Prothrombin: Another Clotting Factor After FV That Is Involved Both in Bleeding and Thrombosis

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Abstract
Clotting factor defects are usually associated with bleeding. About 2 decades ago, 2 polymorphisms, one of FII (G20210A) and another of FV (Arg506Gln), have been shown to be associated with prothrombotic state and venous thrombosis. As a consequence, FII and FV could be considered both as prohemorrhagic factors and prothrombotic conditions. Recently, it has been shown that missense mutations in the prothrombin gene of amino acid Arg596 of exon 14 to Leu596, Gln596, or Trp596 caused the appearance of a thrombophilic state and venous thrombosis. These mutated FII are not associated with bleeding, but only with venous thrombosis. Furthermore, they are all heterozygotes for the mutations. No missense mutation associated with thrombosis has been discovered so far for FV. As a consequence, the prothrombotic activity of FII is the result of a polymorphism and of a missense mutation, whereas that of FV derives only from a polymorphism. The observation that a clotting factor defect may be associated with both bleeding or venous thrombosis depending on the site of the mutation has caused an extensive reevaluation of the blood clotting mechanism.

Keywords
prothrombin, dysprothrombinemias, FV, thrombosis, bleeding, polymorphisms

Clotting proteins have always been associated with either bleeding or thrombosis. The only exception to this was represented by rare dysfibrinogenemias which were known to show thrombosis and bleeding.1 About 2 decades ago, it was demonstrated that FV defects could lead to both a bleeding tendency or a prothrombotic one.2,3 In 1993, it was, in fact, reported that an unknown mutation in FV caused a resistance of the mutated factor toward activated protein C.2 The following year, the polymorphism Arg506Gln (FV Leiden) was recognized to be responsible for the occurrence of the thrombophilic state.3 After these studies, FV defects could be divided as prohemorrhagic (FV deficiency) and prothrombotic (FV Leiden).4 FV has remained safe for the rare dysfibrinogenemias, the only clotting proteins to show this behavior. A few years later, it was reported that prothrombin polymorphism G20210A was associated with thrombophilic state and venous thrombosis.5 The polymorphism is characterized by an increased level of plasma prothrombin activity and antigen, and this was maintained to be the cause for the thrombophilic state.

It has been known for many years that prothrombin plays a fundamental role in blood coagulation. Congenital prothrombin deficiency was discovered by Quick et al in 1946.6 For comparison, hemophilia A and B were surely separated as distinct bleeding conditions only in 1949 to 1952.7,8 Prothrombin defects have always been associated with bleeding, often severe bleeding. Type II forms, namely those characterized by low activity and normal or near normal antigen, have usually shown a milder bleeding tendency as compared with type I defects, namely those with “true” deficiency.9,10 Recently, prothrombin Arg596 mutations, which are type II defects, have been associated with venous thrombosis and no bleeding diathesis.11-15 On the contrary, no such observation has ever been reported for FV. These new findings impose a new classification of prothrombin defects in prohemorrhagic prothrombin defects and prothrombotic prothrombin defects, similarly for what was known for FV.4

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Classification

Prohemorrhagic Prothrombin Defects

Prothrombin defects associated with bleeding may be classified as type I and type II. Homozygous type I defects are characterized by cases of true deficiency in which both prothrombin activity and antigen are low, usually less than 5% of normal. On the contrary, homozygous type II defects are characterized by the presence of low activity, usually around 10% of normal, but normal, near normal, or only slightly decreased prothrombin antigen.9,10

Bleeding is always severe in type I defects, whereas it may be variable in type II defects. Genetically, the defect can be divided into homozygotes, compound heterozygotes, and heterozygotes. The former 2 are always variably symptomatic, whereas the heterozygotes rarely show spontaneous bleeding but may bleed during or after surgical procedures, deliveries, and tooth extractions.16 Prothrombin defects have been described worldwide, but there is a prevalence in patients of Latin extraction.9,10 In this regard, it is interesting to note that prothrombin deficiency in Puerto Rico represents the third most frequent bleeding defect, after the hemophilias and von Willebrand disease.17

Prothrombotic Prothrombin Defects

These could be subdivided into defects due to a polymorphism (common mutation) and defects due to a specific, rare mutation.

