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Factor V, tissue factor pathway inhibitor, and east Texas bleeding disorder

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In a report reading like a fascinating detective story, Vincent and colleagues crack the mysterious case of east Texas bleeding disorder. They show that affected individuals have a mutation in exon 13 of the coagulation *F5* gene that causes increased expression of an alternatively spliced transcript, which encodes a previously unrecognized factor V (FV) isoform they call FV-short. This FV isoform lacks a large portion of the B domain of FV, which is normally released upon the proteolytic activation of FV by thrombin and binds tightly to the coagulation regulator tissue factor pathway inhibitor-α (TFPIα). This interaction leads to an approximately 10-fold increase in the level of TFPIα circulating in plasma and a resultant anticoagulant effect that produces a hemorrhagic diathesis.

The players: TFPIα and FV

Full-length tissue factor pathway inhibitor-α (TFPIα) contains an acidic N terminus followed by three tandem Kunitz-type protease inhibitory domains and a basic C terminus. It regulates coagulation by producing factor Xa–dependent (FXa-dependent) feedback inhibition of the factor VIIa/tissue factor complex (FVIIa/TF) (1), which is responsible for the initiation of coagulation, and by directly inhibiting FXa in a process that is enhanced by protein S (PS) (2, 3). Full-length TFPIα circulates at a low concentration (~0.16 nM) in plasma, but platelets carry approximately 50% of total blood TFPIα and release it at sites of injury where they aggregate.

Plasma levels of TFPIα are reduced in patients with factor V (FV) or PS deficiency (4, 5). Full-length TFPIα (MW 42 kDa) circulating in plasma is bound in two high molecular complexes (MW > 700 kDa) that require the presence of FV and the basic C terminus of TFPIα, which is needed for its interaction with FV (6). These high molecular weight complexes may contain additional constituents (e.g., PS).

The activated form of FV (FVa) is a coagulation cofactor that dramatically accelerates FXa activation of prothrombin to thrombin. FV is a 330-kDa single-chain protein that circulates in plasma at a concentration of approximately 20 nM.

About 20% of the total FV in blood is carried by platelets as fragmented, partially activated forms (7). FV is activated by FXa or thrombin through the proteolytic release of its large, intervening B domain with the ultimate production of the heavy (105 kDa) and light (74 kDa) chains of FVa that associate in a Ca^{2+}-dependent fashion (Figure 1A).

Elegant studies by Bos and Camire demonstrated that the B domain (aa 710–1545) serves to maintain FV in an inactive, procofactor state and that an interaction between a basic region (BR) (aa 963–1008) and an acidic region (AR) (aa 1493–1537) within the B domain are required for this effect (Figure 1A and ref. 8). Two peptides within the BR (aa 983–994 and 997–1008) were shown to be most important for its presumed binding to the AR (Figure 1B). Deletion of either the basic or acidic portions of the B domain produces derivatives of FV with cofactor activity.

The plot

In 2001, Kuang et al. described a large Texas kindred with a moderately severe bleeding disorder that was characterized by bruising, epistaxis, menorrhagia, and hemorrhage following trauma or surgery that frequently required blood transfusion (9). The prothrombin time (PT) and the activated partial thromboplastin time (aPTT) were both prolonged, suggesting an abnormality in the "common" coagulation pathway that consists of FX, FV, prothrombin, and fibrinogen. Assays of all the coagulation factors, however, produced normal results. Autosomal dominant inheritance and a mild inhibitor pattern in coagulation studies in which patient plasma is mixed with normal plasma suggested a gain-of-function mutation that limited coagulation.

One possible explanation of these results was enhanced inactivation of FXa and thrombin by a hyperactive form of the coagulation inhibitor antithrombin. Initial linkage analysis using an intragenic microsatellite marker showed that the disorder indeed mapped to a locus near the antithrombin gene. Sequencing of the antithrombin gene, however, failed to identify a mutation. More defined linkage studies narrowed the involved locus to a 1.5-Mb region (1q24) that was centromeric to the antithrombin gene and contained the gene for FV (*F5*) (9). Subsequent sequencing of the *F5* gene in an affected individual identified a novel A2440G nucleotide alteration in exon 13 that segregated with the disease and produced a S756G substitution in the B domain of FV. Since the B domain of FV is not required for FV activity and FV clotting activity in affected family members was normal, the alteration was felt to represent a private polymorphism within the family and unlikely to be associated with the bleeding disorder. Thus, east Texas bleeding disorder remained unexplained.

