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REVIEW ARTICLE

Peculiar, poorly known, rare congenital bleeding disorders presenting thrombotic events: an understudied chapter of molecular, blood coagulation defects

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ABSTRACT

Very rare, peculiar congenital bleeding disorders are usually dealt with in clinics without giving much importance. We think that this practice is not correct since the disorders may often provide useful information about blood coagulation. In this review, we assess very rare bleeding conditions. We refer to the defects of the fibrinolytic system, alpha 1-antitrypsin Pittsburg, few dysprothrombinemias, east Texas or short FV defect, FIX Padua, and thrombomodulin (TM) abnormality. These defects are usually not included in rare bleeding disorders. Patients were gathered from two sources: personal files and two time-unlimited PubMed searches carried out on February 2010 and July 2019. Combined defects were disregarded. These rare bleeding conditions are often unrecognized even though some of them, such as antiplasmin deficiency, are not that rare with more than 30 cases reported already. The underevaluation of the fibrinolytic defects is due to the decrease in the use of methods capable of detecting increased fibrinolysis in routine laboratory study. The limited use of immunological tests represents a second drawback as in the dysprothrombinemia, east Texas Factor V, and FIX Padua. Finally, the limited use of assays of natural inhibitors such as tissue factor pathway inhibitor and TM has played a role in delaying east Texas FX recognition and TM defect. The study of rare, peculiar bleeding disorders has been very important in clarifying the nature of the defects, and it has even allowed the identification of mutations that may turn them from prohemorrhagic to prothrombotic in some of these proteins. This has greatly contributed to the understanding of the complex relationship existing among clotting defects.

Keywords: Ignored, unrecognized, bleeding, thrombotic disorders.

Introduction

Congenital bleeding disorders are usually divided into 1) common bleeding disorders and 2) rare bleeding disorders. In the former, the hemophilias and von Willebrand disease are usually included. In the latter, FI, FII, FV, FVII, FX, FXI, and FXIII deficiencies are usually dealt with. In recent years, other peculiar bleeding defects have been restudied or newly reported. These are alpha-2 plasmin inhibitor (antiplasmin) deficiency (1,2), plasminogen activator inhibitor-1 (PAI-1) deficiency (3), alpha 1-antitrypsin Pittsburg defect (4,5), the dysprothrombinemia associated with venous thrombosis (6), and the FIX abnormality that shows venous thrombosis and no bleeding (7), east Texas clotting disorder (8,9), and thrombomodulin (TM) mutation disorder (10,11) (Table 1). Due to the fact that most of these conditions have been described and/or duly investigated during the past few years and the fact that those previously described, for example, the fibrinolysis defects, had received, at the time of their discovery, little attention, there is widespread ignorance about these disorders among those in charge of bleeding disorders and, sometimes, even among coagulation experts. The ignorance might have prevented the identification of other similar cases or allowed the mismanagement of some bleeding conditions much to the detriment of the patients

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Table 1. *Approximate number of families with these rare defects reported in the literature.*

Defect	Number of cases	Bleeding	Mutations	Gene name	Gene Symbol	Genotype	Comments
α_1-antitrypsin Pittsburgh	4	Variable	Met358Arg	Serpin family A member 1	*SERPINA1*	Het	The same mutation in all families All patients Het
α_2-antiplasmin	30	Variable	Several mutations	Serpin family F member 2	*SERPINF2*	Hom Het	Three sisters in one family
Plasminogen activator inhibitor-1	15	Variable	Ile120Asp	Serpin family E member 1	*PAI-1* *SERPINE1*	Hom Het	
Dysprohrombinemias	5	None	Arg596Leu Arg596Gln Arg596Trp	Coagulation factor II, thrombin	*F2*	Het	Venous thrombosis
Short FV (east Texas defect)	2	Mild	Ser756Gly Ala863Gly	Coagulation factor V	*F5*	Hom	Two unrelated families, different mutation
F IX Padua	1	None	Arg338Leu	Coagulation factor IX	*F9*	/	Venous thrombosis
Thrombomodulin mutation	2	Mild	Cys537Stop	TM	*THBD*	Het	Same mutation in two unrelated families

Table 2*. Main features of the bleeding conditions dealt with in the present review.*

