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Review

# Clotting Factors that may be responsible for both hemorrhagic or thrombotic Disorders: the genetic plot has become complex for Factors I, II, V, VII and IX

***Antonio Girolami*, Claudia Santarossa, Silvia Ferrari, Elisabetta Cosi, Bruno Girolami and Maria Luigia Randi***

*Department of Medicine, University of Padua Medical School, Padua, Italy*

**Corresponding authors*
**Antonio Girolami**
Department of Medicine
University of Padua Medical School
Via Ospedale 105
Padua, Italy, 35128
Tel. 0039-049-8213026
Fax: 0039-049-657391
**E-mail:** antonio.girolami@unipd.it

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**ABSTRACT**

Clotting factors deficiencies are commonly associated with bleeding. Recent studies have shown that this is not always the case. The first clotting factor defect shown to be associated with both bleeding and thrombosis was fibrinogen or FI. It was shown in fact that the administration of plasma or fibrinogen concentrate could cause in patients with afibrinogenemia massive arterial or venous thrombosis. Dysfibrinogenemias present in about 25% of cases spontaneous thrombotic events involving both veins and arteries. Subsequently it was demonstrated that a polymorphism of FV (FV Leiden) and a polymorphism of FII (G20210A) cause a thrombophilic state with consequent, especially in association with triggering events, appearance of venous thrombosis. About a decade ago it was demonstrated that two mutations of FVII gene, Ala-294Val and Arg304Gln, were associated with a mild bleeding tendency and venous thrombosis. Both these FVII mutations are Type II defects with low activity and normal or near normal FVII antigen. Furthermore in 2009 it was shown that the rare mutation Arg338Leu in exon 8 of the FIX gene was associated with venous thrombosis. Finally in 2012 a dysprothrombinemia due to the Arg596Leu mutation of exon 15 (Prothrombin Yukuhashi) was shown to have no bleeding tendency but, instead, venous thrombosis. In the following years two other mutations of the same residue, Arg596Gln (FII Belgrade) and Arg596Trp (FII Padua 2) were reported. The impact of these defects in the pathogenesis of venous thrombosis is variable, being important for the FII and FV polymorphisms but less significant, because of their rarity, for the FIX and the FII mutations. These studies have considerably contributed to the understanding of blood clotting and the clarification of the mechanisms of the thrombophilic state and of venous thrombosis. With the exception of fibrinogen defects, the role played by other abnormalities in arterial thrombosis seems limited, if any.

**INTRODUCTION**

Clotting factor defects are usually associated with a bleeding diathesis. In the past only fibrinogen had been associated with thrombotic events as exemplified by patients with dysfibrinogenemias or hypodysfibrinogenemia (1). In the early 1990s another clotting factor has become the source of a prothrombotic state, namely a FV polymorphism. The mutation involved, Arg-506Gln (Factor V Leiden) and was discovered in 1994 (2). However, the defect had been suspected one year before under the term of Activated Protein C Resistence (3).

In recent years other clotting factors were suspected or demonstrated to be associated with a thrombotic tendency. The factors are, in chronological order, FVII, FII and FIX.

The discovery that a clotting factor responsible for bleeding might also be associated with thrombotic events has created a vast interest and has spurred greatly our knowledge on the structure-function relation of these clotting factors (4, 5).

The purpose of this review is to deal with these defects in a systematic way. This seems indicated since no similar papers have so far appeared. Furthermore, it is hopeful that the review will further stimulate the study of these "double-faced" clotting factors.

**Classification**

The coagulation factors which have been demonstrated to be associated with both a bleeding tendency and a thrombophilic state are the following: FI (fibrinogen), FII (prothrombin), FV (proaccelerin), FVII (proconvertin), FIX (antihemophilic factor B). The main features of these clotting factor deficiencies will be dealt with, singularly.

The single deficiencies will be dealt according to the common nomenclature of clotting factors, namely FI, FII, FV, FVII and FIX even though this order does not correspond to the order of the discovery of their prothrombotic effect.

