Novel aspects of factor XIII deficiency

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Purpose of review

Here we review recent developments concerning the diagnosis, classification and treatment of factor XIII (FXIII) deficiency and new findings related to the pathogenesis of the disease.

Recent findings

Most recently, the International Society on Thrombosis and Haemostasis, Scientific and Standardization Committee published a guideline for the diagnosis and classification of FXIII deficiencies. Since 2009, three novel mutations causing severe bleeding diathesis were discovered in the FXIII-A gene and one in the FXIII-B gene. A newly described FXIII-A deficiency was of the extremely rare qualitative type II deficiency. The first well established founder effect was reported for a causative FXIII-A mutation. More than a quarter of all FXIII-A deficiencies are due to autoantibody, among them the first case of deficiency caused by anti-FXIII-B autoantibody was reported in the last 2 years. The safety and effectiveness of plasma FXIII concentrate for prophylaxis and treatment is now well established. The new recombinant FXIII product is currently in phase III clinical trial and the preliminary data are promising.

Summary

FXIII deficiency is considered the most underdiagnosed bleeding diathesis. The recommended algorithm for its diagnosis and classification could improve the diagnostic efficiency. The preferred choice for substitution therapy is FXIII concentrate (plasma-derived or, in the future, recombinant).

Keywords

autoantibody, bleeding diathesis, factor XIII, factor XIII deficiency, replacement therapy

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Introduction

Blood coagulation factor XIII (FXIII) is a zymogen-type coagulation factor, the precursor of a transglutaminase. FXIII in the plasma (pFXIII) is a tetrameric complex ($FXIII\text{-}A_2B_2$) of two potentially active A subunits (FXIII-A; Mr ~ 83 kDa) and two protective/carrier B subunits (FXIII-B; Mr ~ 80 kDa). Its cellular form (cFXIII), a homodimer of FXIII-A ($FXIII\text{-}A_2$), is present in the cytoplasm of platelets, monocytes, and the monocyte-derived macrophages including tissue macrophages (histiocytes). pFXIII is synthesized in cells of bone marrow origin, but the exact contribution of megakaryocytes and monocytes/macrophages is not known. FXIII-B is produced by the hepatocytes and the two types of subunits form a complex in the plasma. In normal conditions, all FXIII-A is present in the plasma as part of the pFXIII complex, whereas FXIII-B is present in excess. Approximately 50% of it circulates as a free, uncomplexed protein. FXIII-A, like other transglutaminases, consists of four well defined and sequentially folded domains (beta sandwich, catalytic core, barrel 1 and barrel 2 domains) and an N-terminal activation peptide (FXIII-AP) of 37 amino acids, which is cleaved off upon pFXIII activation. FXIII-B is a mosaic protein that consists of 10 'sushi-domain' repeats also known as complement control protein modules, or short consensus repeats. It prevents the rapid clearance of $FXIII\text{-}A_2$ from the circulation and protects $FXIII\text{-}A_2$ from a slow spontaneous activation that would occur, in its absence, at plasmatic Ca^{2+} concentration.

pFXIII is activated by the concerted action of thrombin and Ca^{2+} in the final phase of the clotting cascade. Fibrin serves as a powerful cofactor of the activation process; it increases the rate of pFXIII activation by 80-fold and the activation occurs on the surface of newly formed fibrin. In this process, first thrombin cleaves the peptide bond Arg37-Gly38 in FXIII-A and releases AP-FXIII from the N terminus, and then, in the presence of Ca^{2+}, the B subunits dissociate and $FXIII\text{-}A_2$ assumes an active configuration ($FXIII\text{-}A_2^*$). The Ca^{2+}-induced structural changes result in a closed to open (extended) structural transformation during which the active-site

cysteine, originally buried within the catalytic core domain of FXIII-A, becomes unmasked and available for reaction with its substrates.

