Rare bleeding disorders: diagnosis and treatment

Roberta Palla, PhD¹, Flora Peyvandi, MD, PhD¹ and Amy D Shapiro, MD²,³

¹A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, and Luigi Villa Foundation, Milan, Italy;
²Indiana Hemophilia & Thrombosis Center, Indianapolis, IN, USA
³Pediatrics Michigan State University, East Lansing, MI, USA

Corresponding author:
Amy D. Shapiro, MD
Medical Director
Indiana Hemophilia and Thrombosis Center
Telephone +1 317 871 0000
Fax: +1 317 871 0010
E-mail: ashapiro@ihtc.org

Keywords
Rare bleeding disorders
Bleeding symptoms
Phenotype
Genotype
Clotting factor concentrates
Abstract

Despite the worldwide prevalence of rare bleeding disorders (RBDs), knowledge of these conditions and their management is suboptimal; healthcare professionals often have little diagnostic and treatment experience with variable access to diagnostic modalities required for accurate identification; therefore patients often experience morbidity and mortality due to delayed diagnosis. As RBDs represent a small potential commercial market, few, if any, specific therapies exist for these conditions. As a result, affected individuals commonly face delayed diagnosis, incomplete laboratory evaluation, and limited treatment options.

Standardization and customization of coagulation assays, full genome sequencing, and global clotting assays will significantly improve diagnosis of patients with rare bleeding disorders. In addition, new therapeutic modalities, both recombinant and plasma derived, are emerging at least in developed countries. Registries and clinical trials have demonstrated decreased bleeding and improved outcomes when patients are appropriately diagnosed and properly treated. Expansion and harmonization of international registries has been initiated to correlate genotype, laboratory and clinical phenotype including bleeding severity to improve the diagnosis and the therapeutic approach. This review focuses on the latest advances in our understanding, diagnosis and treatment of rare bleeding disorders.
Introduction

Rare inherited bleeding disorders (RBDs), including deficiencies of coagulation factors fibrinogen, FII, FV, combined FV and FVIII, FVII, FX, FXI, FXIII, and congenital deficiency of vitamin K-dependent factors (VKCFD), are transmitted as autosomal recessive conditions; some cases of FXI and dysfibrinogenemia may be autosomal dominant. RBDs are reported in most populations with homozygous or a double heterozygous incidence varying from 1 in 500,000 for FVII deficiency to 1 in 2-3 million for prothrombin and FXIII deficiencies (Table 1). Relative frequency varies among populations, being higher where consanguineous or endogamous marriages are common, with increased frequency of specific mutant genes.

The evaluation of the world-wide RBD distribution relies on two large surveys that collected epidemiological data; one is led by the World Federation of Haemophilia (WFH, http://www.wfh.org/, accessed July 28, 2014) and the other within the European Network of the Rare Bleeding Disorders (EN-RBD; http://www.rbdd.eu/, accessed July 28, 2014). The WFH began RBD data collection in ~2004, while the EN-RBD project in 2007. Data from both surveys confirmed FVII and FXI deficiencies as the most prevalent representing ~39 and 26% respectively of the total affected population, followed by deficiencies of fibrinogen, FV and FX (8–9%), FXIII (~ 6%) and combined FV and FVIII (~ 3%); the rarest disorder was FII deficiency (prevalence 1%) (Figure 1). The WFH survey revealed that half of available data originated from Europe underscoring the need for increased efforts to establish accurate diagnosis and improved world-wide data collection systems.

Clinical symptoms

Clinical symptoms among RBD patients vary significantly between disorders, and patients, even when affected with the same disorder. A comparison of the most and least common symptoms among those with severe deficient RBDs is shown in Table 2. Heterozygous individuals commonly do not manifest a bleeding tendency. Mucocutaneous and surgical associated bleeding were reported in 20% of patients while post-traumatic hemarthrosis and hematomas are rarely reported in FVII and FX deficiencies. Menorrhagia, spontaneous abortion and bleeding during vaginal delivery were reported in ~20% of women with all deficiencies.
Women with RBDs require specific attention and care; in addition to the common bleeding symptoms, they may also experience gynecologic bleeding. Beyond menorrhagia, affected females are at increased risk of hemorrhagic ovarian cysts, endometriosis, endometrial hyperplasia, polyps, and fibroids. Pregnancy and childbirth pose particular clinical challenges, and miscarriages, bleeding during pregnancy and post-partum hemorrhage have been frequently reported in some deficiencies. These events impact quality of life and employment.

Classification

RBD rarity has resulted in limited knowledge with decreased focus on etiologic and pathogenetic research and difficulty describing natural history and variants. Each RBD can have several bleeding symptoms ranging from minor post-traumatic to severe episodes appearing at birth or later in life. In some deficiencies, residual coagulant activity is directly related to hemorrhagic risk, yet this is not true for all. The first correlation of residual coagulant activity and clinical RBD bleeding severity was reported by the EN-RBD, based on data from 489 patients representing 13 European treatment centers. Clinical bleeding episodes were classified into four severity categories based on location, potential clinical impact, and bleeding trigger either spontaneous, after trauma or drug induced (Table 3). This study documented a strong association between residual coagulant activity and clinical bleeding severity for deficiencies of fibrinogen, combined FV + VIII, FX and FXIII, with weak association for FV and FVII deficiencies; residual FXI activity did not predict clinical bleeding severity. From the same study it was documented that the minimum level to ensure complete absence of clinical symptoms is different for each disorder (see recommended EN-RBD trough levels in Table 4). Thus RBDs cannot be considered as a single class of disorders; instead studies should focus on evaluation of specific aspects of each individual RBD.