**A) Fibrinogen defects**

Fibrinogen defects are subdivided in Afibrinogenemia, Hypofibrinogenemia, Dysfibrinogenemia and Hypodysfibrinogenemia (1). The first two show a variable bleeding tendency that may be severe in afibrinogenemia with a high prevalence of brain haemorrhage. Thrombotic manifestations, mainly arterial, in afibrinogenemia or hypofibrinogenemia occur after replacement therapy with fibrinogen concentrates or Fresh Frozen Plasma (FFP) (6, 7).

Patients with Dysfibrinogenemia, usually heterozygotes, are asymptomatic in 50% of cases, show a mild bleeding diathesis in 25% of cases and thrombotic events in the remaining 25%. Hypodysfibrinogenemia are compound heterozygotes with hypofibrinogenemia. They usually show, like the Dysfibrinogenemia, a mild bleeding tendency together with a prothrombotic one (1).

The conditions that represent the dual behaviour, prohemorrhagic and prothrombotic, are actually the Dysfibrinogenemias and the Hypodisfibrinogenemia. Dysfibrinogenemias may present only thrombotic events.

The mutation Arg554Lys has been frequently found in families with Dysfibrinogenemia and thrombosis (Paris or Dusart, Nashville, Chapel Hill) (8-10).

Dysfibrinogenemia may be subdivided in those due to 1) impairment of the transformation of fibrinogen to fibrin and 2) impairment of the conversion of fibrin monomers to polymers (1).

Thrombosis in congenital afibrinogenemia or hypofibrinogenemia do not occur spontaneously (1).

**B) Prothrombin defects**

The Prothrombin defects associated with bleeding and thrombosis are two, namely (10) 1) the prothrombin polymorphism G20210A and 2) the dysprothrombinemias due to mutations of residue Arg596 of exon 15 (11).

1) The polymorphism G20210A is widely diffuse, about 2-3% of the population. The carriers have no bleeding tendency but instead present a mild prothrombotic tendency for venous thrombosis and no effect on arterial thrombosis. The prothrombotic defect is mild and is often associated with triggering events or conditions (oral contraceptives, immobilization, trauma, concomitant presence of FV Leiden etc). Spontaneous or idiopathic thrombosis is rare, if any. Recurrences seem also rare (12-18).

2) Prothrombin deficiency is subdivided in two types, namely Type I or cases of true deficiency in which there is a severe, concomitant decrease of both prothrombin activity and antigen and Type II where prothrombin activity is low but prothrombin antigen is normal or near normal (19, 20). The bleeding tendency is more severe in Type I with respect to Type 2 (19, 20).

Very severe Prothrombin deficiency (less than 1%) may be incompatible with life (19, 20). Even heterozygotes for Prothrombin deficiency may present excessive bleeding at delivery or after tonsillectomy or tooth extraction (21).

The Type 2 form, due to mutations in residue Arg596 of exon 15, presents venous thrombosis and no bleeding tendency.

The first dysprothrombinemia with thrombosis was described in 2012 in Japan as Prothrombin Yukuhashi (11).

The proposita was a 12 year old female who had no bleeding tendency and had her first thrombosis at the age of 11. The patient was demonstrated to have a prothrombin abnormality due to an Arg596Leu mutation that possessed a gain of function. The gain of function consisted in an increased resistance towards antithrombin (Figure 1). Immediately after this first patient, other similar cases were reported involving always the AA Arg596 but substituted by Gln or Trp, instead of Leu as in the original case (Prothrombin Belgrade and Prothrombin Padua2) (22-25).

The main features of these dysprothrombinemias are gathered in Table 1. The most important common aspect presented by this group of patients, regardless of the country of origin, Japan, Serbia, Italy, is the occurrence of venous thrombosis at a young age (11-27 years). In addition, they are all heterozygotes and have no bleeding tendency. At about the same time a patient from India was reported (prothrombin Amrita) with a dysprothrombinemia due also to an Arg596Gln mutation. However, this case shows a difference from the previous group, despite the same mutation seen in Prothrombin Belgrade (Arg559Gln), since the venous thrombosis occurred at the age of 60. Furthermore, there is no family study (25).

