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COMMENTARY

F5-Atlanta: Factor V-short strikes again

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Functional interactions between tissue factor pathway inhibitor alpha (TFPIα) and certain forms of coagulation factor V (FV) have recently emerged as new players in the regulation of the initiation of coagulation.[1] Their physiological relevance is illustrated by the bleeding tendency associated with the genetic up-regulation of FV-short, a minor splicing isoform of FV that preferentially binds TFPIα, as in the East Texas[2,3] and Amsterdam[4] bleeding disorders. In this issue, Zimowski et al. describe a third and more extreme example of this bleeding mechanism,[5] providing new information on the regulation of FV-short expression.

In 2001, an autosomal dominant bleeding disorder, characterized by prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT) in the presence of normal levels of all coagulation factors, was described in a large family from East Texas.[2] The disease locus was mapped to a region of chromosome 1 that contains the *F5* gene, encoding FV, the well-known (pro)cofactor of prothrombinase, where an apparently missense variant was identified (*F5* c.2350A>G[a] in exon 13, predicting the p.Ser784Gly substitution in the B-domain). Despite its perfect co-segregation with the disease phenotype, this mutation did not offer a straightforward explanation for the bleeding tendency, because the B-domain is remarkably tolerant of amino acid substitutions and the proband had normal FV clotting activity. Therefore, the involvement of FV was considered unlikely and the East Texas bleeding disorder was almost forgotten for the next 12 years.

In the meantime, evidence started accumulating that FV interacts with TFPIα, the Kunitz-type inhibitor of factors VIIa and Xa. To start with, it was reported that FV-deficient patients have markedly decreased TFPIα levels and that FV immunodepletion removes TFPIα from plasma, suggesting that the two proteins circulate as a complex, which may protect TFPIα from truncation and/or clearance.[6] Around the same time, elegant work on the mechanism of FV activation indicated that FV is maintained in the procofactor (i.e., inactive) state by a high-affinity interaction between a basic and an acidic region in the B-domain.[7] This intra-molecular interaction is broken during the early steps of FV activation, when the B-domain is cleaved at Arg^{709} and Arg^{1018}, generating a partially active intermediate that has lost the basic region, but still retains the acidic region. Intriguingly, the C-terminus of TFPIα turned out to contain a basic region that is highly homologous to the basic region of FV and can bind to the exposed acidic region of partially activated forms of FV, thereby restoring the procofactor state[8] and inhibiting prothrombinase activity.[9] In addition, binding of TFPIα to the acidic region of partially activated FV was found to interfere with cleavage of FV at Arg^{1545}, thereby delaying full FV activation, preventing the loss of the acidic region and effectively maintaining FV activity under the control of TFPIα.[10,11] Collectively, these observations (1) identified the acidic region in the B-domain of FV and the basic region in the C-terminus of TFPIα as the binding sites responsible for the FV/TFPIα interaction; and (2) uncovered an unsuspected role of TFPIα in the regulation of FV activation/prothrombinase activity in the early phases of coagulation. Later, it was also shown that FV enhances the inhibition of factor Xa (FXa) by TFPIα and protein S, both in model systems[12,13] and in plasma.[14]

Capitalizing on this emerging knowledge and taking it further, the East Texas bleeding disorder was eventually unravelled in 2013, revealing a fascinating molecular mechanism.[3] Upon re-evaluation, the *F5* c.2350A>G variant proved to be a splicing mutation that enhances the expression of a previously overlooked splicing isoform of FV, called FV-short, which lacks 702 amino acids of the B-domain, including the basic region (Figure 1). Due to the absence of the basic

[a]All mutations are annotated according to the Human Genome Variation Society (HGVS) nomenclature (http://varnomen.hgvs.org).

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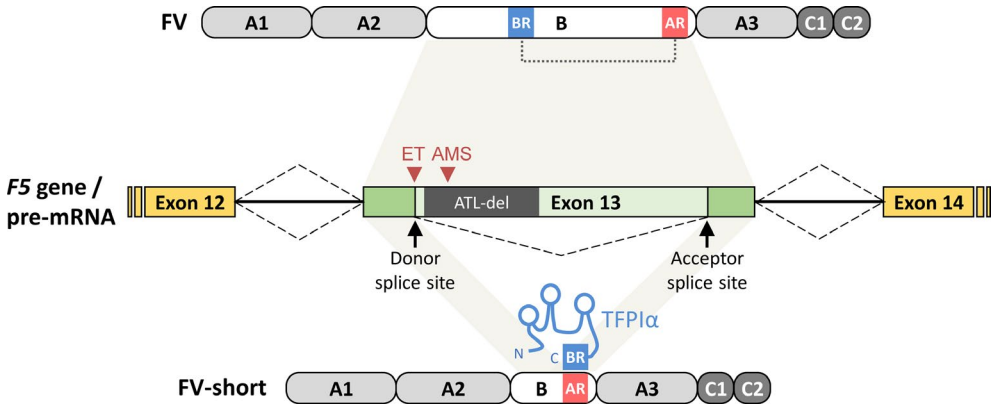