Condition	Bleeding	Thrombosis	Mutation	Gene name	Gene Symbol	Hom	Het	Comments
Dysprothrombinemias	No	Yes	Arg596Leu Arg569Gln Arg596Trp	Coagulation factor II, thrombin	*F2*	No	Yes	Only venous thrombosis
Short FV Defect	Yes	No	Ser756Gly Ala863Gly	Coagulation factor V	*F5*	Yes	Yes	Two forms: FV east Texas and FV Amsterdam
F IX Padua	No	Yes	Arg338Leu	Coagulation factor IX	*F9*	/	/	
α_1-antitrypsin Pittsburgh	Yes	No	Met358Arg	Serpin family A member 1	*SERPINA1*	No	Yes	Bleeding: variable. The same mutations in all 4 families
α_2-plasmin inhibitor (antiplasmin)	Yes	No	Gln del 137; Val384Met; IV56+IG>A, etc	Serpin family F member 2	*SERPINF2*	Yes	Yes	Bleeding: variable
Plasminogen activator inhibitor-1	Yes	No	Ala15Thr Ile120Asp, etc	Serpin family E member 1	*PAI-1* *SERPINE1*	Yes	Yes	Bleeding: variable
TM defect	Yes	No	Cys537Stop	TM	*THBD*	No	Yes	Bleeding is mild Two families with the same mutation

(Table 2). The purpose of the present review is to deal with these peculiar, underestimated, and poorly known molecular defects.

Subjects and Methods

All patients with the following defects have been investigated: 1) defects of the alpha 2-plasmin inhibitor (alpha 2-antiplasmin), 2) defects of alpha 1-PAI-1, 3) alpha 1-antitrypsin Pittsburgh abnormality, 4) dysprothrombinemia with thrombosis and no bleeding, 5) short FV conditions (factor V east Texas and factor V Amsterdam), 6) FIX abnormality with thrombosis and no bleeding (factor IX Padua), and 7) TM with a Cys537 Stop mutation defect. The papers were gathered from two sources: 1) personal files and 2) two time-unlimited searches in PubMed carried out on February 2010 and July 2019. Side tables and mesh words were also evaluated or used as retrieval keys. Once gathered, the original papers were obtained with the help of the Pinali Medical Library of the University. The references of the single papers were duly compared and examined to avoid omissions. Combined defects were excluded from the study. The molecular biology studies with consequent identification of a mutation were recorded whenever available. The bleeding tendency present in these conditions was evaluated and reported as mild, moderate, or severe according to accepted clinical observations. Peculiar bleeding manifestations seen in some of these conditions were duly detailed. The occurrence of thrombotic events in these patients was also recorded whenever present. Such thrombotic events had to be demonstrated by objective methods.

Results

Alpha 2-plasmin inhibitor (antiplasmin) deficiency

Alpha 2-plasmin inhibitor is a single-chain glycoprotein with a molecular weight of approximately 70.000 Da. It is similar in structure to alpha 1-antitrypsin and antithrombin (AT) and belongs to the serine-proteinase inhibitors (serpins). These inhibitors react with their target to form a stable inactive complex. A schematic representation of the fibrinolytic system is shown in Figure 1. There are at least 30 cases of alpha 2-plasmin inhibitor deficiency reported in the literature (1,2,12-18), and therefore, the defect seems relative, more frequent than the other disorders here discussed. This is well documented by the fact that the condition has been the object of two reviews (17,18). The majority of antiplasmin defects belong to type I. Type 2 defects with normal antigens are rare (17,18). The deficiency is associated with a variable bleeding tendency in the homozygotes, whereas heterozygotes are rarely symptomatic. The bleeding occurs a few hours after a wound or surgical procedure. In the beginning, the clot seems normal, but then it becomes friable due to the continuous action of the unchecked plasmin present on the clot surface. Bleeding from the umbilical stump is also common. The type of bleeding is similar to that seen in FXIII deficiency (late bleeding). The homozygotes may present a peculiar form of bleeding, namely, intramedullary bleeding in long bones (13). It is interesting to note that this type of bleeding has not been described in other fibrinolytic disorders (PAI-1). It has been described instead in afibrinogenemia (19). A type 2