More detective work

To determine whether the A2440G alteration in the FV genome detected by Kuang et al. produced an effect on the level or size of the FV protein, Vincent and colleagues (10) analyzed the plasma of family members by Western blotting. They identified a unique 250-kDa band that was prominent in the plasma of affected and barely detectable in the plasma of unaffected family members and confirmed by mass spectrometry that it represented a form of FV. A subsequent search for potential mRNA splicing abnormalities using RT-PCR and mRNA from family members' white blood cells detected the expected *F5* transcript (~2950 bp) and a shorter transcript (~840 bp, encoding
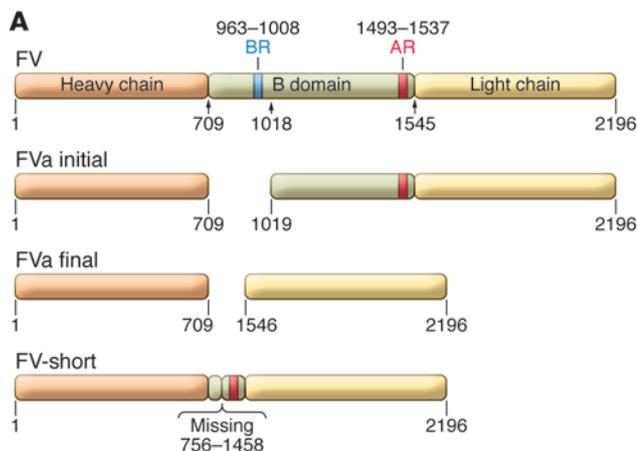
Figure 1
Forms of factor V and alignment of the sequences of the B domain BR and TFPIα. (**A**) Structure of FV. FV: the heavy chain (orange), light chain (yellow) and B domain (green) are shown. The BR (blue) and AR (red) of the B domain are depicted as well as the sites of cleavage produced by FXa and thrombin during the activation of FV. FVa initial: initial cleavage of FV by FXa and thrombin occurs at R709 and R1018. FVa final: cleavage at R1545 is produced by thrombin, but slowly or not at all by FXa, producing the heavy chain and light chains of FVa that associate in a calcium ion–dependent fashion. FV-short: lacks residues 756–1458 of the FV B domain, but contains the AR. (**B**) Alignment of B domain and TFPIα sequences. Two sequences within the BR domain of the FV B domain found to be involved in its presumed interaction with AR are shown: peptide 983–994 (light blue lettering) and peptide 997–1008 (dark blue lettering), with the latter appearing to be much more important (8). The sequence of TFPIα required for its interaction with FV is shown in dark blue lettering (6). Boxed is a region of substantial homology.

FV-short) that predominated in affected individuals and was a minor constituent in unaffected individuals. Thus, the A2440G mutation appeared to dramatically increase (~20-fold) the expression of an alternatively spliced transcript that is normally in low abundance. Sequencing showed that FV-short lacked aa 756–1458 of the B domain of FV (Figure 1A).

The authors then turned their attention to the coagulation abnormalities evident in people with east Texas bleeding disorder. Using a sensitive thrombin generation assay in which coagulation was induced with low levels of TF, they showed that the inhibitory activity in patient plasma was associated with FV-short, but that recombinant FV-short itself did not possess intrinsic anticoagulant activity. As TFPIα had been shown to interact with FV in normal plasma (4, 6), TFPIα levels were measured and found to be approximately 10-fold higher in the affected family members. Immunoprecipitation experiments confirmed that TFPIα was bound to FV-short in patient plasma. Further, in normal plasma, TFPIα appeared to be equally distributed between FV and FV-short even though FV-short is at a greater than 20-fold lower concentration. Therefore the affinity of FV-short for TFPIα is considerably greater than that of FV.

Remaining questions

This report has solved the case of east Texas bleeding disorder, and in doing so, it has identified a previously unrecognized, alternatively spliced isoform of FV, demon-

strated a gain-of-function mutation in the *F5* gene, and shown that increased levels of TFPIα cause bleeding. As is common with a scientific advance, the findings raise additional questions:

What mechanism underlies the tight binding of TFPIα to FV-short? The basic C-terminal peptide of full-length TFPIα (FL-TFPIα) is required for the binding of TFPIα to both FV-short and to FV. FV is maintained in an inactive, procofactor state through an interaction between a BR and an AR within its B domain (8). The BR is not present in FV-short, leaving its AR as a potential site for TFPIα binding (Figure 1A). Consistent with this notion, alignment of the peptides in the BR that affect its interaction with the AR and the C-terminal peptide of TFPIα reveals an area of remarkable homology (Figure 1B). The much lower affinity of TFPIα for FV might be explained by its need to compete with the endogenous BR in FV for AR binding.