Activated FXIII (FXIIIa), a transglutaminase, cross-links peptide-bound glutamine and lysine residues through isopeptide bonds; during the reaction, ammonia is released. The main hemostatic functions of FXIIIa are to cross-link fibrin chains and α_2 plasmin inhibitor (α_2PI) to fibrin. The rapid formation of fibrin γ-chain homodimers and α_2PI–fibrin α-chain heterodimers is followed by the slower progressive cross-linking of fibrin α-chains into high molecular weight polymers. By these biochemical processes, FXIIIa mechanically stabilizes the newly formed fibrin clot and protects it from shear stresses and prompt degradation by the fibrinolytic system. Most recent experiments suggest that FXIII also supports the adhesion, spreading and fibrinogen binding of platelets and clot retraction [1•,2•,3]. Further experiments are required to prove whether a platelet function defect also contributes to the severe bleeding diathesis of FXIII-deficient patients. In addition to its hemostatic functions, FXIII is also involved in wound healing and angiogenesis and is essential for maintaining pregnancy. For more details on the structure, activation and function of FXIII, the most recent reviews should be consulted [4,5•].

Clinical symptoms

FXIII deficiency can be congenital or acquired. Congenital deficiency is a rare bleeding disorder (1 : 2 000 000) transmitted as an autosomal recessive trait, with higher frequency in countries where consanguineous marriages are practised. Severe inherited FXIII deficiency is characterized by life-long bleeding tendency, abnormal wound healing and frequent spontaneous miscarriage in affected women [6–13]. The bleeding tendency of patients with inherited FXIII deficiency is severe in the majority of cases. Delayed umbilical cord bleeding is reported in about 80% of cases and can be considered as diagnostic symptom of FXIII deficiency. The fact that intracranial bleeding is reported in about 30% of cases [14] makes primary prophylaxis mandatory in patients affected with severe FXIII deficiency. Ecchymoses, intramuscular and subcutaneous hematomas, oral cavity, mouth and gingival bleeding and prolonged bleeding following trauma are also characteristic symptoms [14]. The lack of large-scale clinical studies on heterozygous FXIII-deficient patients does not allow drawing of evidence-based conclusions on the prevalence of clinical symptoms in this group of patients. Recently, Mahmoodi *et al.* [15•] reported clinical information on 350 heterozygous individuals with inherited coagulation disorders, and heterozygosity for XIII deficiency seemed to be associated with prolonged or massive bleeding after minor traumas. However, these data need to be confirmed in other cohorts of patients.

Key points

- Factor XIII (FXIII) deficiency has been considered the most underdiagnosed rare hemorrhagic disorder.
- An algorithm is proposed for the diagnosis and classification of inherited and acquired FXIII deficiencies according to the recommendations of the Factor XIII and Fibrinogen Subcommittee of the International Society on Thrombosis and Haemostasis, Scientific and Standardization Committee.
- FXIII deficiency caused by an autoantibody against a FXIII subunit is more frequent than originally thought.
- The recommended substitution therapy for FXIII deficiency is FXIII concentrate; virus-inactivated plasma FXIII concentrate has been proven well tolerated and efficient and recombinant FXIII preparation is expected to be available in the near future.

The uncertainty of most commonly used FXIII activity assays in the low-activity range makes it difficult to obtain accurate correlation between genotype, laboratory phenotype and clinical severity. In the great majority of patients with FXIII-A deficiency, FXIII activity is lower than 5% [14]. Clinical symptoms are rather unpredictable; in some patients, long period of only mild symptoms might be followed by severe bleeding complications [14]. To date, even though the involvement of FXIII in hemostasis has been well characterized, there are still some aspects of FXIII deficiency that need to be clarified. Little information is available on the role of FXIII in pregnancy, particularly in the process of placentation, which could explain the high rate of miscarriages. It is also important to explore why wound-healing disorder could be observed only in a fraction of patients with severe FXIII deficiency.

Classification of FXIII deficiencies

The former classification distinguishing between type I (combined deficiency of FXIII-A and FXIII-B) and type II (FXIII-A deficiency) deficiencies has been outdated by the finding that patients assumed to have type I combined deficiency are defective in the FXIII-B gene [16] and that the lower level of FXIII-A is due to its accelerated clearance from the circulation in the absence of the protective FXIII-B. The combined defect of FXIII-A and FXIII-B genes is highly unlikely; no such case has been published in the literature. This classification causes confusion; it should not be used any more. The classification recommended by the International Society on Thrombosis and Haemostasis, Scientific and Standardization Committee (ISTH SSC), Factor XIII

and Fibrinogen Subcommittee has been published as an official SSC communication [17••] and is shown in Table 1.