Laboratory diagnosis

RBD laboratory diagnosis is initially investigated via coagulation screening tests including the activated partial thromboplastin time (APTT) and prothrombin time (PT) (Table 1). A prolonged APTT with a normal PT suggests FXI deficiency after exclusion of FVIII, FIX, and FXII deficiencies. The reverse pattern is typical of FVII deficiency, whereas the prolongation of both tests directs further analysis towards deficiencies of combined FV and FVIII, FX, FV, prothrombin or fibrinogen. All coagulation tests depending on the formation
of fibrin as the end point are necessary to evaluate fibrinogen deficiency; hence, beside the PT and APTT, the thrombin time (TT) is performed (Table 1). Abnormal screening coagulation tests are followed by mixing studies (50:50) to exclude an inhibitor. When mixing studies correct, specific factor assays are performed to identify the deficiency. Factor antigenic assays are essential for diagnosis of quantitative deficiencies of fibrinogen or FII, to appropriately classify and treat patients with dysfibrinogenemia and dysprothrombinemia, both associated with an increased thrombotic risk. The screening clotting tests (PT, APTT, fibrinogen, platelet count, bleeding time) are normal in FXIII deficiency (Table 1); diagnosis is established via specific assays. Increased clot solubility in 5 M urea, dilute monochloroacetic or acetic acid are not quantitative or standardized, and only detect activity levels <5%, resulting in underdiagnosis. Therefore, FXIII activity should be quantitatively measured via ammonia release during the transglutaminase reaction or incorporation of radioactive amines into proteins. Here, plasma blanking is required to avoid the FXIIIa-independent ammonia release that could lead to incorrect results in the low-activity range (<5-10%). If FXIII activity is decreased, the deficiency subtype, FXIII-A or FXIII-B is determined with an immunological FXIII antigen assay to assure appropriate classification and treatment.

**Molecular diagnosis**

Molecular diagnosis is based on causative mutation identification in genes encoding corresponding coagulation factors. Exceptions are combined FV and FVIII deficiency, caused by mutations in genes encoding proteins involved in FV and FVIII intracellular transport (MCFD2 and LMAN1) and VKCFD, caused by mutations in genes encoding enzymes involved in post-translational modification and vitamin K metabolism (GGCX and VKOR). Inheritance pattern is autosomal recessive for all RBDs, except for some cases of FXI and of hypo- and dysfibrinogenemia. Information on RBD identified mutations is available through the ISTH mutation database (http://www.isth.org/?MutationsRareBleeding). Missense mutations are most frequent, representing 50-80% of identified mutations, except for LMAN1 variants where the most frequent mutations are insertions/deletions (50%). Insertion/deletion mutations represent 20-30% of gene variations of the fibrinogen, FV, MCFD2 and FXIII genes and <15% of remaining coagulation factor mutations. Splicing and nonsense mutations comprise 5-15% of coagulation factor identified mutations, with a maximum rate of 20% in the LMAN1 gene. Variants located in the 3’ and 5’ untranslated regions of the
genes are least frequent (<5%) and found only in fibrinogen, FVII, FXI and FXIII. Despite significant advances in knowledge, 5-10% of affected patients with severe deficiencies have no identifiable genetic defect; here the use of next generation sequencing (NGS), correlated with additional investigation on the deleterious/causative role of identified sequence variations, may elucidate novel genetic pathways.

Over the past twenty years the body of literature on the molecular aspects of RBDs based on naturally occurring mutations has grown. The early work in this area, especially studies highlighting potential genotype/phenotype correlations, largely have been conducted in mouse models and revealed that complete absence of almost all of these coagulation factors resulted in a severe clinical phenotype often incompatible with life or achieving adulthood. These data cannot uniformly be extrapolated to RBD human expression.

**Global hemostasis tests**

Standard coagulation screening tests reveal the basic integrity of the coagulation process; evaluation of coagulation speed and extent are limited by test sensitivity at very low residual factor levels. Tests evaluating global hemostatic capacity (thrombin generation test and thromboelastography) may provide more accurate evaluation of *in-vivo* hemostasis and treatment response, and be better suited to predict clinical phenotype as they more effectively assess rate/total thrombin generated, whole blood clot formation and/or fibrin polymerization. Recently, these tests have been used to evaluate hemostasis in patients with RBDs specifically FV and FXI deficiency. These assays represent an emerging strategy to determine therapeutic effectiveness and to monitor RBD treatment, particularly FXI deficiency where standard assays fail to correlate with bleeding risk. Test standardization to reproduce reliable thrombin generation measurements facilitated by standardized pre-analytical and analytical procedures is required before widespread clinical use.

**Treatment**

RBD treatment is difficult as clinical management information for specific bleeding episodes is often scarce; replacement therapy may require use of fresh frozen plasma (FFP) that may be associated with adverse events, licensed products without the specific indication (e.g. prothrombin complex concentrate (PCC) for prothrombin deficiency), or unlicensed products. In some cases specific replacement therapy is unavailable.
PALLA et al  Rare Bleeding disorders

Treatment mainstay is replacement of the deficient coagulation factor and use of adjunctive therapies (antifibrinolytics, estrogen/progestogen) where appropriate. Unfortunately, safety and efficacy data for the few available products is limited as is experience in optimal use compared to hemophilia. Blood borne infectious agent transmission avoidance is primary in replacement therapy choice. Solvent/detergent-treated plasma is an important source of replacement recommended in many RBDs; virus-inactivated concentrates, when available, are also safe yet may be cost prohibitive in developing economies.

Non-virus-inactivated plasma and cryoprecipitate should be avoided when possible. Virally inactivated specific factor concentrates are available for several deficiencies and preferred when virally inactivated plasma is not available or repeated infusions required, to avoid potential fluid overload or inability to achieve a hemostatic level, especially during surgery or CNS hemorrhage. An updated registry of available clotting factor concentrates is published by the WFH (9th edition, http://www.wfh.org). The patient’s personal/family bleeding history are important management guides. Adjuvant therapies such as antifibrinolytics alone or in combination with replacement therapy, and estrogen-progestin preparations are considered for less severe mucosal tract hemorrhage or heavy menstrual bleeding, and before minor surgeries.