FIGURE 1 Alternative splicing of the *F5* gene. The region of the *F5* gene (or pre-mRNA) spanning exons 12 through 14 is shown in the middle. Inclusion of the whole exon 13 in the mature mRNA leads to the production of full-length factor V (FV; top), whose B-domain contains the basic region (BR) and the acidic region (AR) tightly bound together. In a minor fraction of transcripts the central portion of exon 13 is spliced out of the mature mRNA using the indicated donor and acceptor splice sites. This leads to the production of FV-short (bottom), whose much shorter B-domain contains only the AR and interacts tightly with the BR of TFPIα. The *F5*-East Texas (ET) and *F5*-Amsterdam (AMS) mutations as well as the *F5*-Atlanta deletion (ATL-del) considerably increase the frequency of FV-short splicing

region, FV-short is constitutively active, but its prothrombinase activity is effectively suppressed by TFPIα, which binds with high affinity to its exposed acidic region.[10,11] FV-short, which is expressed at sub-nanomolar levels in all individuals, proved to be the actual carrier of TFPIα in the circulation[3] and to stimulate the inhibition of FXa by TFPIα/protein S 10-fold more potently than FV.[15] Therefore, up-regulation of FV-short by the *F5* c.2350A>G mutation increases both TFPIα antigen (~10-fold) and its anti-FXa activity, explaining the moderate mucosal and post-traumatic bleeding observed in the East Texas family.[3]

In 2015, a similar bleeding disorder associated with elevated TFPIα levels was described in a Dutch mother and son with trauma-related bleeding. Also in this case, a splicing mutation was identified in *F5* exon 13 (c.2588C>G, *F5*-Amsterdam, Figure 1). This mutation induces the expression of another splicing isoform of FV, structurally and functionally similar to FV-short, but absent in normal individuals.[4]

The interesting paper by Zimowski et al. reports on an African-American patient with severe bleeding symptoms and massively elevated plasma levels of FV-short and TFPIα (>20-fold), in the presence of a heterozygous deletion of 832 bp in *F5* exon 13 (c.2413_3244del, *F5*-Atlanta, Figure 1).[5] Although the bleeding mechanism is essentially the same as in the East Texas and Amsterdam disorders, this new case is remarkable for its extreme laboratory and clinical phenotypes and unusual genetic mechanism. The *F5*-Atlanta deletion introduces a frameshift and premature stop codon in exon 13, preventing the expression of full-length FV (by nonsense-mediated decay of its mRNA), but not of FV-short, where the whole deletion is spliced out as part of the FV-short-specific intron. Surprisingly, the patient had high rather than low FV (>200%, as estimated by semi-quantitative western blotting), most of which was FV-short. The strong bias toward FV-short expression was already apparent at the mRNA level, indicating that *F5*-Atlanta influences alternative splicing in favor of FV-short in a novel and unexpected way.

The regulation of alternative splicing is extremely complex, but at the most basic level it relies on signals embedded in the sequence of the primary transcript.[16] These comprise the donor and acceptor splice sites, which define the boundaries of each intron, but also splicing enhancers and silencers, subtler regulators that guide the spliceosome to the intended splice sites. The splicing event that gives rise to the FV-short transcript, that is, the removal of an optional intron embedded in exon 13, occurs at a very low frequency in normal individuals, but is enhanced in the East Texas, Amsterdam, and Atlanta bleeding disorders. While the *F5*-East Texas mutation strengthens the donor splice site of the FV-short--specific intron[3] and *F5*-Amsterdam creates an entirely new donor splice site,[4] *F5*-Atlanta does not affect the donor or acceptor splice sites, but (most probably) alters the regulatory framework of FV-short splicing. In fact, the massive up-regulation of FV-short expression associated with the *F5*-Atlanta deletion strongly suggests that the deleted region contains one or more splicing silencers that normally suppress FV-short splicing.[5] Abolition of these negative regulator(s) would cause relatively more *F5* pre-mRNA transcribed from the *F5*-Atlanta allele to be processed into FV-short mRNA, increasing the FV-short/full-length FV transcript ratio. This could also explain the patient's overall increased FV level, as the biosynthesis and secretion of FV-short, which lacks most post-translational modification sites in the B-domain, is likely to be more efficient than that of full-length FV, resulting in relatively more secreted protein (as observed in recombinant expression systems).

Since the *in silico* prediction of splicing regulatory elements is still challenging, the exact identity and position of the FV-short splicing silencer(s) presently remain elusive. However, as noted by the authors, common genetic variants in *F5* exon 13 (and particularly within the *F5*-Atlanta deletion) may affect these putative regulatory elements, thereby modulating FV-short splicing and causing inter-individual differences in FV-short (and TFPIα) levels. This phenomenon could contribute to the variable bleeding tendency of patients

with haemophilia, in which TFPIα is a major determinant of thrombin generation,[17] and play a role in unexplained bleeding disorders, in which TFPIα level and/or activity are often elevated.[18,19] Although several anti-TFPI agents are currently being developed for the treatment of hemophilia,[20] FV-short, which is both the physiological carrier and a potent cofactor of TFPIα, may also represent an attractive therapeutic target to restore hemostatic balance in various bleeding disorders. In particular, deciphering the splicing code that regulates FV-short expression would allow the design of specific antisense-mediated splicing modulation strategies to down-regulate FV-short.

While over-expression of FV-short (and consequently TFPIα) has been associated with bleeding in three unrelated patients/families, it is presently unknown if and to what extent a deficiency of FV-short would contribute to the risk of venous thrombosis. Unfortunately, the lack of an accurate and high-throughput assay to measure FV-short currently precludes association studies between FV-short levels and clinical endpoints. On the other hand, the East Texas and allied disorders show that much can be learned from experiments of nature. Considering the high degree of genetic polymorphism of *F5* exon 13 and the complexity of the splicing code, it seems at least plausible that additional patients with *F5* gene mutations increasing (or perhaps decreasing) FV-short expression will be identified. So, FV-short might strike again and possibly reveal more secrets in the process.

CONFLICTS OF INTEREST

The author has no conflicts of interest.

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