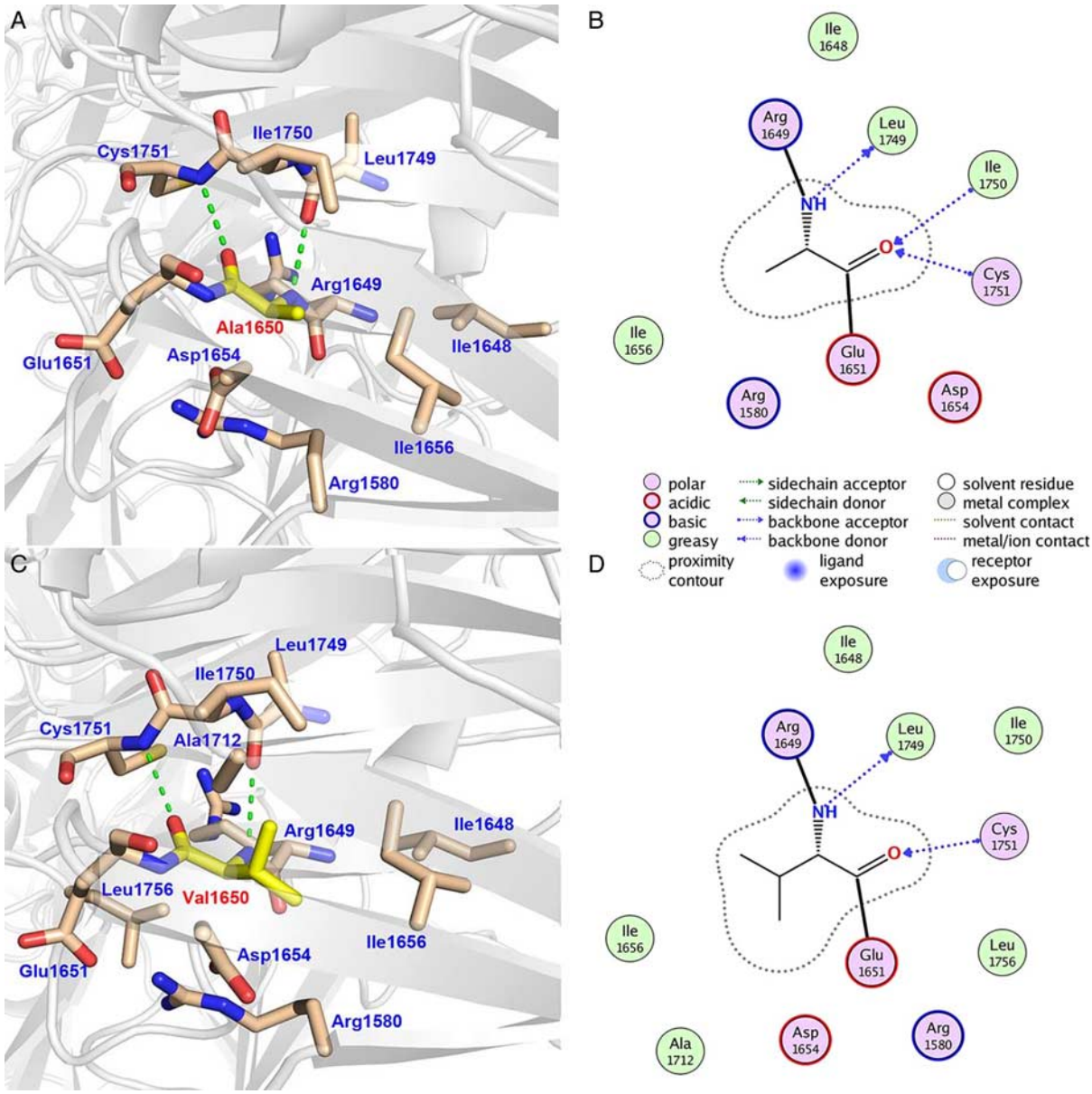


**FIGURE 3.** Interaction between residue 1650 and surrounding residues in wild-type FV (A and B) and the A1650V variant (C and D).

in hemophilia patients.[4] FVD can occur in individuals of any race, with a slightly higher prevalence in Middle Eastern Jews and non-Jewish Iranians, especially those from consanguineous marriages.

In summary, a set of twins had FVD with low activity levels of factors V, VIII, and XII, and genetic analysis revealed *FV* mutations. The current research suggests that hereditary FVD is generally caused by *FV* gene defects, which is in line with our research results. If a combined deficiency of FV and factor VIII is present, the condition could be caused by a disordered mutation of the endoplasmic reticulum-Golgi intermediate compartment gene *ERGIC-53*, which is also known as *LMAN1*. However, the condition may be caused by a mutation in the *MCFD2* gene, a cofactor of the LMAN1 protein. The NGS results of the patients did not reveal the mutation site. If factor VIII deficiency is observed, the mutation site could be the long arm of the X chromosome (Xq-28) and inherited from the sex chromosome. In addition to the mutation site not matching these sites, the genetic characteristics described in this study did not match the mutant characteristics. Therefore, the possibility that the children had a combined FV and factor VIII deficiency and hemophilia A was excluded. NGS technology is currently commonly used for DNA sequence detection, but this technique may not provide direct evidence that can be used to diagnose the disease type. The reasons that FVD was considered were that, first, based on the activity of coagulation factors (FV was significantly decreased, while factor VIII was decreased to a lesser degree) and the patient's 4 coagulation results, the PT and APTT were both prolonged, which is often observed with severe deficiency of factors II, V, or X or combined deficiency of factors V and VIII. The other reasons included the genetic characteristics of the patient's family and finally the

patients' second-generation sequencing results. We analyzed the reasons for the reduction in factor VIII activity. Because FV and factor VIII are both coagulation cofactors, it cannot be ruled out that the drastic reduction in FV affected the content of factor VIII in the coagulation cascade. The twins suffered from the disease at the same time, and both had the exon 14 c.4949C > T (p.A1650V) and the exon 15 c.5113A > C (p.S1705R) missense mutations on the *FV* gene. Analysis of specific mutation sites revealed that both variants are located in FV domain A3, an integral part of the light chain (A 3-C1-C2). Mutations in domain A3 alter the protein conformation and inactivate FV. Bioinformatics software analysis is more likely to detect the cause of the disease, and the probands had an abnormal phenotype. The maternal variation, c.4949C > T, is included in the dbSNP database, but the pathogenicity is unknown, especially since the mother is a carrier with a normal phenotype. Animal studies are needed to further investigate this mutation, especially given its low probability of being pathogenic. Similarly, the paternal variation, c.5113A > C, has not been reported in any database, but the father has a normal phenotype. According to the ACMG guidelines, a single variant is classified as having "unknown clinical relevance." In this case, these 2 mutations are likely pathogenic, given the pedigree analysis and copresence of both mutations. In short, the pedigree reflects autosomal recessive inheritance with 2 heterozygous mutations that enrich the genetic mutation spectrum of hereditary FVD.

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