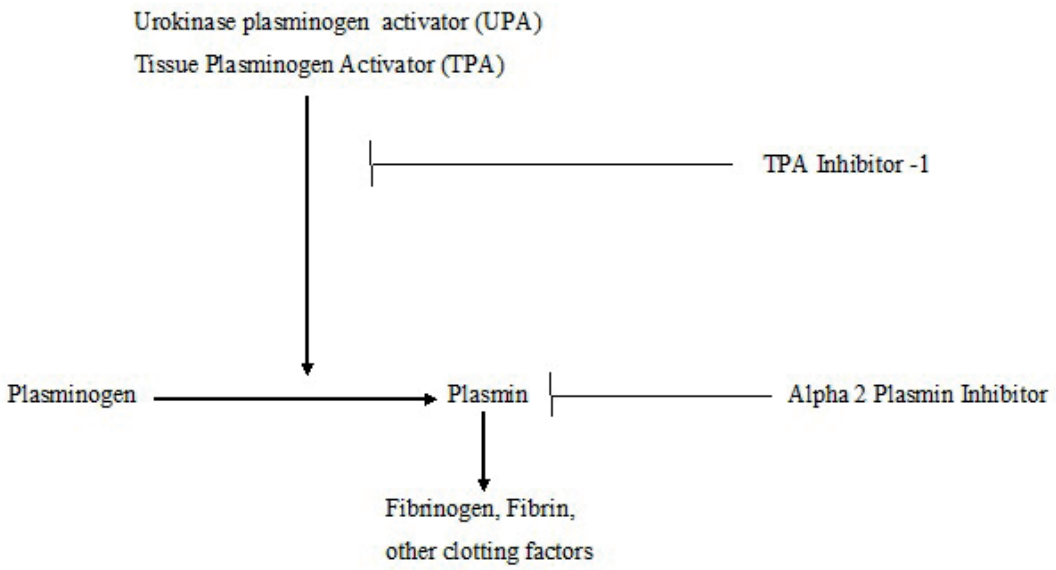

Figure 1. *Schematic and simplified representation of fibrinolytic system.*

variant of alpha 2-plasmin inhibitor (alpha 2-antiplasmin Enschede is characterized by a mutation G-G insertion in exon 10) renders the protein unable to inhibit plasmin and becomes a substrate for plasmin instead. The complex that is formed is not stable so that plasmin is liberated and is responsible for a mild bleeding tendency in the homozygote (20). Other mutations have been described in other families with antiplasmin deficiency. They are type I defects, namely, they show concomitant low activity and antigen. These are antiplasmin Okinawa (glutamic acid deletion), antiplasmin Nara (frameshift mutation), antiplasmin Paris (G to A splicing site mutation), and a few others (17,18).

Plasminogen activator inhibitor 1

Plasminogen activator inhibitor 1 is a serine protease inhibitor that inhibits both tissue-type and urokinase-type plasminogen activators. It is released mainly by platelets, and it forms stable complexes with plasminogen activator, thereby playing an important role in the control of fibrinolysis. Congenital PAI-1 was first described in 1989 (21). A few additional cases have been reported after that year (22-28). The congenital deficiency is rarer than that of antiplasmin deficiency. Only about 15 cases have been described so far, including a few heterozygotes who are also slightly symptomatic. The defect is accompanied by a mild bleeding tendency, which usually occurs 1 or 2 hours after the clot formation. Delayed bleeding may also occur in FXIII and antiplasmin deficiencies. Furthermore, these patients show prolonged wound bleeding. This condition could also be confused with FXIII deficiency, but it is usually less severe.

The basis for the bleeding resides in the lack of control of the plasminogen activators on the surface of the clots. This causes an increased local fibrinolysis due to plasmin formation. The molecular studies in these patients are rare. In one family, a homozygous frameshift mutation was reported in patients of Amish extraction together with several heterozygotes. The mutation caused the complete absence of PAI-1 (23). The administration of epsilon aminocaproic acid has been employed for the control of the bleeding. Fresh frozen plasma (FFP) was also used during pregnancy (23). Prognosis is usually good.