Inherited FXIII deficiencies are classified as FXIII-A and FXIII-B deficiencies; subtypes I and II of FXIII-A deficiency represent quantitative and qualitative defects, respectively. The gene coding FXIII-A (*F13A1*) is located on chromosome 6 at the 6p25.3–p24.3 position and contains 15 exons producing a 3.9-kb mRNA, whereas the FXIII-B gene (*F13B*) is located on chromosome 1 at the 1q31–32.1 position and comprises 12 exons producing a 2-kb mRNA. FXIII deficiency is of autosomal recessive inheritance; patients with severe disease are homozygotes or compound heterozygotes. Causative mutations have been found in FXIII-A and FXIII-B genes and are listed in different databases (www.f13-database.de, www.med.unc.edu/isth/mutations-databases and www.hgmd.cf.ac.uk). More than 70 mutations within the FXIII-A gene and four mutations in the FXIII-B gene were reported until 2009. The location of these mutations is summarized in another study [14]. Since then several interesting reports have been published on inherited FXIII deficiency. Morange *et al.* [18] reported a novel homozygous four bases insertion in exon 14 (c.2116insAAGA) introducing a frameshift that after seven altered amino acids results in a stop codon and a protein with a truncated second β-barrel domain (p.Pro675TyrfsX7). Interestingly, the deficiency is of the extremely rare type II variant. The mutant protein lost its activity, but the plasma FXIII antigen level was at the lower limit of the reference interval. This finding suggests that the C-terminal part of β-barrel 2 is essential for the expression of FXIII activity. Ivaskevicius *et al.* [19•] also described a novel homozygous mutation resulting in a stop codon in the same area of β-barrel 2 (c.1994G>A; p.Trp664X). Unfortunately, in this case, FXIII antigen level was not reported and the subtype of deficiency could not be established. Another novel mutation in β-barrel 2 (p.Arg703Trp) occurred in compound heterozygous arrangement. Interestingly, its appearance seems to constitute a de-novo event, the first one reported in the FXIII-A gene [20]. It was shown in two parallel publications that a small insertion (c.869insC) is the dominant mutation in Tunisian FXIII-A-deficient patients, which is due to an ancient founder effect [21•,22]. In addition to the five previously described mutations in the FXIII-B gene that led to homozygous or compound heterozygous FXIII-B deficiency, a new homozygous case with duplication in exon 7 (c.1155_1158dupACTT) was reported [23•]. The mutation resulted in a protein lacking the last five sushi domains.

Acquired FXIII deficiencies consist of two main groups: the autoantibodies against a FXIII subunit and the other moderate FXIII deficiencies caused by decreased synthesis of a FXIII subunit due to impaired bone marrow function or liver disease, by consumption of FXIII or by dilution coagulopathy. Discussion of the latter moderate deficiencies is beyond the scope of this review. Up to 2009, 36 cases of FXIII deficiencies due to an anti-FXIII-A autoantibody were reported in the literature, whereas in the last 2 years, 14 additional cases were reported [24••,25•,26•,27], which suggests improved laboratory diagnostics and/or clinical awareness. In supplemented patients with inherited FXIII deficiency, the development of an antibody against FXIII is extremely rare. In about one third of the patients, the anti-FXIII autoantibody develops in patients with autoimmune disease, most commonly with systemic lupus erythematosus. However, in a significant portion of the patients, the autoantibody is idiopathic and develops in elderly individuals. Autoantibodies against FXIII-A could be of neutralizing or nonneutralizing type. The former interferes with the activity of FXIIIa or with FXIII activation, whereas the latter type of autoantibody forms an immune complex with the respective