Most RBD treatment is “on-demand”, defined as soon as possible after bleed onset. In clinically severe cases or specific deficiency states, prophylactic treatment is considered. Dosage and treatment frequency depend on the required minimal hemostatic level of each coagulation factor, its plasma half-life and the type of bleeding episode treated or to be prevented. Use of prophylaxis is related to bleeding frequency, risk of severe spontaneous bleeding, and associated long-term disability despite on-demand treatment. General treatment recommendations are summarized in Tables 4 and 5.

While recombinant technology and viral inactivation methods have virtually eliminated the risk of blood-borne infection, other potential concentrate related adverse events persist (e.g., inhibitor development, thrombosis, hypersensitivity reactions). Cases of autoantibodies have been reported in fibrinogen, FII, FVII, FXI and FXIII deficiencies following replacement therapy. In FXI deficiency, 41% of patients homozygous for null mutations developed an inhibitor following exposure to exogenous FXI (plasma, FXI concentrates, or anti-RhD immunoglobulin).
Surveillance systems such as the Universal Data Collection (UDC) by the Centers for Disease Control and Prevention (CDC) in the United States\textsuperscript{37}, and the European Haemophilia Safety Surveillance system (EUHASS) in Europe\textsuperscript{38} register and monitor treatment and complications. These two programs highlight the importance to conduct multinational, multicenter data collection for long-term post-registration surveillance and to analyze large numbers of homogeneous/standardized data, to overcome the rarity of these disorders.

**OVERVIEW OF SPECIFIC RBDS**

**Fibrinogen deficiency\textsuperscript{9,39}**

Fibrinogen deficiency is heterogeneous with two main phenotypes distinguished: plasma and platelet protein levels are not measurable or very low, leading to afibrinogenemia and hypofibrinogenemia, whereas, low clottable fibrinogen with normal or moderately reduced fibrinogen antigen results in dys- and hypodysfibrinogenemia. Fibrinogen is hepatically produced from three homologous polypeptide chains, $\alpha$, $\beta$ and $\gamma$, and assembled to form a 340-kDa hexamer. The three genes encoding fibrinogen $\beta$ ($FGB$), $\alpha$ ($FGA$) and $\gamma$ ($FGG$), ordered from centromere to telomere, are clustered in a region of approximately 50 kb on chromosome 4.

Dys- and hypofibrinogenemic patients are usually asymptomatic or intermittently symptomatic, while those with afibrinogenemia exhibit bleeding commonly manifesting in the neonatal period, with 85% presenting with umbilical cord bleeding.\textsuperscript{40,41} Table 2 lists other less frequent symptoms. Women may experience menometrorrhagia, but some have normal menses. First-trimester abortion is common in afibrinogenemic women but less common in dysfibrinogenemia. Reports of thromboembolism in afibrinogenemia exist either with or without replacement therapy, likely related to fibrinogen’s binding of excess thrombin. Some mutations predict clinical phenotype, particularly in dysfibrinogenemia where some gene variations are associated with bleeding while others with thrombotic risk.\textsuperscript{9}

Conventional treatment is on-demand, but effective long-term secondary prophylaxis with fibrinogen administration every 7–14 days has been described after CNS hemorrhage. High-level replacement, e.g. during pregnancy, should be moderated by the reported occurrence of thromboembolic events. Depending
on the country, patients may receive FFP, cryoprecipitate or fibrinogen concentrate, with the latter being the
treatment of choice.\textsuperscript{42-44}

**Prothrombin deficiency\textsuperscript{10,39}**

Prothrombin deficiency is the rarest inherited coagulation disorder, with a prevalence of \( \approx 1 \) in 2 million. Two
main phenotypes are distinguished: hypoprothrombinemia with both decreased activity and antigen levels,
and dysprothrombinemia with normal synthesis of a dysfunctional protein. Hypoprothrombinemia associated
with dysprothrombinemia was also described in compound heterozygotes. Prothrombin, a vitamin-K
dependent glycoprotein hepatically synthesized, is the zymogen of the serine protease \( \alpha \)-thrombin and is
encoded by a gene of approximately 21 kb located on chromosome 11.

Severe prothrombin deficiency (plasma levels <5\%) in either homozygous or double heterozygotes, is
uniformly characterized by severe bleeding (Table 2). Dysprothrombinemia manifests as a variable less
severe bleeding tendency, while heterozygous subjects are usually asymptomatic; occasionally, excessive
bleeding after surgical procedures has been observed. In homozygous females menorrhagia is frequent.

Replacement therapy is needed only in severe deficiency, for bleeding or to ensure adequate prophylaxis
before major procedures. As no prothrombin concentrate exists, FFP, PCC or both are used. In case of
severe bleeding or major surgery, higher prothrombin levels are achieved with PCCs without risk of FFP
associated volume overload.\textsuperscript{45} PCCs contain other vitamin-K dependent coagulation factors, which could
potentially induce thrombotic complications, therefore, patients require close monitoring.

**Factor V deficiency\textsuperscript{11,39}**

FV has a dual role in coagulation: it is a protein cofactor required by the prothrombinase complex for
thrombin generation, and contributes to the proteins C/S anticoagulant pathway by down-regulating FVIII
activity. FV is mainly hepatically secreted, with some evidence that it is also synthesized in the
megakaryocyte/platelet lineage. FV protein is encoded by a large (80 kb) and complex (25 exons) gene
located on chromosome 1.