Ding et al. have recently reported a family in which there are two patients with an Arg382His mutation and an important bleeding tendency, one of whom had a post-partum deep vein thrombosis (DVT) and pulmonary embolism (PE) (26).

| Authors (Year) | Age, Sex | FII Act | FII Ant | Bleeding | Venous Thrombosis (Age at First Episode) | Mutation | Genotype | Eponym | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Miyawaki et al (2012) | 17, F | 37,6 [a)] | 63,8 [a)] | No | Yes (11 years) | Arg596Leu | Het | Prothrombin Yukuhashi | Patient from Japan |
| Djordeyevic et al (2013) | | | | | | | | | |
| Fam I | n.r., F | n.r. | n.r. | n.r. | Yes (17 years) | Arg596Gln | Het | Prothrombin Belgrade | Six patients in two families |
| Fam II | 27, F | 46 [a)] | 144 [a)] | No | Yes (16 years) | Arg596Gln | Het | | |
| Sivasundar et al (2013) | 60, M | n.r. | n.r. | No | Yes (60 years) | Arg596Gln | Het | Prothrombin Belgrade (Amrita) | Patient from India |
| Kishimoto et al (2016) | 23, F | n.r. | n.r. | No | Yes (15 years) | Arg596Gln | Het | Prothrombin Belgrade | Patient from Japan |
| Bulato et al (2016) | | | | | | | | | |
| Fam I | 47, M | 54 | 80 | No | Yes (38 years) | Arg596Trp | Het | Prothrombin Padua 2 | Seven patients in two families |
| Fam IIt | 29, F | 29 | 89 | No | Yes (27 years) | Arg596Trp | Het | | |

**Table 1:** Cases of Dysprothrombinemias associated with a gain of function toward Antithrombin with consequent appearance of a thrombophilic state.
Abbreviations: Het, heterozygote; NR, not reported.
[a)] Data supplied in "Correspondence". N Engl J Med 2012; 367: 1069-1070 and in Ref. 49 of the present paper.

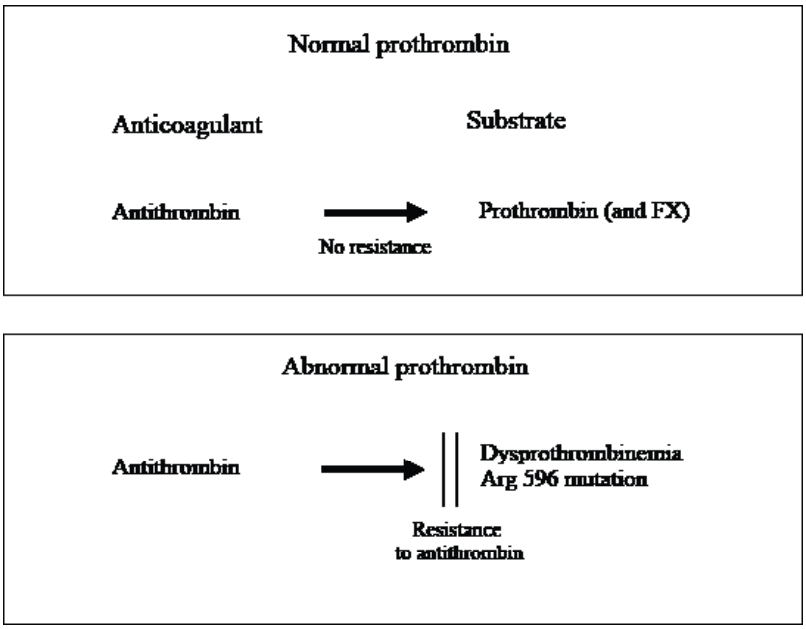


**Figure 1:** Probable mechanism of action for the occurrence of thrombosis in Arg596 mutations in Exon 15 of the prothrombin gene

The interesting peculiarity of this new family is that the proposita and her sister are homozygous for the defect (FII activity 1%; FII antigen normal). All the other previous patients were heterozygous. The proposita is a 30 year old Chinese who had a DVT and PE 28 days post-partum and was treated with low molecular weight heparins and fresh frozen plasma. It is doubtful that this patient may be similar to the previous cases because the patient had received during pregnancy, because of bleeding, 600 Units of a prothrombin complex concentrate (PCC) every week, beginning on the sixt week of gestation for an approximate total of 20400 Units. This prolonged replacement therapy could have created on the long run a thrombophilic state with consequent thrombosis. The patient never had other thrombosis. Equally, her sister never had thrombotic events.