Polymorphisms. The possibility that prothrombin could be related to a hypercoagulable state was first proposed by Poort et al who showed that a G to A 20210 polymorphism of the noncoding region of the prothrombin gene was associated with increased prothrombin activity and antigen levels.5 Subsequently several studies have shown that this polymorphism is a risk, usually a mild one, for venous thrombosis.18-24 That this so is well demonstrated by the observation that homozygotes do not appear to be more severely affected than heterozygotes23 and that the condition is not associated with an increase incidence of recurrences.19,22 Furthermore, it does not seem to represent a sure risk factor for the first episode of venous thrombosis.21,24 The polymorphism seems important when associated with other congenital (FV Leiden) or acquired thrombotic risk factors (oral contraceptive therapy, immobilization, surgery, etc).21 Since the polymorphism is widely even though unevenly diffuse (average about 3%-4% of the population), it may be implicated in many patients with a thrombotic event even though that thrombotic event might be the result of another cause. The polymorphism has no proven role in arterial thrombosis.24

Prothrombotic dysprothrombinemias. In 2012, Miyawaki et al11 reported a family with a dysprothrombinemia and venous thrombosis and no bleeding tendency. The defect was called prothrombin “Yukuhashi” and was due to the heterozygous mutation Arg596Leu. The mutation causes a resistance to antithrombin with consequent appearance of a thrombophilic state.11 During the following years, a few other families were discovered with mutations always involving the Arg596 amino acid, namely prothrombin Belgrade (Arg596Gln) and prothrombin Padua 2 (Arg596Trp).12,13 Furthermore, other families with the Arg596Gln mutation were also found in Japan and India.14,15 All these patients were heterozygous and manifested venous thrombosis at a young age (10-27 years). The patient from India (Arg596Gln) showed instead venous thrombosis at the age of 60 and had no family study.15 The description of these patients has radically changed our approach to the understanding and study of prothrombin defects (Table 1).

Prohemorrhagic FV Defects

Congenital FV deficiency may also be divided into type I and type II defects. The former is much more frequent than the
latter.\textsuperscript{25-27} The condition is accompanied at the homozygous or compound homozygous state by a variable bleeding tendency. It is not clear yet whether heterozygotes present a bleeding tendency or not. The defect has been described all over the world, but it is still considered a rare defect with a prevalence of 1:1 500 000. Only about 60 mutations have been described so far and this indicates that molecular biology studies of this defect have lagged.\textsuperscript{28}

**Prothrombotic FV Defects**

**Polymorphisms.** The common polymorphism Arg506Gln in exon 10 known as FV Leiden is associated with a thrombophilic state.\textsuperscript{2,25} The mutated FV is resistant to activated protein C (protein C resistance) and causes a persistence of aFV in the circulation. Such delayed neutralization of aFV and of FVIII results in a prothrombotic state that causes mainly venous thrombosis. The effect on arterial thrombosis is limited, if any. The impact of FV Leiden on venous thrombosis seems more important than that of the prothrombin polymorphism. Other less frequent mutations in FV, such as FV Hong Kong (Arg306-Gly), FV Cambridge (Arg306Thr), and FV Bristol (Ile359Thr), have been reported to cause similar effects.\textsuperscript{29-31}

**Prothrombotic Abnormal Factor V Defects.** No dysform of FV has so far ever been maintained to have associated with thrombosis. A few patients with FV deficiency have been reported to have thrombosis, venous or arterial,\textsuperscript{52} but no specific link with a mutation has never been established (Table 1).\textsuperscript{26,28}

