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COMMENTARY

Better or worse than the original

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See also Pezeshkpoor B, Castoldi E, Mahler A, Hanel D, Müller J, Hamedani NS, Biswas A, Oldenburg J, Pavlova A. Identification and functional characterization of a novel F5 mutation (Ala512Val, FV_{Bonn}) associated with activated protein C resistance. This issue, pp 1353–63.

The discovery of resistance to a main inhibitor of coagulation [1] and the finding of a single factor V mutation (Arg506Gln, FV Leiden) affecting an activated protein C (APC) cleavage site in a significant proportion of thrombophilic patients of Caucasian origin [2] have been major advances for the clinical coagulation laboratory and for the genotyping of congenital thrombophilia. Hundreds of thousands of patients have been genotyped, and > 4500 articles investigating FV Leiden-related issues have been published.

The concept that other frequent genetic mutations affecting FV properties could, in combination with FV Leiden, contribute to the thrombophilic tendency promoted further FV-focused investigation. A frequent, ancient and functional FV haplotype encoding FVR2 [3] was found to be associated with reduced FV levels and modest APC resistance, which might exacerbate the FV Leiden phenotype in compound heterozygotes. However, the reduced ability of FVR2 to predict a significant proportion of risk for thrombosis made it of limited use for diagnosis. Common susceptibility alleles with modest contributions to venous thromboembolism (VTE) risk were, indeed, common findings in several powerful candidate single-nucleotide polymorphism or genome-wide association studies [4].

On the other hand, the search for rare FV mutations able to produce APC resistance by affecting APC cleavage sites other than Arg506 was fruitful. The FV Arg306Thr mutation (FV Cambridge) was found in a family with thrombophilia [5], and confirmed by expression studies to produce mild APC resistance.

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The presence of FV Leiden only in Caucasians promoted the investigation of alternative genetic bases of APC resistance phenotypes in other populations. The Arg306Gly mutation, affecting the same residue mutated in FV Cambridge and conferring an undefined risk for thrombosis, was found in the Hong Kong Chinese population [6], and, more recently, the Glu666Asp mutation was reported [7] in a Chinese family with APC resistance and VTE.

Through a different mechanism, the Ile359Thr mutation (FV Liverpool) [8] reduces cleavage at Arg306 and also causes equally low APC cofactor activity as FV Leiden. This substitution creates a new consensus sequence for N-linked glycosylation, the large bulky carbohydrate side chains of which could interfere with binding sites distant from the affected residue 359.

In patients with an Amerindian ethnic background and APC resistance, the Met443Leu, Glu461Gln and Gly493-Glu mutations were detected in exon 10, which encodes Arg506 [9]. The information available on the phenotypes produced by these mutations is not yet sufficient to enable their functional roles to be established.

The detection of FV mutations associated with APC resistance is now favored by efficient and cheap sequencing tools. Unfortunately, the selection of cases for sequencing is not fully supported by current APC resistance phenotype screening, which is optimized to demonstrate resistance to APC resulting from the FV Arg506Gln mutation. These plasma assays may not have sufficient sensitivity to pick up mild APC resistance with other causes, as suggested by the marginally increased APC resistance observed for FVR2 and FV Liverpool.

As compared with mutations clustered in the FV cleavage site protein regions, the Trp1920Arg mutation (FV Nara) [10], which was recently discovered in a Japanese boy with a low level of plasma FV activity and deep vein thrombosis, was located in a distant domain (C1). This module is involved in the spike-like structures that are able to bind membrane phospholipids. However, FV Nara and wild-type (WT) FV appear to bind phospholipids with similar affinity.

Table 1 FV Bonn versus FV Leiden

FV variant	Localization	Biosynthesis	APC-mediated inactivation	APC cofactor activity	Thrombin generation (without APC)	Residual activity of the Arg506-cleaved FVa	Initial rate of FV activation by thrombin	Ethnic origin
Arg506Gln FV Leiden	APC cleavage site	Normal	Reduced	Reduced	Normal	Not cleaved	Normal	Caucasian
Ala512Val FV Bonn	APC cleavage loop	Normal	Intermediate	Intermediate	Increased	87%	Increased	Caucasian
Wild-type FV	–	Normal	Normal	Normal	Normal	56%	Normal	–

APC, activated protein C; FVa, activated factor V.