Severe deficiency typically presents early in life, nonetheless, FV deficiency is clinically heterogeneous as
despite lower FV levels, severe patients may not bleed as expected. Recent observations point to a pivotal
role for platelet FV providing new insight into this inconsistency. Megakaryocytes can synthesize FV,
however, the majority of platelet FV is endocytosed from the plasma. Following endocytosis FV is modified intracellularly; these changes appear to provide the cofactor with unique physical and functional characteristics rendering it more procoagulant compared to its plasma counterpart. Platelet degranulation and release of platelet FV at the site of vascular injury is a critical contributor to local FV concentration. Furthermore, there is evidence that platelet FV locally released in high concentrations is less susceptible to inhibition supporting normal hemostasis.

Symptomatic patients usually present with umbilical stump bleeding, skin and mucosal tract hemorrhage; epistaxis and menorrhagia are relatively frequent, even with measurable FV levels (Table 2). In patients with mild-to-moderate deficiency, therapy with antifibrinolytic agents is sufficient to control epistaxis, menorrhagia, or other non–life-threatening mucosal bleeding. Menorrhagia can also be managed using hormonal suppressive therapy, progestin-containing intrauterine devices, endometrial ablation, or hysterectomy when required.

FV replacement is accomplished through FFP, preferably virus inactivated, as currently no FV concentrate is available. A study of FV concentrate developed for clinical use in deficient patients is ongoing in preparation for orphan drug designation application to the European Medicine Agency (EMA) and the Food and Drug Administration (FDA). Platelet concentrates are an alternative source of FV that have been used in combination with FFP.46

**Combined factor V and factor VIII deficiency**12,39

Combined FV and FVIII deficiency (F5F8D) is characterized by concomitantly low levels (usually 5–20%) of both coagulant activity and antigen. Interestingly, the concomitant deficiency of these two coagulation factors does not enhance the hemorrhagic tendency observed in each separate defect. F5F8D is causally associated with mutations in *LMAN1* gene, encoding lectin mannose-binding protein (previously named *ERGIC-53*), a 53-kDa type 1 transmembrane protein that acts as a chaperone in the intracellular transport of both factors and with mutations in *MCFD2* gene, encoding multiple coagulation factor deficiency (MCFD)2 protein, which acts as a cofactor for LMAN1, specifically recruiting correctly folded FV and FVIII in the endoplasmatic reticulum. Recent studies have failed to identify additional components of the LMAN1–MCFD2 receptor complex, supporting the concept that F5F8D might be limited to *LMAN1* and *MCFD2*. 
Phenotypes associated with mutations in *MCFD2* and *LMAN1* are indistinguishable and manifested only by FV and FVIII deficiency, although a selective secretion delay of the cargo protein procathepsin C has been observed in HeLa cells overexpressing a dominant-negative form of LMAN1.

Symptoms are usually mild, with a predominance of easy bruising, epistaxis, and bleeding after dental extractions (Table 2). Menorrhagia and postpartum bleeding are reported in affected women. Because of the mild clinical pattern, bleeding is usually on-demand and does not require regular prophylaxis. Affected individuals can be treated with desmopressin and FFP. More severe cases require FVIII concentrate. Sources of both FV and FVIII are required and differential plasma half-lives (FV 36 hours, FVIII 10–14 hours) must be considered.

**Factor VII deficiency**

FVII deficiency is the most common autosomal recessive coagulation disorder (1 in 500,000), typically clinically heterogeneous, ranging in severity from lethal to mild, or even asymptomatic. FVII is hepatically synthesized and encoded by the FVII gene (*F7*) located on chromosome 13, 2.8 kb upstream of the FX gene. Both coagulant and antigenic plasma FVII levels are influenced by genetic and environmental factors (sex, age, cholesterol and triglyceride levels); FVII levels are also modulated by *F7* polymorphisms.

FVII deficiency is phenotypically variable: some patients do not bleed despite very low FVII activity, while others with similar levels experience frequent bleeding. The most frequent symptoms are epistaxis and menorrhagia, with life- or limb-threatening bleeding relatively rare (Table 2). However, CNS hemorrhage was reported to have a high incidence (16%) in a series of 75 patients. Thrombotic episodes have also been reported in 3–4% of FVII deficient patients, particularly associated with surgery and replacement therapy, but spontaneous thrombosis may also occur. It can be inferred that patients with FVII deficiency are not protected against thromboembolism.

Various therapeutic options are available for FVII deficiency, including FFP and PCCs (FVII concentration in both are low, therefore not optimal treatments); plasma-derived FVII concentrates; and recombinant FVIIa. Recombinant FVIIa is genetically engineered and considered the optimal replacement therapy as utilized at a low dose (10–20 µg/kg). Prophylaxis is debated in FVII deficiency, but has been utilized in those with severe bleeding. Infused FVII short half-life contributes to difficulty establishing standardized prophylactic
The occurrence of frequent menorrhagia is almost invariably associated with chronic iron deficient anemia in women with severe deficiency. Pregnancy alone does not require special precautions and uncomplicated delivery is possible without prophylaxis. However, all cases of reported postpartum hemorrhage occurred with FVII coagulant activity <15% not receiving prophylaxis. Therefore, delivery should occur under the coverage of short-term replacement.50

**Factor X deficiency**14,39

Factor X deficiency14,39

FX is a glycoprotein pivotal in the coagulation cascade, as the first enzyme in the common pathway of thrombin formation. FX is mainly hepatically synthesized and encoded by the FX gene (*F10*), comprising 22 kb and located on chromosome 13, a few kilobases downstream of the *F7* gene.

In FX deficiency, the bleeding tendency appears at any age, although the more severely affected (<1% activity) present early in life with umbilical-stump, CNS or GI bleeding (Table 2). Patients with severe deficiencies commonly experience hemarthroses and hematomas. Common symptoms reported at all severity levels include epistaxis and menorrhagia. Heterozygous patients have been reported with postpartum bleeding requiring treatment.