Alpha 1-antitrypsin Pittsburgh defect

Alpha 1-antitrypsin is an inhibitor of trypsin. It belongs to the serpins group and normally has no sure role in blood coagulation. The condition was first described in 1978 by Lewis et al. as a form of AT defect (4). Subsequently, it was clarified that a Met358Arg mutation in the alpha 1-antitrypsin molecule had transformed it in an AT (5). Only five patients with a deficiency of alpha1-antitrypsin have been described so far, and all are due to the same mutation (5,29-31). The patients described seem to be all heterozygotes and show a variable bleeding tendency, which becomes more pronounced after trauma. This is explained by the increase of alpha 1-antitrypsin that occurs after a trauma or acute phase reactions (Figure 2). The mechanism underlying the bleeding tendency is due to a potent, heparin-independent, AT activity of the mutated alpha 1-antitrypsin (5). Since the mutated alpha 1-antitrypsin also inhibits plasminogen activation and plasmin, the bleeding may be mitigated (5). The mutated alpha 1-antitrypsin also inhibits protein C activation, and this could also contribute to reducing the bleeding tendency by decreasing the inhibition of FVa and FVIIIa (5). All clotting tests are severely prolonged, but the defect is corrected by the addition of normal plasma, contrary to what happens in the plasma of patients treated with heparin. The therapeutic approach, when needed, is based on FFP administration.

Conditions with Increased Plasminogen Activator Level

For the sake of completeness, a mention is due to the few patients reported to have a bleeding tendency secondary to increased plasminogen activators levels. Unfortunately, the first case has no family history and appears an acquired condition secondary to dyslipidemia (32), as seen in hypothyroid patients (33). The second case (34)

Figure 2. *Schematic representation of the action of alpha 1-antitrypsin Pittsburg. The bleeding occurs mainly after trauma when there is an acute reaction-induced surge in the production of the mutated alpha 1-antitrypsin.*

refers to a family with patients who showed increased plasminogen activator levels and a mild bleeding tendency. There are doubts about this family, too, since no genetic studies could be carried out. The observation that no further cases have been reported strongly suggests that the cases were not congenital.

Dysprothrombinemias (AT Resistance) with Thrombosis

Congenital prothrombin deficiency is one of the rarest coagulation disorders. Homozygotes or compound heterozygotes with FII levels of less than 10% of normal present a severe bleeding tendency. The complete absence of prothrombin is incompatible with life (35,36). Heterozygotes with FII activity levels around 50% of normal may present occasional bleeding during surgery or tooth extractions (37). No thrombotic event has ever been reported in prothrombin deficiency. Recently, a few cases of prothrombin abnormalities (dysprothrombinemia) have been associated with a thrombotic tendency due to AT resistance (6,38-41). AT is a small glycoprotein of a molecular weight of 58,000 Dalton produced by the liver and circulates at a concentration of about 0.12 mg/ml. When coupled with heparin, it exerts mainly an anti-FII and anti-FX activity. Without heparin, the activity of AT is markedly reduced. The increased resistance of these abnormal prothrombins to the action of AT creates a condition of prolonged thrombin activity, which may cause thrombosis. This is a new clinical entity characterized by a relative decrease of AT activity due to the presence of abnormally resistant prothrombins (thrombins), which have mutations in a special region of the molecule, encoded by exon 14, that is supposed to interact with AT. Due to these mutations, the generation of the complex thrombin (T)-AT is defective, whereby thrombin persists in the circulation, and a thrombophilic state ensues.

The first prothrombin abnormality responsible for this effect was reported in Japan in 2012 (Prothrombin Yakuhashi) (6). Subsequently, other similar cases were published in Serbia, India, and Italy (38-41). The five families involve three different mutations on the same amino acid, an Arginine: Prothrombin Yakuhashi Arg596Leu (6), Prothrombin Belgrade Arg596Gln (38), and Prothrombin Padua 2, Arg596Trp (39). Another prothrombin abnormality seen in Japan is identical to prothrombin Belgrade, namely Arg596Gln (40), and prothrombin Amrita was seen in India which also has an Arg596Gln mutation (41). It is still unknown at what level of the codon sequence a mutation can cause the shifting of a bleeding condition into a thrombophilic one. Upstream, we have prothrombin Perija (Gly591Ala) (42). Downstream, after a.a.596, we have prothrombin Scranton (heterozygote for Lys599Thr) (43). It is interesting to note that all these patients are heterozygotes for the mutation.