FV Nara points to different mechanisms for producing APC resistance, including the alteration of both FV biosynthesis and FV structure, which were also documented for FVR2, including the functional Asp2194Gly mutation in the FV C2 domain. The structural changes induced by mutations in these C-terminal modules could lead to reduced interaction with APC and/or protein S.

By significantly decreasing both susceptibility to APC and APC cofactor activity, FV Nara appeared to be more resistant than FV Leiden, and thus to be potentially more prothrombotic. Although evaluation of the penetrance of rare mutations cannot be properly addressed, the two Japanese patients were homozygous for the Trp1920Arg mutation, and their heterozygous parents were asymptomatic. Interestingly, the affected members of the single family with FV Liverpool were heterozygous for a FV null allele, and thus pseudohomozygous for the Ile359Thr mutation. This mechanism was found to substantially enhance the risk for thrombosis conferred by FV Leiden [11] carriers. Overall, these genetic observations support a rule of thumb: the absence of WT FV increases the thrombotic risk associated with APC resistance variants by approximately one order of magnitude, as compared with the heterozygous condition. As a consequence, the chance of observing patients with compound or homozygous conditions substantially increases.

In this issue, Pezeshkpoor *et al*., from the University Clinic of Bonn, Germany, and from Maastricht University, The Netherlands, report a novel and stimulating finding, i.e. the Ala512Val mutation (FV Bonn), detected in the heterozygous condition in six unrelated patients and their affected family members [12]. APC resistance was described in plasma from the six probands. This article reports a valuable functional characterization of the recombinant FV Bonn variant, mutated in the loop where the Arg506 APC cleavage site and FV Leiden are located.

The comparison with WT FV, and particularly with FV Leiden, provides interesting information about gain-of-function properties of close mutations (Table 1). The kinetic analysis of APC-catalyzed inactivation demonstrated that activated FV (FVa) Bonn is inactivated more slowly than WT FVa, potentially because of a three-fold slower rate of cleavage at Arg506. On the other hand, FVa Bonn was inactivated faster than FVa Leiden and expressed APC cofactor activity that was slightly reduced, but higher than that of FV Leiden.

Taking into account these features, FV Bonn could represent only a mild form of FV Leiden conferring a modest risk for thrombosis. Interestingly, it was observed that FV Bonn is more procoagulant than WT FV and FV Leiden in the absence of APC. Clearly increased thrombin generation and a shorter lag time were found, and, at very low concentrations, FV Bonn still promoted considerable thrombin generation,which was much higher than that promoted by WT FV and FV Leiden. This observation can potentially be explained by the initial rate of FV Bonn activation by thrombin, which is higher than that of WT FV and FV Leiden. An increased activated FX (FXa) cofactor activity of FVa Bonn, possibly via enhanced interaction with FXa, is also a plausible hypothesis, and is supported by functional and structural observations. However, with the assay limitation, the residual activity of the FV Bonn cleaved at Arg506, which could reflect FXa-binding affinity, was higher than that of WT FVa. Moreover, the mutated Ala512 bridges residues (Arg 510, Ala511, and Asp513) that contribute to an extended FXa-binding surface. The increased affinity between the FVa variant and FXa could represent a new mechanism producing APC resistance, favored by the competition between APC and FXa for the FV protein region mutated in FV Bonn. Other FV mutations found in thrombophilic patients [9], which at present are poorly characterized, could share a similar mechanism and increase FXa affinity.

In conclusion, among the thrombophilic FV variants, the Ala512Val mutation produces pleiotropic effects resulting in a new combination of functional correlates: (i) increased resistance to APC inactivation, enhancing the availability of FVa; (ii) decreased APC cofactor activity, enhancing the availability of activated FVIII; and (iii) improved FXa cofactor activity, a specific feature of

FV Bonn, supporting the prothrombinase complex even better than WT FV.

In addition to providing new insights into the roles and interactions of FV, this functional pattern might explain the unusually high penetrance of this gain-of-function mutation, which has been associated with recurring thrombotic events in all heterozygous carriers identified so far, and thus appears to have worse clinical behavior than FV Leiden. We are eager to know further biochemical and genetic details, and particularly the frequency of the FV Bonn mutation in patients and controls from the regions, or valleys, of Germany and Serbia, where the six patients come from.

Disclosure of Conflict of Interests

The author states that he has no conflict of interest.

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