Data from the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) registry demonstrated that the proportion of FX deficient patients requiring treatment is higher than other RBDs. Therapy usually is administration of PCC. A recently developed freeze-dried human coagulation FIX/FX concentrate with specified FIX/X content has facilitated prophylaxis.51 Pharmacokinetics of a new high-purity FX concentrate has been recently performed (ClinicalTrials.gov identifier: 00930176) and is ongoing in children. Therapeutic options for control of menorrhagia are both medical (e.g. antifibrinolytics, hormonal suppressive therapy, levonorgestrel intrauterine device, clotting factor replacement) and surgical (e.g. endometrial ablation and hysterectomy if required). Even if FX plasma concentration increases in pregnancy, women with severe deficiency and a history of adverse pregnancy outcomes (e.g. abortion, placental abruption or premature birth), may benefit from continuous replacement therapy.

**Factor XI deficiency**15,39

The estimated prevalence of severe FXI deficiency in most populations is ~1 in 1 million, but higher in
Ashkenazi Jews where heterozygosity approaches 8%. FXI is mainly hepatically synthesized, although small transcript quantities are detected in megakaryocytes and platelets. The protein is encoded by the FXI gene, comprising 23 kb located on chromosome 4. In some cases, missense mutations were shown to exert a dominant-negative effect through heterodimer formation between the mutant and wild-type polypeptides, resulting in dominant transmission. The existence of a platelet FXI transcript, originating from the skipping of exon 5, was hypothesized but subsequently not confirmed. Although recent data support FXI transcripts undergoing alternative splicing leading to FXI isoform synthesis, their physiological role and importance require elucidation.

The most frequent symptoms are oral and postoperative bleeding, occurring in >50% of patients (Table 2). FXI deficient women are prone to menorrhagia. Case series of severely deficient women revealed that 70% of pregnancies were uneventful without prophylactic treatment. The relationship between plasma FXI levels and the bleeding tendency is not as clear-cut as in other RBDs. Bleeding phenotype is not correlated with genotype but rather the site of injury. Injury in an area of high fibrinolytic activity (e.g., urogenital tract, oral cavity after dental extraction or tonsillectomy), increases bleeding risk (49–67%) as compared to sites with less fibrinolytic activity (1.5–40%). Usually, patients with severe deficiency (1% or less) are mildly affected with most manifestations injury-related. Patients with more moderate (low but detectable) FXI levels are also mild bleeders. Therefore, phenotypes are not strikingly different in these two groups. Patients with similarly reduced FXI antigen and activity levels exhibit variable bleeding tendencies; some are asymptomatic even after trauma, while others display bleeding with trauma, or delayed bleeding beginning several hours to days following injury. Neither FXI antigen nor activity correlate with clinical bleeding risk, and APTT assays are not predictive. Attempts to differentiate FXI deficient patients with or without a bleeding tendency have focused on plasma thrombin generation characteristics; conflicting results are reported. Recently, new plasma clot formation assays were demonstrated to be useful to distinguish phenotypes, with bleeders exhibiting significantly reduced fibrin network density and clot stability, suggesting that these parameters are determinants of bleeding risk, but are not wholly dependent on plasma FXI levels.

Treatment is based on antifibrinolytic agents, FFP, and FXI concentrate. Recombinant FVIIa was
successfully used in surgery. Care should be taken to reduce complications including thrombosis, especially with FXI concentrate, volume overload, and hypersensitivity reactions. Inhibitory antibodies can develop in severe deficiency. Patients with FXI deficiency without a bleeding history despite appropriate challenges do not require prophylactic treatment.

**Factor XIII deficiency**

FXIII is a transglutaminase functioning to cross-link the $\alpha$ and $\gamma$ fibrin chains, resulting in increased clot strength and fibrinolytic resistance. FXIII consists of two catalytic A subunits (FXIII-A) and two carrier B subunits (FXIII-B). FXIII-A is synthesized in cells of bone marrow origin, while FXIII-B is hepatically produced. The corresponding genes are located on chromosomes 6 and 1. FXIII deficiency, together with prothrombin deficiency, are the rarest of recessively transmitted RBDs occurring in 1 in 2 million. In inherited FXIII deficiency, FXIII-A plasma levels measured as functional activity or immunoreactive protein are usually extremely reduced, whereas the FXIII-B subunit is reduced but at measurable levels.

Patients with FXIII-A deficiency have a bleeding tendency that is usually severe, with early onset of life-threatening symptoms (e.g. umbilical-cord and CNS bleeding) in up to 80% and 30% respectively. Table 2 reports other common or less frequent clinical symptoms. In women of reproductive age, miscarriage and intraperitoneal bleeding are often reported. These symptoms collectively lead to early diagnosis, and prophylactic treatment. Prophylaxis is feasible as 2-5% FXIII plasma levels are sufficient to prevent severe bleeding, and the long *in-vivo* half-life, 11–14 days, requires infrequent replacement (1 month or longer). Importantly, the recent EN-RBD study revealed that only patients with FXIII:C >30% remain asymptomatic. Prophylactic FXIII infusions are recommended in FXIII-A-deficient pregnant women to prevent fetal loss.

When FXIII concentrate is not available, FFP and cryoprecipitate should be considered, the latter being preferable due to higher FXIII content. Plasma derived FXIII has been used for several years and shown to be safe and effective; a new recombinant FXIII-A$_2$ concentrate (rFXIII-A$_2$) is available and a phase III clinical trial (ClinicalTrials.gov identifier:00713648) recently completed, demonstrated that rFXIII is safe and effective in bleed prevention in congenital FXIII-A subunit deficient patients. rFXIII was recently approved for the treatment of FXIII-A deficiency in Australia, Canada, the European Union, Switzerland and the US.