Table 1 Laboratory diagnosis/classification of factor XIII deficiencies

	Plasma FXIII				Platelet FXIII	
	Activity	A_2B_2	A_2	B	Activity	A_2
Inherited deficiency						
FXIII-A deficiency						
Type I	↓↓↓	↓↓↓	↓↓↓	>30%	↓↓↓	↓↓↓
Type II	↓↓↓	↓-n	↓-n	>30%	↓↓↓	↓-n
FXIII-B deficiency	↓↓	↓↓↓	↓↓	↓↓↓	n	n
Autoantibody against FXIII						
Anti-FXIII-A antibody						
Neutralizing	↓↓↓	↓-n	↓-n	>30%	n	n
Nonneutralizing	↓↓↓	↓↓↓	↓↓↓	>30%	n	n
Anti-FXIII-B antibody						
Nonneutralizing	↓↓↓	↓↓↓	↓↓↓	↓↓↓	n	n
Other acquired deficiency	↓	↓	↓	↓-n	na	na

↓↓↓, highly decreased activity/concentration usually below 30%; ↓↓, considerably decreased activity/concentration usually 5–10%; ↓, slightly decreased activity usually in the range of 20–70%; n, normal; na, nonapplicable. Reproduced with permission from [17••].

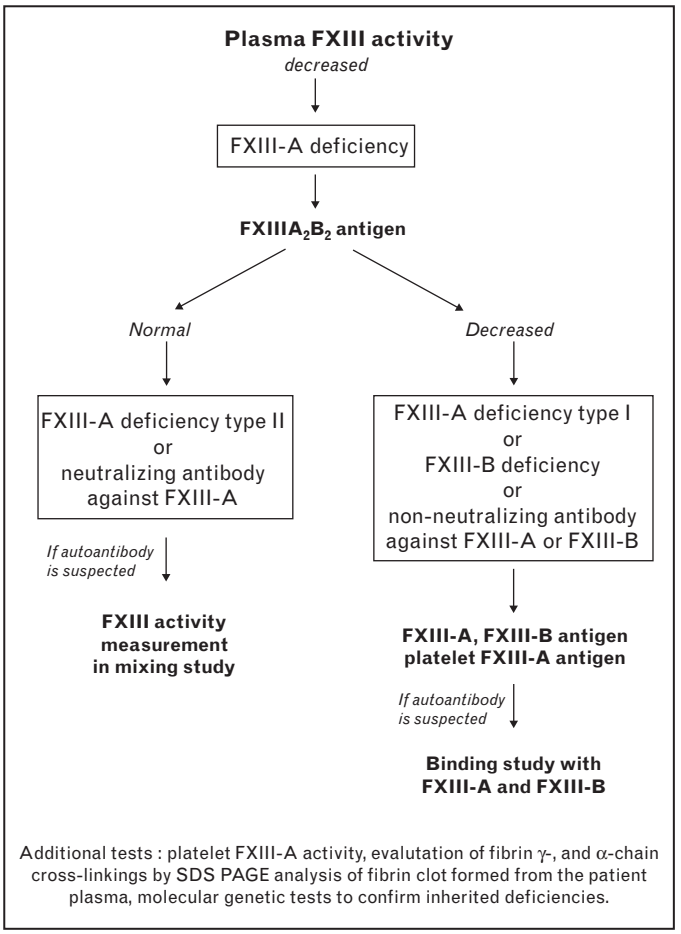
complexed (or free) FXIII subunit, which is then rapidly cleared by the reticulo-endothelial system. Earlier publications mostly report neutralizing anti-FXIII-A autoantibodies; this, however, could be due to the diagnostic difficulties in detecting nonneutralizing autoantibodies. In a most recent Japanese study, 11 patients with nonneutralizing anti-FXIII-A autoantibodies were reported as opposed to five with neutralizing antibodies [24••]. An autoantibody against FXIII-B was first reported in 2009 [28]. It formed a complex with FXIII-B and FXIII-A_2B_2 and highly accelerated their clearance from the circulation causing life-threatening bleeding complications. Another less characterized case was reported most recently [24••].

