Factor V Deficiency with a Thrombotic Clinical Phenotype

Meera Sridharan, MD, PhD1,2  Lea M. Coon, MS, LCGC2 Dong Chen, MD, PhD2  Rajiv K. Pruthi, MBBS1,2

1Division of Hematology, Department of Internal Medicine, Mayo Clinic Rochester, Rochester, Minnesota
2Division of Hematopathology and Laboratory Genetics, Department of Laboratory Medicine and Pathology, Mayo Clinic Rochester, Rochester, Minnesota

Address for correspondence Rajiv K. Pruthi, MBBS, Division of Hematology, Department of Internal Medicine, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905 (e-mail: pruthi.rajiv@mayo.edu).


Coagulation factor V (FV) is a large 330-kD glycoprotein mainly synthesized in the liver and megakaryocytes. Eighty percent of blood FV circulates in plasma while the remaining 20% is stored within platelet α granules.1 In its active form (FVa), it is an essential cofactor for factor Xa, assisting the conversion of prothrombin to thrombin. The procoagulant activity of FVa is regulated by activated protein C (APC), which cleaves multiple peptide bonds in FVa. Cleavage of FV by APC at residue p.Arg534 (“Arg506” in legacy nomenclature) converts FV into an anticoagulant protein (FVac) that lacks procoagulant activity and serves as a cofactor to APC, which in synergy with protein S mediates degradation of FVIIIa.1 FV is also a carrier of, and cofactor to, the coagulation inhibitor tissue factor pathway inhibitor.2,3 Defects in FV may therefore result in either hemorrhagic or thrombotic phenotypes. The FV Leiden mutation (“Arg506Gln” in legacy nomenclature; p.Arg534Gln in NM_000506.4) is the most common defect in FV leading to a thrombotic phenotype, while severe FV deficiency, an autosomal recessive condition due to point mutations in the FV gene (F5), can be associated with mild or severe bleeding.4

Though defects in FV may cause either bleeding or thrombosis, it is rare for FV deficiency per se to predispose to thrombosis. Here, we present a case of an individual with recurrent episodes of thrombosis who was found to have FV deficiency. This 33-year-old white male, who had previously undergone hemostatically uneventful hypospadias repair and pilonidal cyst removal, experienced two episodes (3 and 1 years prior to current presentation) of superficial phlebitis of the right lower extremity which were managed conservatively (anti-inflammatory agents and compression stockings). He presented to our institution for management of bilateral segmental and right lobar pulmonary emboli (PE; documented on CT angiography) and soleal vein thrombosis. The thrombosis was felt to be provoked by ankle trauma that occurred 1 month prior to the thrombosis. Management consisted of initial enoxaparin, with transition to rivaroxaban for 3 months (after which CT angiography demonstrated resolution of PE), and then aspirin 81 mg daily was initiated.

While on enoxaparin, thrombophilia testing demonstrated normal activities of antithrombin and protein C, as well as normal free protein S antigen. Activated protein C resistance ratio (APC-R), a ratio calculated based on APTT obtained with and without added APC (both diluted in FV-deficient plasma), was indeterminate, but he did not have the factor V Leiden mutation. He also did not have the prothrombin “G20210A” thrombosis-susceptibility variant in F2, NM_000506.4: c.97G > A. Follow-up testing off anticoagulation demonstrated a slightly prolonged prothrombin time (PT) of 13.7 seconds (s) (reference range: 10.3–12.8 seconds) and activated partial thromboplastin time (APTT) of 42 seconds (reference range: 26–36 seconds). APTT corrected with equal volume mixing study with normal plasma. Reflexive testing demonstrated factor V activity (FV:C) of 20% (reference range: 70–165%).

Approximately 2 years later, he underwent a left arthroscopic shoulder capsular shift and labral repair with no hemostatic support, and no bleeding complications were reported. Two weeks later, evaluation of left arm pain documented presence of thrombosis in the brachial and cephalic veins. He received 3 months of rivaroxaban; follow-up upper extremity ultrasound demonstrated resolution of thrombus. Approximately 18 months later, he presented with left calf pain with swelling and shortness of breath. He was found to have an acute occlusive thrombus in the posterior tibial, peroneal, and soleal veins. He also had bilateral PE. Unlike the prior episodes of deep vein thrombosis (DVT) and PE, no provoking etiology could be identified. He was started on warfarin with plans for indefinite treatment.