Only a few cases of inherited FXIII-B deficiency are reported with 16 causative mutations identified; FXIII-B
deficiency bleeding symptoms appear milder than FXIII-A-deficient patients.

**Vitamin K-dependent coagulation factors deficiency (VKCFD)**

Vitamin K-dependent coagulation factors, FII, FVII, FIX and FX, require glutamic acid residue γ-carboxylation at Gla domains to enable calcium binding and attachment to phospholipid membranes. The process is catalyzed by hepatic γ-glutamyl carboxylase (GGCX) and its cofactor, reduced vitamin K (KH2). During the reaction, KH2 is converted to vitamin K epoxide (KO), which is recycled to KH2 by the vitamin K epoxide reductase (VKOR) enzyme complex. Heritable dysfunction of GGCX or the VKOR complex results in the secretion of undercarboxylated vitamin K-dependent coagulation factors, leading to a combined deficiency. γ-carboxylation of glutamic acid residues is required for activity of proteins C, S and Z; despite decreased proteins S/C levels in VKCFD, there are no reports of venous/arterial thrombosis. The effect of VKCFD is clearly hemorrhagic. The GGCX and VKOR proteins are encoded by two corresponding genes: GGCX (13 kb, 15 exons) located on chromosome 2 and the unusually small VKORC1 (5126 bp, three exons) located on chromosome 16; the latter gene was so named because of evidence suggesting that VKOR is a multi-subunit complex.

VKCFD commonly presents early in life with intracranial hemorrhage or umbilical stump bleeding (Table 2); routine vitamin K administration may delay neonatal diagnosis. Severely affected children may present with skeletal abnormalities including nasal and distal digital hypoplasia, epiphyseal stippling and mild conductive hearing loss. Older patients can present with easy bruising and mucocutaneous or postsurgical bleeding. Treatment with oral or parenteral vitamin K1 should be promptly started. Some patients demonstrate inadequate response. Limited data exist on the effectiveness of 10 mg vitamin K1 weekly prophylaxis. Massive parenteral doses do not always correct activity levels with persistence of undercarboxylated molecules; here factor replacement via PCC or alternatively a virally inactivated FFP could be used in acute bleeding episodes or prior to surgery.

**Concluding remarks**

Understanding the pathophysiology, presentation and treatment options for RBDs is critical to facilitate genetic counselling, optimal patient management and improved long-term outcomes. RBD rarity limits in-depth individual deficiency analysis and contributes to increased risk of misdiagnosis and poor and
sometimes fatal consequences. Although many efforts have been made to address these gaps in RBDs knowledge, further multinational collaborative efforts are required. Despite valuable findings obtained by current registry efforts, accurate knowledge of the true world-wide incidence of each RBD, the pattern and frequency of their bleeding episodes and the minimum residual coagulation factor level required to ensure normal hemostasis remain. These gaps can only be addressed by international global efforts.

Ongoing efforts continue to promote international data harmonization. In the United States, the American Thrombosis and Hemostasis Network (www.athn.org) is expanding its data collection system to assure inclusion of individuals with RBDs followed through the US federal Hemophilia Treatment Center Network. In Europe, the PRO-RBDD project (http://eu.rbdd.org), through the development of an international network of care providers and a web-based database, aims to better identify the number of affected individuals worldwide and to prospectively collect clinical and laboratory data to evaluate the frequency of clinical manifestations, their sequelae, and document consumption of treatment products and define related complications.

The Rare Coagulation Disorders Resource Room website is another step in global initiatives enhancing information dissemination, ongoing research and registry efforts, and fosters collaboration among a growing international network. This resource provides readily available first-line education for both healthcare providers and affected individuals and will serve as a platform for world-wide researchers and clinicians to exchange information, share experiences, while fostering data collection and collaboration on clinical trial design.

**Participation in PRO-RBDD Registry**

PRO-RBDD is open to Hemophilia Treatment Centers worldwide; to join, visit http://eu.rbdd.org and contact info@rbdd.eu. Project staff will assist you to join the network including required documentation. Currently studies on fibrinogen and factor XIII deficiencies are ongoing. Participants may utilize the database and propose future new studies.

**Acknowledgment**
The authors thank Marzia Menegatti (Università degli Studi di Milano, Milan, Italy) for the assistance in the preparation of the manuscript.

FP is recipient, as leader of the work package dedicated to the prospective study of rare bleeding disorders within the project “European Haemophilia Network (EUHANET)” funded by the commission of the European Union – the Executive Agency for Health and Consumers.

Conflict-of-Interest Statements

RP declares no competing financial interests. FP has received honoraria for participating as a speaker at satellite symposia and educational meetings organized by Novo Nordisk, CSL Behring, LFB, Grifols, Bayer and Baxter, and she has received unrestricted research grant funding from Novo Nordisk, Biotest and Kedrion Biopharma. ADS reports no conflicts of interest and no funding related to topics discussed in the manuscript.
References


36. Keeling D, Tait, C, Makris M. Guideline on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. A United Kingdom Haemophilia Center


60. Brenner B, Kuperman AA, Watzka M, Oldenburg J. Vitamin K-dependent coagulation factors
Figure Legend

Figure 1. Worldwide distribution of rare bleeding disorders derived from World Federation of Haemophilia (WFH) and European Network of the Rare Bleeding Disorders (EN-RBD).
**Table 1.** General features of autosomal recessive deficiency of coagulation factors.