The main laboratory and clinical features of these patients are shown in Table 3. All patients had venous thrombosis;

Table 3. *Cases of dysprothrombinemias due to Arg596 mutations associated with a gain of function towards AT with consequent appearance of a thrombophilic state. Other cases with the Arg596Gln mutation have been reported in Japan and India. The other two mutations have not been seen yet. Arg596Leu and Arg596Trp in other families. Recently, another prothrombin mutation, Tyr434His in Exon 11 has been reported to be associated with venous thrombosis (51). In this case, the underlying mechanism is not antithrombin resistance but increased activity toward fibrinogen and other proteins.*

Authors (Year)	Age, Sex	FII Act	FII Ant	Bleeding	Venous Thrombosis (Age at First Episode)	Mutation	Gene name	Gene Symbol	Genotype	Eponym	Comments
Miyawaki et al.	17, Female	37.6[a]	63.8[a]	No	Yes (11 years)	Arg596Leu	Coagulation factor II, thrombin	*F2*	Het	Prothrombin Yukuhashi	Patient from Japan
Djordjevic et al. Fam I	NR, Female	NR	NR	NR	Yes (17 years)	Arg596Gln	Coagulation factor II, thrombin	*F2*	Het	Prothrombin Belgrade	Six patients in two families
Fam II	27, Female	46[a]	144[a]	No	Yes (16 years)	Arg596Gln			Het		
Bulato et al. Fam I	47, Male	54	80	No	Yes (38 years)	Arg596Trp	Coagulation factor II, thrombin	*F2*	Het	Prothrombin Padua 2	Seven patients in two families
Fam II	29, Female	29	89	No	Yes (27 years)	Arg596Trp			Het		

[a]Data supplied in "Correspondence." N Engl J Med 2012; 367:1069-70 and in Ref. 49 of the present paper.
Het, heterozygote; NR, not reported.

there is no information about arterial thrombosis. Clinical suspicion should arise from the following observations: 1) venous thrombosis in a young patient known to have no other known prothrombotic defects, 2) slightly decreased or borderline low prothrombin activity level, 3) prothrombin antigen level higher than the activity counterpart, and 4) positive family history of venous thrombosis. Genetic analysis is needed to confirm the suspicion. The occurrence of thrombosis at a young age is of paramount importance. The mean age, excluding the case from India, is 20.1 (range 11-38). The patient from India had a venous thrombosis at the age of 60 years (41).

The therapeutic approach consists of the administration of Coumarin drugs. Should heparin be used, higher than normal dosages are needed because of the "resistance" caused by the prothrombin Arg 596 mutation.

Short FV or East Texas Bleeding Disorder

In 2001, a mild bleeding condition was reported, as an autosomal dominant disorder, in a large kindred from east Texas. The patients had a mild bleeding tendency, but no diagnosis was reached (8). The nature of the defect was clarified several years later (9). It was due to the presence of a short FV that lacked a.a.756-14580 of the B domain. This short FV has a great avidity for tissue factor pathway inhibitor (TFPI). As a consequence, the levels of TFPI in these patients are about 10 times greater than in normal subjects. The high level of TFPI retards or inhibits the function of the tissue factor-FVII complex and, indirectly, FX activation, and this explains the mild bleeding tendency. The condition is also known as "short FV defect" (9). The mutation in exon 13 responsible for the defect is Ser756Gly. Since the B domain is not required for FV activity, this explains the normal levels of FV present in these patients. However, the B domain is essential in maintaining FV in an inactivated state. Once it is removed or reduced, the resulting FV shows a great avidity for TFPI (44). The gain of function of this abnormal FV refers to its binding to TFPI. This leads to the prolongation of TFPI life and its increased plasma levels with a consequent bleeding tendency. In other words, the high TFPI levels interfere with the a FVII-tissue factor formation and activity with a consequent appearance of a hypocoagulable state. Recently, another similar FV defect was reported (FV Amsterdam) (45). This is also a short FV but is different from east Texas FV, where only 63 aa. of the B domain is missing. The number of missing aa. in FV East Texas is 702. In this case, the deletion of most of the B domain results in a greatly increased binding of TFPI. In the case of FV Amsterdam, the increase of TFPI is less pronounced (45), but the clinical and laboratory picture is similar. The mutation is Ala863Gly, always in exon 13.

