Introduction

Congenital factor V (FV) deficiency is a rare autosomal recessive bleeding disorder (prevalence ~1:1 000 000).1 Individuals heterozygous for this disorder are usually asymptomatic.1,2 However, the bleeding phenotype in individuals with undetectable levels of FV antigen and activity (<1%) in their plasma varies dramatically.1,2 Although the majority of the total FV pool circulates in plasma, ~20% to 25% is stored in platelet α-granules (4600-14 000 molecules per platelet).3 This platelet-derived FV pool originates solely from megakaryocyte endocytosis of the plasma procofactor through a process that results in the formation of a partially proteolytically activated cofactor (FV/Va)2 and phenotypically alters it to a more procoagulant phenotype.4,10

The most common treatment of individuals with symptomatic FV deficiency is administration of fresh frozen plasma (FFP) to temporarily maintain plasma FV at minimally hemostatic levels (20% to 30%).11 Its effect on platelet-derived FV/Va concentrations is unknown. In the current investigation, an individual with undetectable levels of both plasma- and platelet-derived FV/Va,3 who receives fresh frozen plasma (FFP) transfusions to control gastrointestinal (GI) bleeding, was studied.

Study design

Patient history

A 67-year-old man with congenital FV deficiency (<1% plasma- and platelet-derived FV antigen and activity)3 was recruited and consented according to a protocol approved by the University of Vermont Committee on Human Research. The patient experienced recurrent epistaxis and prolonged bleeding after dental surgery and underwent left hip hemiarthroplasty and right knee total arthroplasty for end-stage arthropathy caused by recurrent hemarthroses. Both surgeries required subsequent revision. When studied initially (February 2005), the patient was receiving 2 units of FFP per week to prevent GI bleeding. At follow-up (August 2008 and October 2012), he only required 2 units of FFP every 2 weeks.

Whole genome sequencing

Whole genome sequencing and subsequent analyses were performed by the University of Vermont Advanced Genome Technologies Core Facility. DNA, isolated from peripheral blood, was sequenced on an Illumina HiSeq1000 sequencer (average of 25 ± 5 reads). The data were analyzed using the Genome Analysis Toolkit. Variants were evaluated for biological relevance with Polymorphism Phenotyping v2 and Scale-invariant feature transform algorithms.
Assessment of plasma-derived FV antigen and activity

FV antigen was determined by a competitive radioimmunoassay. Plasma-derived FV levels between 0 and 2 hours of FFP administration were extrapolated based on the FV turnover rate in a nonhuman primate model assuming a starting plasma volume of 3200 mL (hematocrit = 36%) and a constant transfusion rate (3.75 mL plasma/min).

Western blotting analyses of platelet- and plasma-derived FV

Plasma and platelet-derived FV/Va was visualized by western blotting as detailed previously. For quantitative western blotting analyses, platelet lysates were treated with thrombin (2 U/mL, 10 minutes, 37°C) to convert all platelet-derived FV/Va to FVa. The density of the platelet-derived FVa heavy and light chains was compared with a standard curve prepared from an unaffected control presumed to have ~10,000 molecules FV per platelet.

Whole blood coagulation

Tissue factor (TF)-initiated whole blood clotting assays and quantification of serum thrombin-antithrombin complex (TAT) formation were performed as described. Whole blood clotting reactions contained: (1) TF (5 pM) and corn trypsin inhibitor (CTI) (100 μg/mL); (2) TF, CTI, and FV (2 nM); (3) TF, CTI, and protease activated receptor (PAR) 1 (100 μM) and PAR4 (500 μM) agonist peptides; and (4) CTI alone.

Measurement of TFPI

Plasma tissue factor pathway inhibitor (TFPI) was quantified using Quantikine Human TFPI Immunoassay (R&D Systems, Minneapolis MN).

Results and discussion

Whole genome sequencing identified 167 variants in the patient’s F5 gene. Two variants, rs6027 (A6755G mutation causing an Asp2194Gly substitution in the F5 gene) and L90S (an A to G mutation at chr1:169541563 causing a Leu90Ser [Leu62Ser] substitution in the F5 A1 domain), were classified as damaging, having been shown previously to be associated with FV deficiency. The patient is heterozygous at both loci, which may explain his complete absence of FV; however, other variants may play a role.

Following FFP administration, the patient’s plasma FV concentration increased from undetectable (t = 0 hours) to 1.3 μg/mL (2 hours) (Figure 1A, circles), declined rapidly, and was undetectable by 96 hours. These data were confirmed by western blotting (Figure 1B). In contrast, quantifiable levels of platelet-derived FV/Va were observed prior to FFP administration (~124 molecules per platelet) (Figure 1A, triangles; Figure 1C), which presumably represented platelet-derived FV/Va remaining from the previous transfusion. Platelet-derived FV/Va nearly doubled by 6 hours, peaked at 24 hours post–FFP administration (609 molecules per platelet) (Figure 1A, triangles), with a substantial amount remaining (434 molecules per platelet) 96 hours posttransfusion (Figure 1A, triangles). A follow-up study confirmed that the rapid acquisition of FV by the patient’s platelets was the result of megakaryocyte and not platelet endocytosis of the plasma molecule (supplemental Figure 1; available on the Blood Web site), and that following endocytosis, the patient’s platelet-derived FV/Va was proteolytically processed normally (supplemental Figure 2).