**Discussion**

This evaluation of the spectrum of prothrombin defects demonstrates that the assumption that these defects were associated only with bleeding was inaccurate.\textsuperscript{9,10,33} The prothrombotic role of the G to A 20210 polymorphism represented the first suggestion that genetic changes in the prothrombin molecule could predispose to thrombosis rather than to bleeding.\textsuperscript{3} However, the defect was mild and its significance sometimes questioned.\textsuperscript{21,23} The description of the dysprothrombinemias due to the Arg596 mutations has allowed stronger conclusions about the prothrombotic effect of some prothrombin mutations. It is interesting to note that all the 3 mutations Arg596Leu (Yukuhashi), Arg596Gln (Belgrade), and Arg596Trp (Padua 2) were heterozygous, showed no bleeding tendency, and were associated with venous thrombosis, but for one case, at a young age.\textsuperscript{11-15} This suggests that homozygotes, so far never reported, could present a very severe thrombotic clinical picture. This, indirectly, confirms the known fact that homozygotes with true deficiency and prothrombin levels of less than 5% show a very severe bleeding tendency. Cases with complete absence of prothrombin are maintained to be incompatible with life.\textsuperscript{9,10} Recently, another case of dysprothrombinemia with bleeding and venous thromboembolism has been reported.\textsuperscript{34} The condition was due to an Arg382His mutation which has no relation with the Arg596 group. The proposita had deep thrombosis and postpartum pulmonary embolism after she had received 600 U of prothrombin complex concentrate (PCC), every week, starting on the sixth week of pregnancy for bleeding prophylaxis for a total of approximately 20 400 U. Furthermore, the patient was homozygous for the defect and had an important bleeding tendency. These observations clearly indicate that the patient has no similarities with the Arg596 group patients who had no bleeding tendency. This case is probably a case of dysprothrombinemia with PCC and pregnancy-induced thrombophilic state and thromboembolism.\textsuperscript{35}

Taken all together, these new observations indicate that prothrombin, after FV, should be considered as clotting factors potentially involved with both bleeding and thrombosis. Finally, they suggest that, in conformity with what has occurred for FV and FII, other clotting factors might show a similar pattern.

The demonstration that prothrombin, besides FV, may be involved both in bleeding and thrombosis indicates the need of a comparison between the 2 clotting factors. The double role played by FV is due to a polymorphism and not due to a missense mutation.\textsuperscript{4} Furthermore, in the case of FV Leiden, we have resistance of the mutated FV to the downregulating effect of activated protein C. The contrary, in the case of prothrombin, we have 2 mechanisms, namely an increase in the level of this factor due to the G20120A polymorphism and a missense mutation in a specific area of the protein (Arg596) encoded by exon 14 which renders the prothrombin resistant to antithrombin\textsuperscript{71} while there is no increase in prothrombin activity. So far there is no known missense mutation in FV associated with thrombophilic state. The few FV abnormalities (type II defects) described have never been associated with thrombophilic state and thrombosis.\textsuperscript{26,27} The 2 “short” FV defects recently described (East Texas FV and FV Amsterdam) have only a mild bleeding tendency but are not associated with venous or arterial thrombosis.\textsuperscript{36,37}

Nothing is known about the prevalence of the prothrombin Arg596 mutations in the general population. A recent study by Wu et al has demonstrated the presence of 14 heterozygotes among 267 Chinese patients with idiopathic venous thromboembolism.\textsuperscript{38} There is a certain parallelism between the Arg596 Prothrombin abnormalities and Arg338Lys FIX Padua.\textsuperscript{39} The latter condition is due to a mutation in the FIX protein associated with greatly increased levels of activity (about 10 times the wild type), no bleeding, and a prothrombotic tendency that causes venous thrombosis. This similar behavior of FIX and FII, despite the differences in factor activity, is of interest because of the different genetic pattern (X-linked vs autosomal recessive) shown by the 2 factors despite their similar vitamin K dependency.

The demonstration that at least 1 (FII) or 2 (FII and FIX) clotting factors, in addition to the known case of FV, originally considered to be associated only with bleeding, may, given certain polymorphisms or specific missense mutations, be also associated with thrombotic event has represented a major change in our approach to clotting factor deficiencies. The
possibility that other clotting factors might undergo the same revision cannot be completely excluded.

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