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Estimated prevalence*</th>
<th>Gene (chromosome)</th>
<th>Laboratory diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>1 in 1 million</td>
<td>FGA, FGB, FGG (all on 4q28)</td>
<td>Afibrinogenemia: TT ↑↑, APTT ↑↑, PT ↑↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dys- and Hypodisfibrinogenemia: TT ↑, APTT ↑, PT ↑↑</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>1 in 2 million</td>
<td>F2 (11p11–q12)</td>
<td>TT normal, APTT ↑, PT ↑</td>
</tr>
<tr>
<td>Factor V</td>
<td>1 in 1 million</td>
<td>F5 (1q24.2)</td>
<td>TT normal, APTT ↑, PT ↑</td>
</tr>
<tr>
<td>Combined factor V and VIII</td>
<td>1 in 1 million</td>
<td>LMAN1 (18q21.3–q22)</td>
<td>TT normal, APTT ↑, PT ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCFD2 (2p21–p16.3)</td>
<td></td>
</tr>
<tr>
<td>Factor VII</td>
<td>1 in 500 000</td>
<td>F7 (13q34)</td>
<td>TT normal, APTT normal, PT ↑</td>
</tr>
<tr>
<td>Factor X</td>
<td>1 in 1 million</td>
<td>F10 (13q34)</td>
<td>TT normal, APTT ↑, PT ↑</td>
</tr>
<tr>
<td>Factor XI</td>
<td>1 in 1 million</td>
<td>F11 (4q35.2)</td>
<td>TT normal, APTT ↑, PT normal</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>1 in 2 million</td>
<td>F13A1 (6p24–p25)</td>
<td>TT normal, APTT normal, PT normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F13B (1q31–q32.1)</td>
<td>Specific assays required</td>
</tr>
<tr>
<td>Vitamin-K dependent</td>
<td>Reported in &lt; 50</td>
<td>GGCX (2p12)</td>
<td>TT normal, APTT ↑, PT ↑↑</td>
</tr>
<tr>
<td>coagulation factors</td>
<td>families</td>
<td>VKORC1 (16p11.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Including dysfunctional proteins.
### Table 2. Clinical symptoms in severe RBDs

<table>
<thead>
<tr>
<th>Rare Bleeding Disorder</th>
<th>Afibrinogenemia; Hypo- &amp; Dysfibrinogenemia</th>
<th>Prothrombin deficiency</th>
<th>FV deficiency</th>
<th>Combined FV &amp; FVIII deficiency</th>
<th>FVII deficiency</th>
<th>FX deficiency</th>
<th>FXI deficiency</th>
<th>FXIII deficiency</th>
<th>Vitamin K dependent factors deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main bleeding symptoms for severe deficiencies</strong></td>
<td><strong>Afibrinogenemia:</strong></td>
<td><strong>Common:</strong> umbilical cord epistaxis, menorrhagia, postoperative hematomas</td>
<td><strong>Common:</strong> subcutaneous and muscle hematomas, prolonged post-injury, mucosal tract, hemorrhagia</td>
<td><strong>Common:</strong> easy bruising, epistaxis, gingival, postoperative, post-dental, extraction, post-circumcision, menorrhagia, postpartum</td>
<td><strong>Common:</strong> umbilical cord, epistaxis, menorrhagia, hemorrhagia, post-trauma, postoperative</td>
<td><strong>Common:</strong> oral cavity, postoperative, menorrhagia</td>
<td><strong>Common:</strong> umbilical cord, ecchymosis, subcutaneous, hematoma, oral cavity, after trauma, menorrhagia, miscarriage &amp; intrauterine</td>
<td><strong>Common:</strong></td>
<td><strong>Common:</strong> intracranial (at birth) umbilical cord, retroperitoneal soft tissue, easy bruising, mucocutaneous, postoperative</td>
</tr>
<tr>
<td></td>
<td><strong>Less common:</strong> skin, GI, genito-urinary tract, CNS, Menorrhagia</td>
<td><strong>Less common:</strong> umbilical cord, menorrhagia, postoperative</td>
<td><strong>Less common:</strong> umbilical cord, hemorrhagia</td>
<td><strong>Less common:</strong></td>
<td><strong>Less common:</strong></td>
<td><strong>Less common:</strong></td>
<td><strong>Less common:</strong></td>
<td><strong>Less common:</strong> wound healing, hemorrhagia, muscle, hematomas, epistaxis, GI, postoperative</td>
<td><strong>Common:</strong> intracranial (at birth) umbilical cord, retroperitoneal soft tissue, easy bruising, mucocutaneous, postoperative</td>
</tr>
<tr>
<td></td>
<td><strong>Uncommon:</strong> musculoskeletal</td>
<td><strong>Uncommon:</strong></td>
<td><strong>Uncommon:</strong></td>
<td><strong>Uncommon:</strong></td>
<td><strong>Uncommon:</strong></td>
<td><strong>Uncommon:</strong></td>
<td><strong>Uncommon:</strong></td>
<td><strong>Uncommon:</strong></td>
<td><strong>Children may show skeletal abnormalities</strong></td>
</tr>
<tr>
<td><strong>Risk of thrombosis</strong></td>
<td><strong>Afibrinogenemia:</strong> reported</td>
<td>Inherited dysprothrombinemia due to G20210A mutation and linked to slightly increased levels of circulating prothrombin, there is a significantly higher risk to develop thromboembolic diseases</td>
<td>—</td>
<td>—</td>
<td>Thrombotic episodes, particularly deep vein thrombosis, post-treatment reported (3-4% of patients). Spontaneous thrombosis may occur</td>
<td>—</td>
<td>Cases of myocardial infarction and venous thrombosis reported (idiopathic or after FXI infusion)</td>
<td>—</td>
<td>Although proteins S/C levels decreased, no reports of venous/arterial thrombosis</td>
</tr>
</tbody>
</table>
### Table 3. Categories of clinical bleeding severity

<table>
<thead>
<tr>
<th>Clinical bleeding severity</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>No documented bleeding episodes</td>
</tr>
<tr>
<td>Grade I bleeding</td>
<td>Bleeding that occurred after trauma or drug ingestion</td>
</tr>
<tr>
<td>Grade II bleeding</td>
<td><em>Spontaneous minor bleeding:</em> bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis and menorrhagia</td>
</tr>
<tr>
<td>Grade III bleeding</td>
<td><em>Spontaneous major bleeding:</em> hematomas, hemarthrosis, CNS, GI, and umbilical cord bleeding</td>
</tr>
</tbody>
</table>

CNS = central nervous system, GI = gastrointestinal
**Table 4.** Replacement therapy of RBDs.