As there is no geographical or familial relationship between these two families because of the different mutations, it is highly probable that they represent separate founder effects. From a diagnostic point of view, the most important suspicion should arise when a mild familiar bleeding tendency is not explained by any known defect, even if rare as antiplasmin deficiency. The suspicion has to be confirmed by the finding of high levels of TFPI and the correct diagnosis clinched by genetic studies. The significance of an elevated level of TFPI had never been suspected of playing a role in the diagnosis of a bleeding tendency in humans (44,45). TFPI is a polypeptide that has two isoforms such as TFPI and TFPI1 which show different inhibitory activities. TFPI congenital deficiency has never been described in humans, but the inhibitor is, at present, the object of great scientific interest (46). Since the bleeding tendency is mild, there are no specific therapeutic problems. FFP may be used.

FIX Defects

FIX deficiency is responsible for hemophilia B. This is a sex-linked severe bleeding disorder. The defect is also subdivided into type I and type II. The FIX Padua abnormality is due to the hemizygous mutation Arg338Leu in exon 8 (7). The propositus has highly elevated FIX activity, whereas the FIX antigen is only mildly increased. There is no bleeding diathesis but a venous thrombosis. A brother is similarly affected but asymptomatic. The mother is a carrier and asymptomatic. The clinical significance of this mutation in the pathogenesis of inherited thrombosis is limited since the abnormality seems rare (47,48). It has a great scientific impact instead, since it demonstrates, as it is true for the dysprothrombinemia that a clotting factor, depending on the site of the mutation, may cause either bleeding or thrombosis.

Thrombomodulin Defects

Thrombomodulin (TM) is a transmembrane glycoprotein composed of 557 amino acids. It has a great affinity for binding to thrombin and to transform it from a coagulant to an anticoagulant compound (44,48). The complex T-TM (T-TM) has two main actions: 1) it activates protein C, which, in turn, once activated, downregulates FVa and FVIIIa, namely, it behaves as an anticoagulant and 2) it also activates the T activable fibrinolysis inhibitor (TAFI) (49). Activated TAFI inhibits the adherence of plasminogen to the surface of a fibrin clot, thereby decreasing the effect of plasmin-induced fibrinolysis. As a consequence, TM plays an important role in regulating the structure and resistance of the fibrin clot (46). Congenital TM deficiency has never been described. In 2014 and 2015, two families were with a mild bleeding tendency, but normal coagulation tests were reported (10,11). Extensive investigations demonstrated that the defect was due to greatly elevated levels of soluble TM. The genetic analysis of the TM gene showed the presence of the same mutation, namely Cys537Stop, in both the families. Such a mutation is responsible for the gain of function effect of TM. It has to be remembered that another TM mutation (Asp468Tyr) has been associated with venous thrombosis (50). This observation has not

been confirmed yet. Should it be, TM defect should be included among the coagulation defects that may cause both bleeding and thrombosis. A schematic representation of the action of this abnormal TM is shown in Fig. 3. Bleeding is usually mild, but it may become severe after trauma or surgical procedures that generate tissue factor (10,11).

The exact mechanism of bleeding is still not fully explained, but it seems that, in these patients, the effect of the T-TM complex on FVa and FVIIIa (potentially hemorrhagic action) prevails on the effect of TAFI (potentially procoagulant action). The assay method used to evaluate circulating TM is an enzyme-linked immunosorbent assay (ELISA) method. The continuous shedding of TM into the circulation, once bound to thrombin, causes persistent activation of Protein C with secondary down-regulation of FVa, FVIIIa, and inhibition of thrombin generation within the hemostatic clot that, as a consequence, is weak. These patients also show decreased prothrombin consumption, which has not been explained (10,11). The bleeding tendency is mild, and FFP could be given if needed. The description of these two families with the same mutation has considerably spurred the interest in the T-TM complex activities (46). Further studies will clarify the exact features of the defect. Table 4 shows that the mutations seen in these conditions have been added at the end of the description of the single defects.