Lupus anticoagulant was assessed at six different times during the patient’s clinical course, both on and off anticoagulation. STACLOT-LA was indeterminate when patient was on warfarin but negative when tested off warfarin.
Venous thromboembolism (VTE) is a major cause of morbidity and mortality worldwide and some individuals are found to have an underlying genetic predisposition to thrombophilia. VTE is the most common inherited cause of PE and 40% to 50% do not have the factor V Leiden (F5G20210A, variant of uncertain significance) mutation. No further molecular studies of the proband or his children.

One was heterozygous for FV Leiden and had a FV:C of 50% and PE thought to be related to oral contraception. She had a negative D-dimer test for VTE and was discharged. She delivered a healthy child vaginally and was discharged. She was lost to follow-up.

Of his two children, one was heterozygous for FV Leiden and had a FV:C of 46% and did not have the factor V Leiden mutation or prothrombin F12 mutation. No further molecular studies of the proband or his children.

FV Leiden pseudohomozygotes showed a pattern similar to pseudo-homozygous FV Leiden (c.1001G>A, p.Arg334Gly, FV Hong Kong, legacy nomenclature). Both mutations affect the same cleavage site responsible for complete loss of FVa procoagulant activity.

The causative role of c.4906G>A, p.Glu1636Lys in the setting of FV deficiency was felt to be due to pseudo-homozygosity of FV deficiency (Table 2). For example, one case of previously reported cases of heterozygosity of FV deficiency was felt to be due to pseudo-homozygosity.

In silico prediction Overall prediction

<table>
<thead>
<tr>
<th>Gene (chromosome)</th>
<th>cDNA alteration</th>
<th>Amino acid alteration</th>
<th>Intron or exon</th>
<th>MSCPP2 impact prediction</th>
<th>M5CSF2 impact prediction</th>
<th>Mutation taster</th>
<th>Align GVHD</th>
<th>Overall prediction</th>
<th>Classification via ACMG criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F5 Chr1(GRCh37)</td>
<td>c.6528 + 3A&gt;C</td>
<td>NA</td>
<td>Intron 24</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Loss of function (F5 deficiency)</td>
<td>VUS</td>
</tr>
<tr>
<td>2 F5 Chr1(GRCh37)</td>
<td>c.6322C&gt;T</td>
<td>p.Arg2108Cys</td>
<td>Exon 23</td>
<td>Probably damaging: score 1.00</td>
<td>Deleterious (score: 0.01, median: 0.35)</td>
<td>Disease causing (p-value: 0.988)</td>
<td>Class CO (GV: 353.86-GD: 0.00)</td>
<td>Gain of function, increased activation of procoagulant qualities</td>
<td>VUS</td>
</tr>
<tr>
<td>3 PROCR Chr20(GRCh37)</td>
<td>c.655A&gt;G</td>
<td>p.Ser219Gly</td>
<td>Exon 4</td>
<td>Benign: score 0.02</td>
<td>Tolerated (score 0.4)</td>
<td>Polymorphism (p-value: 0.998)</td>
<td>Class CO (GV:205.53-GD: 28.37)</td>
<td>Disease-associated polymorphism</td>
<td>VUS</td>
</tr>
<tr>
<td>4 FGG Chr4(GRCh37)</td>
<td>c.318C&gt;T</td>
<td>Synonymous substitution</td>
<td>Exon 4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Benign</td>
<td>Benign</td>
</tr>
</tbody>
</table>

Table 1 Next-generation sequencing results for our case

Abbreviations: ACMG, American College of Medical Genetics and Genomics; VUS, variant of uncertain significance.