Laboratory diagnosis of FXIII deficiencies

Figure 1 presents an algorithm for the classification of FXIII deficiencies and Table 1 summarizes the results expected in the different subtypes. The algorithm for the diagnosis and classification of FXIII deficiencies crystallized out in the last years [14,17••] and reagent kits required for full classification are now available commercially (see supplementary material in [17••]). Still, FXIII deficiency remained the most underdiagnosed coagulopathy, partly due to the practise of using clot solubility in concentrated urea (alternatively in diluted monochloroacetic acid or acetic acid) solution as the screening test for FXIII deficiency. This test is now obsolete, is poorly standardized and detects only very severe FXIII deficiency with FXIII activity below 1–5%.

The screening test, which establishes the diagnosis of FXIII deficiency, should be a FXIII activity assay. FXIII activity assays are based on two principles: they measure the ammonia released during the transglutaminase reaction by the NAD(P)H-dependent glutamate dehydrogenase reaction spectrophotometrically at 340 nm or measure the amount of a small molecular weight labeled amine substrate covalently linked to a protein. In the latter case, the free and bound radiolabeled, fluorescent or biotinylated amine should be separated. A detailed

Figure 1 Algorithm for the diagnosis and classification of factor XIII deficiencies



For details on the mixing study and binding assay see supplementary material in [17••].

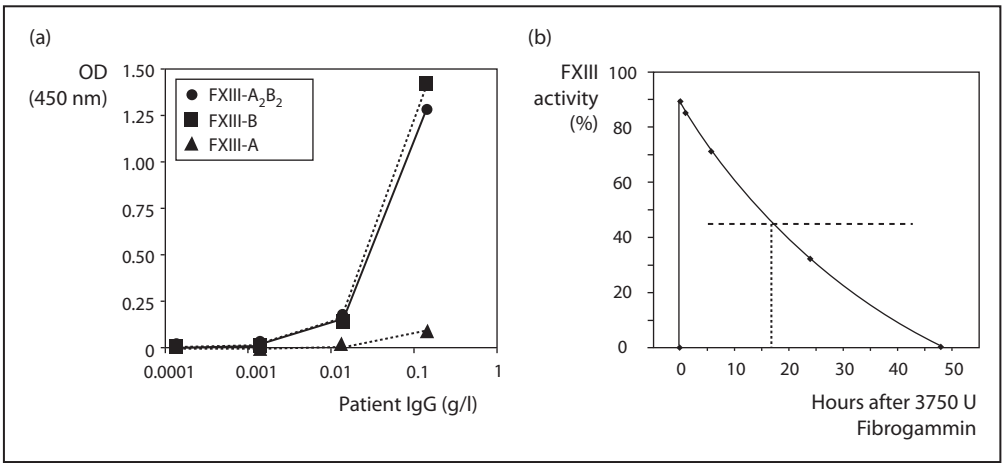
review and comparison of the two types of methods is given in [14,17••]. Briefly, the ammonia release methods are quick kinetic tests, are easy to perform and can be automated [29••], but their sensitivity in the low-activity range (below 5% FXIII activity) should be improved. Most recently, earlier recommendation [30] on the deduction of plasma blank measured in the presence of a FXIIIa inhibitor has been confirmed [29••]. Without plasma blank deduction, due to FXIIIa-independent NAD(P)H consumption, the ammonia release assays overestimate FXIII activity in the low-activity range. The amine incorporation tests are more sensitive, but they are laborious, nonkinetic tests and cannot be applied to automatic analyzers. The diagnostic tools used for the classification of FXIII deficiencies include FXIII-A_2B_2, FXIII-A and FXIII-B antigen determinations from the plasma, FXIII activity and FXIII-A antigen measurement from the platelet lysate produced by a nonionic detergent. Study of fibrin cross-linking by SDS PAGE analysis of washed plasma clot is a useful addition and genetic analysis confirms the diagnosis.