<table>
<thead>
<tr>
<th>Deficient factor</th>
<th>Plasma half-life</th>
<th>Recommended trough levels</th>
<th>On demand dosages</th>
<th>Recommended trough levels to maintain asymptomatic state after publication of the EN-RBD results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>2–4 days</td>
<td>0.5–1g/L</td>
<td>Cryoprecipitate (15-20 mL/kg) SD-treated plasma (15–30 mL/kg) Fibrinogen concentrate (50–100 mg/kg)</td>
<td>1 g/L</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>3–4 days</td>
<td>20–30%</td>
<td>SD-treated plasma (15–25 mL/kg) FIX concentrate and PCC (20–40 units/kg)</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>Factor V</td>
<td>36 hours</td>
<td>10–20%</td>
<td>SD-treated plasma (15–25 mL/kg)</td>
<td>10%</td>
</tr>
<tr>
<td>Factor V and factor VIII</td>
<td>FV 36 hours, FVII 10–14 hours</td>
<td>10–15%</td>
<td>As for FV</td>
<td>40%</td>
</tr>
<tr>
<td>Factor VII</td>
<td>4–6 hours</td>
<td>10–15%</td>
<td>FVII concentrate (30–40 mL/kg) PCC (20–30 units/kg) rFVIIa (15–30 μg/kg every 4–6 hours)</td>
<td>&gt;20%</td>
</tr>
<tr>
<td>Factor X</td>
<td>40–60 hours</td>
<td>10–20%</td>
<td>SD-treated plasma (10–20 mL/kg) PCC (20–30 units/kg) FX/FIX concentrate (10–20 units/kg)</td>
<td>&gt;40%</td>
</tr>
<tr>
<td>Factor XI</td>
<td>50 hours</td>
<td>15–20%</td>
<td>SD-treated plasma (15–20 mL/kg) FXI concentrate (15–20 units/kg)</td>
<td>15-20%</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>9–12 days</td>
<td>2–5%</td>
<td>Cryoprecipitate (2–3 bags) SD-treated plasma (3 mL/kg) FXIII concentrate (till 50 units/kg for high hemorrhagic events) rFXIII-A (35 units/kg)</td>
<td>30%</td>
</tr>
<tr>
<td>Vitamin K dependent</td>
<td></td>
<td></td>
<td>Vitamin K (10 mg) IV, or SC, for minor bleeding PCC (20–30 units/kg) with vitamin K (5–20 mg) for severe bleeding or major surgery FFP 15–25 ml/kg is an alternative to PCC</td>
<td>No data available</td>
</tr>
</tbody>
</table>

PCC, prothrombin complex concentrate; rFVIIa, recombinant activated FVII; SD, solvent/detergent
Table 5. General recommendations for long-term prophylaxis.²,³⁵

<table>
<thead>
<tr>
<th>Deficient factor</th>
<th>Recommended trough levels</th>
<th>Reported dose schedule for successful long-term prophylaxis</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>0.5–1 g/L</td>
<td>Cryoprecipitate: 1 unit; 3 units; Fibrinogen concentrate: 30–100 mg/kg</td>
<td>Afibrinogenemic patients with recurrent life-threatening bleedings or undergoing surgeries.</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>20–30%</td>
<td>PCC: 20–40 units/kg; SD-treated plasma: 10–15 mL/kg</td>
<td>Prevention of bleeding during surgery or in children with recurrent hemarthrosis or CNS.</td>
</tr>
<tr>
<td>Factor V</td>
<td>10–20%</td>
<td>SD-treated plasma: 10–15 mL/kg; rFVIIa: 20–40 µg/kg</td>
<td>Prevention of bleeding during surgery or in children with recurrent hemarthrosis or CNS.</td>
</tr>
<tr>
<td>Factor V and factor VIII</td>
<td>10–15%</td>
<td>SD-treated plasma: 10–15 mL/kg; rFVIIa: 20–40 µg/kg</td>
<td>Prevention of bleeding during surgery or in children with recurrent hemarthrosis or CNS.</td>
</tr>
<tr>
<td>Factor VII</td>
<td>10–15%</td>
<td>SD-treated plasma: 10–15 mL/kg; pdFVII: 10–40 units/kg; rFVIIa: 20–40 µg/kg</td>
<td>Prevention of bleeding during surgery or in children with recurrent hemarthrosis or CNS.</td>
</tr>
<tr>
<td>Factor X</td>
<td>10–20%</td>
<td>PCC: 20–40 units/kg; FX/FIX concentrate: 20–40 units/kg</td>
<td>Patients with recurrent life-threatening bleedings or undergoing surgeries.</td>
</tr>
<tr>
<td>Factor XI</td>
<td>15–20%</td>
<td>No data</td>
<td>—</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>2–5%</td>
<td>Cryoprecipitate: 2 units; SD-treated plasma: 15–20 mL/kg</td>
<td>Highly recommended in severe patients.</td>
</tr>
<tr>
<td>Vitamin K dependent</td>
<td></td>
<td>Vitamin K: 5–20 mg</td>
<td>1/week orally</td>
</tr>
</tbody>
</table>

PCC, prothrombin complex concentrate; pdFVII, plasma-derived FVII; rFVIIa, recombinant activated FVII; SD, solvent/detergent.
Rare bleeding disorders: diagnosis and treatment

Roberta Palla, Flora Peyvandi and Amy D. Shapiro