Known risk factor for thrombosis, MAF: 8.4% overall per 1,000 genomes. Causes a missense mutation in the transmembrane domain. Based on a large random-effects meta-analysis, odds of venous thromboembolism increased by a factor of 1.22 (95% CI: 1.11–1.33, p < 0.001) for every additional copy of the G allele, but no evidence was found for an association with myocardial infarction (Dennis et al).
NGS allowed the identification of two heterozygous variants in F5, one in intron 24 and one in exon 23, which may be contributing to FV deficiency and thrombotic clinical phenotype, respectively. In-silico splicing predictors (MaxEntScan, NNSPLICE 0.9, Human Splicing Finder [HSF 2.4.1], SpliceSiteFinder-like, GeneSplicer) available through variant visualization software Alamut Visual were used to predict the variant’s impact on splicing. Though further in vitro analysis is required, our in silico analysis suggested that the intron variant c.6528 + 3A > C could lead to FV deficiency. This variant is predicted to abolish or weaken splicing at the downstream canonical splice, which would lead to retention of intron 24 (∼Table 1). A premature stop codon would occur at c.6528 + 34, leading the transcript to be subject to nonsense mediated decay. Other studies have demonstrated that intron retention is associated with lower protein level, and those transcripts that escape nonsense-mediated decay are not actively translated.9 Of note, the c.6528 + 3A > C alteration is known to the large population database gnomAD,10 where it was found in the heterozygous state in 2/55,723 Europeans and in no other population (0/67,185 individuals screened).

The other alteration detected in our case, c.6322C > T, p.Arg2108Cys, is a missense variant leading to substitution of arginine with cysteine in the FV C2 domain, which is essential for binding the light chain to the phosphatidyl serine-rich membranes of activated platelets. The C2 domain of FV contains three adjacent loops or “spikes” (Ser21-Trp31, Asn39-Asn45, and Asp93-Cys94), which are critical for this interaction. The substitution of arginine with cysteine in this region may alter the interaction with the phosphatidyl serine-rich membranes, potentially leading to a loss of function or acquired resistance to anticoagulant therapy.

Table 2 FV deficiency and clinical thrombosis as reported in the literature

<table>
<thead>
<tr>
<th>Publication (n = number of individuals reported)a</th>
<th>Age (gender)</th>
<th>Genetic variants in F5b</th>
<th>Clinical manifestations</th>
<th>FV activity</th>
<th>Family study</th>
</tr>
</thead>
</table>
| Zehnder and Jain, 1996 (n = 1)                    | 41 (M)      | • Heterozygous for FV Leiden  
• No other genetic analysis performed | Recurrent VTE          | 50%         | Yes          |
| Lunghi et al, 2005 (n = 3)                        | M, n = 2    | • Heterozygous E1608 K  
(HGV5: p.E1636K (n = 3)  
• Heterozygous for FV Leiden (n = 2)  
• FVHR2 haplotype (n = 1) | DVT                  | 55%, 20%, NA | Yes          |
| Simioni et al, 2005 (n = 5)                       | NA          | • Heterozygous for FV Leiden (n = 5)  
• Q1894X (HGVS: p.Q1922X)  
• G2049X (HGVS: p.G2077X)  
• G2112D (HGVS: p.G2140D)  
• P1224X (HGVS: p.P1252X)  
• Y1702 (HGVS: p.Y1730C) | VTE                  | 45–61%       | Yes          |
| Nishiya et al, 2009 (n = 1)                       | 13 (M)      | • Homozygous mutation W1920R (HGVS: p. W1948R) | DVT                  | 10%         | Yes          |
| Janicki et al, 2013 (n = 1)                       | 26 (F)      | • Homozygous R2102H (HGVS: p.Arg2130His), associated with FV deficiency  
• Homozygous R513K (HGVS: p. R541K), associated with thrombosis  
• Homozygous D107H (HGVS: p.D135H), associated with miscarriages | Bleeding episodes and miscarriages | Severe deficiency | Yes          |
| Manotti et al, 1989 (n = 1)                       | 62 (M)      | No genetic testing      | DVT and PE            | 12%         | Yes          |
| Reich et al, 1976 (n = 1)                         | 36 (F)      | No genetic testing      | Thrombophlebitis and PE | 14%         | Yes          |
| Miller, 1960 (n = 1)                              | 32 (F)      | No genetic testing      | Primarily bleeding but one episode of clotting | <2%         | Yes          |
| García-Noblejas et al, 2005 (n = 1)              | 36 (M)      | No genetic testing      | PE after receiving FFP for FV deficiency and hemarthrosis | 1%          | No           |