Does the TFPIα:FV-short complex have FV cofactor activity? The studies of Bos and Camire suggest that FV-short should display FV cofactor activity (8). It is conceivable that, by interacting with the AR, TFPIα functions as a surrogate for the BR, thereby keeping FV-short in a procofactor state by interfering with its binding to FXa.

Is east Texas bleeding disorder simply caused by increased circulating TFPIα, or does the TFPIα:FV-short complex possess intrinsic anticoagulant activity? The presence of FVa in the prothrombinase complex increases TFPIα inhibition of FXa by producing a 20-fold decrease in the Ki of the TFPIα-

FXa encounter complex (11). This effect is completely abrogated by the presence of the substrate prothrombin (12). It is possible that the TFPIα:FV-short complex has enhanced anti-FXa activity that is less sensitive to the presence of prothrombin. The inhibitory activity of such a complex would presumably be circumvented by thrombin cleavage of FV-short at R1545, releasing the AR and TFPIα (Figure 1A).

What is the effect of FL-TFPIα on forms of FV that are produced normally during coagulation and that, like FV-short, lack the BR of FV? Proteolysis of FV by FXa and thrombin involves cleavage at R709, R1018, and R1545 (Figure 1). Initial cleavages by both FXa and thrombin occur at R709 and R1018 and produce a form of FV that lacks the BR and would exhibit cofactor activity. This form of FV, and perhaps the partially proteolyzed forms of FV released by platelets, would be potential targets of TFPIα until cleavage at R1545 releases the AR from the activated FV molecule. An important difference in the activation of FV by FXa and by thrombin, however, is that cleavage at R1545 occurs much more slowly or not at all in the case of FXa (13). This implies that FXa-activated FV would be considerably more susceptible to an effect of TFPIα. Whether FXa generated by FVIIa/TF or thrombin produced weakly by FXa in the absence of FVa is responsible for the initial activation of FV during hemostasis is not known. TFPIα released at relatively high local concentrations by platelets that adhere and aggregate at a site of injury could limit thrombin generation that was

dependent on FXa-activated FV. Once generated, thrombin would counter this effect of TFPIα by producing forms of activated FV that have been cleaved at R1545 and lack the potential TFPIα-binding site AR.

Is there a role for PS? PS is known to interact with FXa, FV(a), and TFPIα and is also released by stimulated platelets. Thus, its potential role in the reactions discussed above warrants investigation.

Most important to the affected members of the east Texas family, however, is that the elucidation of the underlying pathophysiology suggests that TFPI inhibitors currently in development may provide a means of treatment (14, 15).

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A "*Tric*" to tighten cell-cell junctions in the cochlea for hearing

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Tricellulin is a tricellular tight junction–associated membrane protein that controls movement of solutes at these specialized cell intersections. Mutations in the gene encoding tricellulin, *TRIC*, lead to nonsyndromic deafness. In this issue of the *JCI*, Nayak et al. created a gene-targeted knockin mouse in order to mimic the pathology of a human *TRIC* mutation. Deafness appears to be caused either by an increase in the K$^+$ ion concentration around the basolateral surfaces of the outer hair cells or, alternatively, by an increase in small molecules such as ATP around the hair bundle, leading to cellular dysfunction and degeneration. Furthermore, the mice have features suggestive of syndromic hearing loss, which may have implications for care and treatment of patients harboring *TRIC* mutations.

Millions worldwide suffer from a debilitating hearing loss (1). In many regions of the world in which health care conditions and public health are less developed or in populations with high rates of consanguinity, the number of people affected is extremely high. Understanding the mechanisms leading to hearing loss may help widen the current scope of therapeutic options, which are currently restricted to hearing aids and cochlear implants. The causes of hearing loss are manifold, including both genetic and environmental factors. Hereditary hearing loss is caused by mutations in a wide variety of genes that encode the proteins associated with the transduction of sound waves from the external ear to the middle and inner ear and finally to the brain. To date, hereditary hearing loss has been linked to over 60 genes (2).

The cochlea, located in the inner ear, is responsible for the conversion of sound to a neural electric excitatory signal (3). Two distinct fluids, the endolymph and perilymph, are contained within the cochlea. While the perilymph has an ionic composition similar to that of the general extracellular fluid, the endolymph is characterized by a high K$^+$ concentration and endocochlear potential (EP), which are essential for hearing (4). An epithelial cellular sheet covering the cochlea creates a barrier that allows these fluids to maintain their composition.

Tight junctions are essential for sound conduction

Tight junctions (TJs) provide a strong epithelial barrier function within the cochlea, preventing the leakage of solutes through