The Arg382His mutation does not appear to belong to the classic group which involves Arg596Leu or Gln or Trp mutations. Due to the different site of the mutation, the condition has to be considered a dysprothrombinemia with both bleeding and thrombosis secondary to intensive replacement therapy. A contributory effect of other risk factors cannot be excluded (27).

### C) Factor V defects may also be divided in FV polymorphism and FV deficiency (28)

The FV polymorphism Arg506Gln (FV Leiden) is responsible for a prothrombotic state that causes venous thrombosis (2). Similar polymorphic mutations have been described: FV Cambridge (29); FV Hong Kong (30) and FV Bristol (31).

FV Leiden is similar, even though a little more severe, to the G20210A prothrombin polymorphism. The condition is widely present in the general population (3-4%) with the exception of population of Japan and other Asian Countries, in which the de-

fect is rare. The prothrombotic effect involves venous thrombosis, whereas there is no sure effect on arterial thrombosis.

Factor V deficiency is also subdivided in Type I and Type II. The conditions are both associated with a variable bleeding tendency (32-34). Contrary to FII, no Type 2 FV defect has been shown so far to be associated with venous thrombosis.

This applies also to the two "short FV" defects (East Texas bleeding disorder and FV Amsterdam) which show only a mild bleeding tendency (35, 36).

**D) FVII defects**

FVII defects are also subdivided in Type I and Type II. Bleeding tendency is variable and not always strictly related to the FVII activity level (37). Heterozygotes do not bleed (38). Patients with Type II defects seem to have, often, only a mild bleeding tendency. These are the patients who sometimes present thrombotic events. The mutations frequently involved with thrombotic events are Arg304Gln (FVII Padua) and Ala294Val (39). These are both Type II defects. According to some Authors, about 3% of patients with FVII deficiency have thrombotic manifestations (37). Venous thromboses prevail and this is in sharp contrast with FXI deficiency in which the opposite occurs with a clear prevalence of arterial thrombosis (40).

It has been demonstrated also that the majority of patients with FVII deficiency and thrombosis have concomitant triggering events such as immobilization, replacement therapy, oral contraceptives, surgical procedures, pregnancy (41).

**E) FIX Defects**

FIX deficiency is responsible for hemophilia B. This is a sex linked severe bleeding disorder. The defect is also subdivided in Type I and Type II. A special form of Type 2 defect (FIX Padua) is due to the hemizygous mutation Arg338Leu in exon 8 (42). The proposita had highly elevated FIX activity while the FIX antigen was only mildly increased. There was no bleeding diathesis but a venous thrombosis. A brother was similarly affected but was asymptomatic.

The clinical significance of this mutation in the pathogenesis of inherited thrombosis is limited since the abnormality seems rare (43, 44). It has instead a great scientific impact since it demonstrates, as it is true for the dysprothrombinemias, that a clotting factor, depending on the site of the mutation, may cause either bleeding or thrombosis (11).

There are other FIX variants due to several mutations, FIX BM and FIX Leiden, but these variants are not associated with thrombophilia (43, 44). Hemophilia BM is a variant of Hemophilia B that is associated with a slight prolongation of the P.T. The prolongation is particularly evident when ox-brain tissue thromboplastin is used in the assay system (45, 46).

The finding is due to an inhibitory effect exercised by the abnormal FIX on the Tissue Factor + FVII complex. Hemophilia BM has to be considered a special form or variant of Hemophilia B+ (Type 2). Hemophilia B+ does not show the inhibitory activity.

Hemophilia B Leiden is characterized by an increase of FIX activity as the age of the patient increases (47). In these two FIX variants no thrombosis has ever been described.

**Discussion**

Prothrombin, FVII and FIX are vitamin K dependent clotting factors. On the contrary Fibrinogen and Factor V have little in common. The only two factors that share the same function are Fibrinogen and FXIII in the sense, that the latter, when activated by thrombin, stabilizes the fibrin monomers thereby supplying solidity to the clot.