The ability of the patient’s platelets and platelet-derived FV/Va to support thrombin generation 10 days after FFP administration was assessed in a TF-dependent, contact pathway-suppressed, whole blood clotting assay following platelet activation with PAR1 and PAR4 agonist peptides (supplemental Figure 3). Simultaneous addition of TF and the agonist peptides had little effect on whole blood clotting and thrombin formation in an unaffected individual (supplemental Figure 4). Although no thrombin was generated in the absence of added FV (Figure 2, closed squares), the simultaneous addition of PAR1 and PAR4 agonist peptides to the patient’s blood resulted in platelet clumping by 3.8 minutes and clot formation by 7.8 minutes. Thrombin generation was robust (37.5 nM thrombin/minute) with a maximum level of thrombin equal to 369.4 nM (Figure 2, closed circles). In comparison, FV addition (2 nM) to the patient’s blood shortened the clot time (~2.8 minutes) but affected thrombin generation at a nearly identical rate (40.6 nM thrombin/minute) and amplitude (360.9 nM thrombin) (Figure 2, open circles). When the effects of the agonist peptides on the whole blood clotting profiles of the patient (Figure 2, closed circles) and 2 unaffected individuals (Figure 2, triangles) were compared, only the durations of the initiation phases were substantially different (~7.8 minutes vs ~2.8 ± 0.35 minutes).

Following its endocytosis by megakaryocytes, FV is retrograded to form a physically distinct molecule that exhibits an increased procoagulant potential. Because of its localized release from the platelets’ α-granules at vascular injury sites, platelet-derived FV/Va is the predominant cofactor in thrombin generation at the platelet surface. Thus, these combined observations suggest that despite a complete absence of a plasma-derived FV and the presence of ~7% normal levels of platelet-derived FV/Va, the persistence of the highly procoagulant cofactor in the patient’s platelets confers hemostatic competence. The importance of platelets and platelet-derived FV/Va in sustaining normal hemostasis is supported by several studies. A patient with a neutralizing
inhibitor to plasma- but not platelet-derived FV showed no bleeding tendency following extensive surgical challenge.10 In contrast, individuals with platelet-derived FV/Va inhibitors exhibit severe GI bleeding.17,18 Other reports describe the success of platelet transfusions in the cessation of severe bleeding resulting from FV deficiency,19,20 or FV inhibitors.21-23 In a recent study, Duckers et al described 3 individuals with severe plasma-derived FV/Va deficiency (<1% activity) but expression of detectable platelet-derived FV/Va antigen and activity (1.7% to 6.4%) who exhibited only a mild bleeding diathesis.24 The authors speculate that this residual platelet-derived FV/Va coupled with the decreased TFPI levels observed in these individuals allows for sufficient thrombin generation to prevent fatal bleeding.24 Indeed, our patient’s plasma TFPIo level (5.9 ± 0.65 ng/mL) was dramatically lower than that observed in a normal plasma pool (13.0 ± 0.95 ng/mL) consistent with previous observations made in FV-deficient individuals.24,25

Acknowledgments

The authors thank Dr Robert Hondal who helped with peptide synthesis, Dr Matthew Whelihan for his assistance with the whole blood clotting assays, Dr Jolanta Krudysz-Amblo for her assistance with assay of thrombin-antithrombin III, Fatema Walji for her assay of platelet prothrombinase activity, and Dr Kenneth Mann for his generous donation of anti-human FV #9 and #17.

This work was supported by an American Heart Association Scientist Development Grant (0635048N), the University of Vermont College of Medicine Internal Grant Program (B.A.B.), and the National Institutes of Health National Heart, Lung, and Blood Institute grants HL46703 (Project 3 [P.B.T.] and Project 5 [K.E.B.-Z.]), HL91111 (B.A.B.), T32HL007594 (J.C.), and award UC2 HL103010 (P.D.).

Authorship

Contribution: B.A.B. performed research, analyzed data, and prepared the manuscript; J.C., K.E.B.-Z., and P.D. performed research, analyzed data, and critically reviewed the manuscript; N.S.K. oversaw the participation and clinical management of the patient and critically reviewed the manuscript; and P.B.T. conceived of the research, analyzed data, and critically reviewed the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


Platelets and platelet-derived factor Va confer hemostatic competence in complete factor V deficiency

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