Discussion

The defects dealt with in the present review are very rare and poorly recognized. They involve the fibrinolytic system (antiplasmin and PAI), another inhibitor alpha 1-antitrypsin (antitrypsin Pittsburgh), FII, Factor V, Factor IX, and TM. Therefore, several aspects or steps of the blood coagulation system are affected. The approximate number of families with these defects and their main features are shown in Table 1. Unfortunately, not all these defects have been thoroughly investigated. For example, there is a paucity of molecular studies about fibrinolysis-related disorders. The fact that only a few of these cases have been reinvestigated by means of molecular biology techniques betrays the limited interest that these defects had originated among coagulation experts. This represents a clear drawback since the opportunity to investigate the molecular basis of the fibrinolytic system, due to their rarity, may have gone lost. The available studies in this regard concern mainly the patients with antiplasmin deficiency (17,18). An exception is represented by the story of α_1-antitrypsin Pittsburgh. The original patient was considered as a case of AT defect (4). It was subsequently demonstrated that a peculiar antitrypsin mutation (5) had transformed the protein in such a way that it behaved as potent AT (5). It is interesting to know that all patients so far reported are heterozygotes.

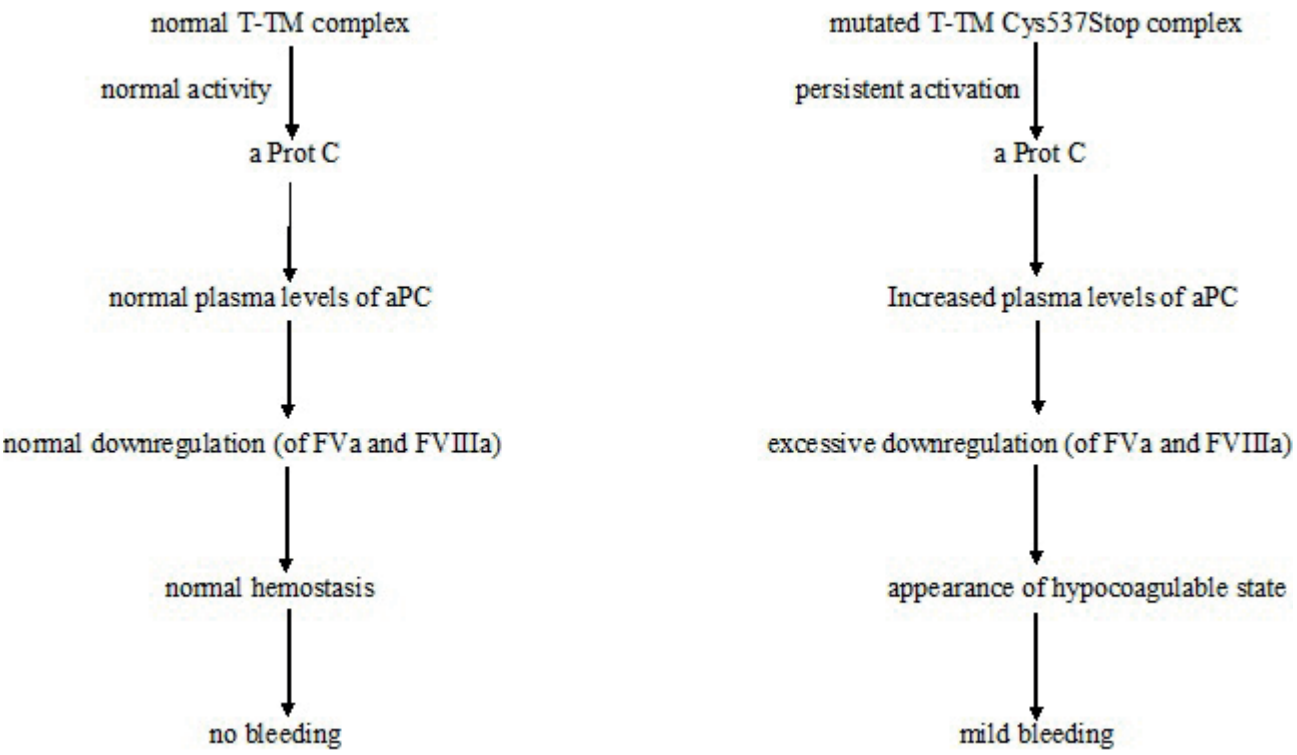

Figure 3. *Schematic representation of mutated TM (Cys537Stop) on the clotting mechanism. The result is the appearance of a mild bleeding tendency, which may warden after trauma or surgery, which increase tissue factor and thrombin function.*