Abbreviations: DVT, deep vein thrombosis; F, female; FFP, fresh frozen plasma; FV, factor V; M, male; NA, not available; PE, pulmonary embolism; VTE, venous thromboembolism.

aIn studies including more than one family member, only the proband was reported.
bAll variants are reported using Human Genome Variation Society nomenclature based on F5 transcript NM_000130.4, GRCh37; if the original article reported legacy nomenclature, then that is also provided.
and Gly75-Tyr84) that are primary membrane binding sites. Spikes 1 and 3 are capped by hydrophobic residues that may immerse themselves in the polar membrane core. Arginine 2108, a basic amino acid, is at the apex of “spike” 2 and makes contact with the negatively charged phosphate groups of the membrane. The substitution of cysteine (which is hydrophobic) for arginine (which is basic) would create another site of membrane immersion that might strengthen the binding between the membrane and FV, or help it bind to more platelet membranes that have a lower phosphatidyl-L-serine concentration. As noted earlier, in vitro analysis would need to be performed to prove these hypotheses. Additionally, our analysis could not determine whether c.6322C>T is in cis or trans to c.6528+3A>C. In gnomAD, F5 C.6322C>T was found only in the heterozygous state in 1/7,651 Africans, 1/8,620 East Asians, 1/15,389 South Asians, and 1/55,650 Europeans, and in no other population group (0/35,593). No phenotypic data of these individuals are publicly available, and per American College of Medical Genetics and Genomics (ACMG) guidelines further evidence is needed to reclassify these variants of uncertain significance and pathogenic or benign variation.

In addition to the F5 variants, our patient also had heterozygous variants in FGG and PROCR. The variant in FGG causes a synonymous amino acid substitution with no predicted impact on splicing and is benign per ACMG guidelines. The PROCR variant is likely a disease-associated polymorphism, and other studies have demonstrated that it is a known risk factor for thrombosis. Additional cases of clinical thrombosis and FV deficiency in patients without the FV Leiden mutation have been described; however, many do not detail patient follow-up. For example, direct sequencing of the F5 gene in a 13-year-old boy with DVT and FV deficiency demonstrated a novel homozygous mutation: c.5842T>C, p.Trp1948Arg. Sequencing of this patient’s parents demonstrated that they were heterozygous for this mutation. Another study performed F5 NGS in a patient with severe FV deficiency, as well as in her asymptomatic parents and newborn baby. The proband was homozygous for three separate missense mutations in the coding sequence of F5. Only one of the three detected missense mutations was described previously in the setting of FV deficiency (c.6305G>A, p.Arg2102His) and it was suggested that this variant was causative for FV deficiency. The other two variants were associated with thrombosis or increased risk of percutaneous delivery. Our review of the literature also identified additional family studies describing cases of FV deficiency and clinical thrombosis; however, these do not present genetic testing data.

Given the rarity of its presentation, there are no guidelines for management of clinical thrombosis in patients with FV deficiency. Antithrombotic treatment is based on clinical expertise and adaptation of guidelines for nonbleeding patients as well as guidelines that are currently applied for classical hemophilia patients. Management is more challenging in these patients is the fact that clinical symptoms do not correlate with FV:C as some individuals with FV:C <1% do not have severe bleeding manifestations. It has been hypothesized that residual platelet FV:C is a determinant of the severity of clinical bleeding a patient with FV deficiency will experience. Due to the variable clinical phenotype that occurs in patients with FV deficiency, specific management of clinical thrombosis needs to be individualized. Our patient had no hemorrhagic episodes; therefore, he was treated with standard doses of anticoagulation. He tolerated anticoagulation with no complications and continues on warfarin. He has not had a recurrent thrombosis while on anticoagulation.

In conclusion, this case highlights the clinical variability that can be seen in individuals with defects in the F5 gene. Though FV Leiden is the most common inherited disorder of thrombophilia, other variants in F5 have been implicated in clinical thrombosis. Management of a person with FV deficiency should be based on clinical symptoms and not on FV:C levels due to the variability in clinical presentation.

Conflicts of Interest
None.

References
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