However FXIII has been associated with bleeding, never with thrombosis.
No close relation is also evident between Prothrombin and FV. Prothrombin (Thrombin) initiates the clot by splitting a part of fibrinogen α or β chains. The FV polymorphism is instead resistant to activated Protein C. Despite this lack of similarities, they have the ability to be related to both bleeding and thrombosis.

The mechanisms causing thrombosis may be different but the end result is the same. Are there differences in the structure of the thrombus depending on the cause? Studies in this regard are scanty and inconclusive. There are, however, some essential clinical and laboratory differences.

The thromboses of patients with disprothrombinemia due to the Arg596 mutations seem only venous since no arterial thrombosis has been so far described. The same seems true also for FV Leiden and for FIX Padua (28, 42).

On the contrary in fibrinogen defects (afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, hypodysfibrinogenemia) both arterial and venous thrombosis have been described even though arterial ones seem to prevail (1, 6). In afibrinogenemia thrombosis usually follows fibrinogen concentrate or plasma replacement therapy. The clinical picture may be dramatic with massive large arteries occlusions (6, 7). Spontaneous thrombosis is very rare, if any in afibrinogenemia and hypofibrinogenemia.

On the contrary in Dysfibrinogenemia and in Hypodysfibrinogenemia thrombosis is often spontaneous or at least it appears to be so (1).

On the contrary, venous thromboses prevail in FVII deficiency. Thrombosis in this case seem to occur more frequently in patients with a Type 2 defect, namely in patients with cross reacting material (CRM+) in their plasma. It has been postulated that the normal or near normal FVII antigen may create a sort of hypercoagulable state that may transform in thrombosis when the patient is exposed to triggering events (41).

| Defect | Only Arterial | Mainly arterial | Only venous | Mainly venous | Associated causes |
|---|---|---|---|---|---|
| Afibrinogenemia Hypofibrinogenemia | No | Yes | No | No | Usually due to replacement therapy |
| Dysfibrinogenemia | Yes | No | No | No | Usually spontaneous |
| Hypodysfibrinogenemia | No | Yes | No | No | Usually due to replacement therapy |
| Dysprothrombinemias | No | No | No | Yes | Usually spontaneous |
| FII polymorphism (G20210A) | No | No | Yes | No | Usually after triggering events |
| FV polymorphism (FV Leiden) | No | No | Yes | No | Usually after triggering events |
| FVII defects | No | No | No | Yes | Usually caused by triggering events |
| FIX abnormality (FIX Padua) | No | No | Yes | No | Spontaneous |

**Table 2:** Tentative present day status of arterial and venous thrombosis in Fibrinogen defects, Dysprothrombinemias, FII and FV polimorfisms, FVII deficiency and FIX abnormality

It has been demonstrated in fact that almost all patients with FVII defect who develop venous thrombosis after exposure to triggering events (trauma, immobilization, oral contraceptive, replacement therapy) belong to Type 2 defects (39, 41). A common culprit is replacement therapy with activated FVII concentrates (41, 48).

Thromboses due to FV polymorphisms are mainly venous and are frequent because of the widespread, even though variable, diffusion of the defect among the World population (low in Asia and Africa, relatively high in Europe).

It is estimated that the polymorfism is present in about 3-4% of the general population. Such a large prevalence justifies the frequent finding of the defect in patients with venous thrombosis. Its role in arterial thrombosis is limited, if any.

The five clotting defects cause therefore discrepant rates of arterial vs venous thrombosis (Table 2).

The exclusive association of dysprothrombinemias and FIX abnormality with venous thrombosis is limited to the present period. It cannot be excluded that the follow-up of these patients could demonstrate also the occurrence of an arterial thrombosis or that the future may reveal the appearance of an arterial thrombosis in new patients.

This review has demonstrated the complexity of blood clotting defects. The original thought that a defect of a given clotting factor may be associated only with bleeding has to be abandoned. A clotting defect that usually causes bleeding may also, given certain mutations in its structure, be responsible for a prothrombotic tendency. This is a surprising and spectacular achievement which is in contrast with what observed for natural anticoagulants, such as antithrombin, Protein C and Protein S. These proteins have so far remained only antithrombotic in the sense that no mutation in their structure has ever been associated with bleeding.

## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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