Due to the varied relations existing between alpha 1-antitrypsin Pittsburgh with other clotting proteins (contact phase factors, Protein C) (5), it is not surprising to admit that the presence of such abnormal proteins at the homozygous levels could represent a complex derangement of the clotting system. The discovery of the prothrombotic dysprothrombinemias, FIX Padua, Short FV, and TM defects has complicated the laboratory investigation and diagnosis of blood coagulation disorders. The dysprothrombinemias due to Arg596 mutations show slightly reduced FII levels and no bleeding and instead show venous thrombosis and an unrecognized combination (6). Recently, another prothrombin abnormality (prothrombin Kio) due to a different but always heterozygous mutation, Tyr434His in exon 11, was also associated with venous thromboembolism and no bleeding (51). Should this finding be confirmed, we will have to conclude that two prothrombin abnormalities, the Arg596 mutations and the Tyr434His one, are capable of turning the molecule into a thrombophilic protein. In the latter case, the mechanism of action seems due to increased activation of fibrinogen and other clotting proteins and not to AT resistance (51). The "short" FV defects show normal clotting tests, including normal FV activity together with a mild bleeding tendency and, for the first time, increased levels of a natural inhibitor, the TFPI that had been in the past only of little importance, if any (9). The patients with abnormal FIX (FIX Padua) show no bleeding but venous thrombosis (7).

Finally, a mutation in TM, always considered only a protein capable of binding thrombin, is found to be responsible for a mild bleeding tendency due to increased plasma levels of soluble TM. TM, a transmembrane protein, plays a role also in plasmatic clotting tests and becomes responsible for a mild bleeding tendency (10,11). Due to these considerations, the diagnostic approach to blood coagulation studies has to be modified. The assays of natural anticoagulants (TFPI) or soluble TM have to be included. These assays are so far only immunological, ELISA test assays. It is likely that "activity" assays could be developed in the future. This would eliminate the possibility that discrepancies might exist between "activity" and protein assays. The worsening of bleeding after trauma or surgery in TM disorders is due to the liberation of TF and T (10,11). This causes a continuous activation of protein C with consequent persistent and extensive downregulation of FVa and FVIIIa, namely a neutralization of their procoagulant activity.

As a result of this protracted downregulation of FVa and FVIIIa, a hypocoagulable state appears together with bleeding. The study on the "short" FV and TM mutations has underscored the importance of natural inhibitors in blood coagulation. So far, only AT, antiplasmin, and plasminogen activator inhibitor-1 had a clear, clinical significance. Now TFPI, TAFI, and soluble TM have entered the play. Furthermore, the central role of protein C has received confirmation (46). Finally, a transmembrane protein as TM has been demonstrated to play a double role, both on the endothelial level and circulating plasma. Now, it occurs that natural anticoagulants due to a mutation in a clotting factor ("short" FV) or a mutation in the protein TM may be responsible for bleeding. The exact prevalence of these defects as a whole is still ill-defined. The antiplasmin deficiency seems relatively more frequent since at least 30 cases have been reported (17,18). On the other side, alpha 1-antitrypsin Pittsburgh, the dysprothrombinemias, Short FV defect, FIX Padua, and the thrombomodulin abnormality seem extremely rare. So far, only two forms of short FV due to different mutations have been described (FV East Texas and FV Amsterdam) (14,15). Furthermore, the five families with alpha 1-antitrypsin Pittsburgh show the same mutation (31). Finally, the thrombomodulin defect is limited to two unrelated cases, but they also show the same mutation (10,11). In the dysprothrombinemias and FIX Padua, venous thrombosis was reported. A thrombosis was also reported in a patient with a TM mutation (50), but this has not been confirmed so far. The thrombotic events in all these patients were always venous. All these conditions probably represent a small portion of bleeding conditions in comparison with the Hemophilias, von Willebrand disease, and even with respect to FVII or FXI deficiencies. However, they should be taken into consideration for patients with normal routine clotting tests. Unfortunately, fibrinolysis tests are seldom used in routine laboratory work. This is wrong since antiplasmin is not as rare as the other defects. The widespread use of automatic clotting instruments for routine use does not allow an evaluation of the clot, and therefore, the increased fibrinolysis may escape detection.

Conclusion

This general survey has demonstrated that the study of rare coagulation disorders played an important role in the understanding of the complexity of the coagulation system. In particular it has demonstrated that a bleeding disorder (prothrombin deficiency) may become a thrombotic one if a mutation occurs in a specific point of its molecule.

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