For the detection and quantification of a neutralizing anti-FXIII-A antibody, a mixing study according to an adaptation of the Nijmegen modification of the Bethesda assay is recommended (see supplementary material in [17••]). Demonstration of the binding of the patient's IgG (IgM) to isolated FXIII subunits and FXIII complex in an ELISA or dot blot arrangement is required to diagnose the presence of a nonneutralizing anti-FXIII autoantibody [24••,28]. To verify the accelerated clearance of FXIII from the circulation, the decrease of FXIII activity/antigen is to be followed by serial measurements following supplementation [27,28]. An example for the diagnosis of non-neutralizing autoantibody is shown in Fig. 2.

Replacement therapy (prophylaxis, on demand treatment)

Primary prophylaxis (10/20 U/kg FXIII every 4–6 weeks) is recommended for patients with severe FXIII deficiency in order to prevent spontaneous severe bleedings, abnormal wound healing and recurrent miscarriages in women. Although fresh frozen plasma (FFP) and cryoprecipitate contain FXIII, highly purified and heat-treated FXIII concentrate is preferred for long-term prophylaxis [31•]. The half-life of FXIII is the longest among coagulation factors (11–14 days). It has been suggested that a level of 5% is sufficient to prevent spontaneous bleeding [32]. According to more recent analyses, a level higher than 10% is needed to reduce the occurrence of bleedings significantly, but still leaving 10% of patients with cutaneous bleeding (EN-RBD; http://www.rbdd.eu). Before the introduction of FXIII concentrate, cryoprecipitate and FFP were the choice of FXIII supplementation. Now, fibrogammin P, a purified pasteurized concentrate, is available for prophylaxis in a recommended dosage of 10–20 U/kg once every 4–6 weeks. In major surgery, 20–30 U/kg per day should be administered to achieve a level above 5% until healing is complete; in minor surgery, a dose of 10–20 U/kg per day for 2–3 days is recommended, whereas in spontaneous bleeding, the treatment varies from 10–20 to 20–30 U/kg per day, depending on the severity of bleeding, until bleeding stops [14,32–35]. Replacement therapy throughout pregnancy is essential for the prevention of

Figure 2 The detection of anti-FXIII-B autoantibody



(a) The binding of the patient's biotinylated IgG to purified FXIII-A_2, FXIII-B and FXIII-A_2B_2 coated to a microtiter plate. The reaction was developed by avidin-biotinylated peroxidase complex. (b) Plasma FXIII activity at various intervals following the administration of 3750 U plasma FXIII (Fibrogammin-P, CSL Behring, Marburg, Germany) to the patient. The horizontal broken line represents half-maximal FXIII activity; the vertical dotted line shows the time when half of the added FXIII activity was eliminated from the circulation. The half-life of FXIII in the patient's circulation was 17 h, as opposed to the normal half-life of approximately 12 days. Reproduced with permission of the American Society of Hematology [27].

abortion and pregnancy loss in severe FXIII-deficient women. Limited data on prophylaxis during pregnancy are available; a plasma FXIII level above 10% seems to be sufficient for successful pregnancy. Asahina *et al.* [9] have reported that 250 IU per week were sufficient to maintain this level in the early period of gestation, and 500 IU per week is recommended after the 23rd week of gestation. The same authors propose that plasma FXIII level should be higher than 30% during labor and a booster dose of 1000 IU is recommended before labor to prevent severe obstetrical hemorrhagic complication. The use of treatments raises the problem of development of autoantibodies; however, in patients treated with FFP, cryoprecipitate or plasma-derived FXIII concentrate, the incidence of inhibitor development was extremely low [36]. To date, no severe bleedings and no serious adverse events have been reported in patients treated with fibrogammin P [34,37••].

A new human recombinant FXIII-A_2 (rFXIII-A_2) (Novo Nordisk, Bagsvaerd, Denmark) product has been developed for FXIII substitution therapy. Safety and pharmacokinetics of a single administration of rFXIII was investigated in a phase I escalating-dose study [38]. No serious adverse event and no development of specific autoantibodies were observed during the study. rFXIII formed a complex with endogenous FXIII-B and the half-life of administered rFXIII was similar to that of FXIII tetramer. It was effective in restoring clot strength and resistance to fibrinolysis. rFXIII is currently in phase III clinical trials. At the 52nd American Society of Hematology Annual Meeting, Inbal *et al.* [39••] reported the experience with 41 FXIII-deficient patients treated with rFXIII-A2. During the treatment, five bleeding episodes were observed and all five occurred after trauma. Four patients developed transient, nonneutralizing, low-titer anti-rFXIII antibodies. These antibodies appeared to be clinically insignificant and no patients developed anaphylactic or allergic reactions. However, the importance of such nonneutralizing anti-FXIII antibodies still needs to be investigated.

The treatment of FXIII deficiency caused by anti-FXIII autoantibody might be extremely difficult and in most cases requires the use of a whole armory of therapeutic tools. Apart from substitution with FXIII concentrate, these include sometimes aggressive, immunosupressive therapy, plasmapheresis, the administration of IgG preparation, anti-CD20 or recombinant FVIIa [24••,25•, 26•,28]. In one patient, spontaneous regression was also described [27].

Conclusion

FXIII deficiency has been considered the most underdiagnosed rare hemorrhagic disorder. The most recent ISTH SSC recommendation for its diagnosis and classification aims to improve the diagnostic efficiency. Recent studies explored novel mutations in the FXIII-A and FXIII-B genes and the first well established founder effect was reported. Recent reports suggest that FXIII deficiency caused by an autoantibody against a FXIII subunit is more frequent than originally thought. The preferred choice for substitution therapy is FXIII concentrate. The plasma-derived concentrate is well tolerated and efficient and a recombinant product is expected to be available in the near future.

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Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 378–379).

1 Kasahara K, Souri M, Kaneda M, *et al.* Impaired clot retraction in factor XIII A • subunit-deficient mice. Blood 2010; 115:1277–1279.
Platelet abnormality in factor XIII-deficient mice.

2 Magwenzi SG, Ajjan RA, Standeven KF, *et al.* Factor XIII supports platelet • activation and enhances thrombus formation by matrix proteins under flow conditions. J Thromb Haemost 2011; 9:820–833.
Impaired platelet function in FXIII deficiency.

3 Jayo A, Conde I, Lastres P, *et al.* New insights into the expression and role of platelet factor XIII-A. J Thromb Haemost 2009; 7:1184–1191.

4 Muszbek L, Bagoly Z, Bereczky Z, Katona E. The involvement of blood coagulation factor XIII in fibrinolysis and thrombosis. Cardiovasc Hematol Agents Med Chem 2008; 6:190–205.

5 Komaromi I, Bagoly Z, Muszbek L. Factor XIII: novel structural and functional • aspects. J Thromb Haemost 2011; 9:9–20.
A most recent review on the structure and function of FXIII.

6 Inbal A, Lubetsky A, Krapp T, *et al.* Impaired wound healing in factor XIII deficient mice. Thromb Haemost 2005; 94:432–437.

7 Dardik R, Loscalzo J, Inbal A. Factor XIII (FXIII) and angiogenesis. J Thromb Haemost 2006; 4:19–25.

8 Asahina T, Kobayashi T, Okada Y, *et al.* Maternal blood coagulation factor XIII is associated with the development of cytotrophoblastic shell. Placenta 2000; 21:388–393.

9 Asahina T, Kobayashi T, Takeuchi K, Kanayama N. Congenital blood coagulation factor XIII deficiency and successful deliveries: a review of the literature. Obstet Gynecol Surv 2007; 62:255–260.

10 Burrows RF, Ray JG, Burrows EA. Bleeding risk and reproductive capacity among patients with factor XIII deficiency: a case presentation and review of the literature. Obstet Gynecol Surv 2000; 55:103–108.

11 Koseki-Kuno S, Yamakawa M, Dickneite G, Ichinose A. Factor XIII A subunit-deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages. Blood 2003; 102:4410–4412.

12 Muszbek L, Bagoly Z. Fibrin formation disorders and pregnancy loss. Thromb Res 2007; 119:S69–S70.

13 Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. Semin Thromb Hemost 2003; 29:171–174.

14 Karimi M, Bereczky Z, Cohan N, Muszbek L. Factor XIII deficiency. Semin Thromb Hemost 2009; 35:426–438.

15 Mahmoodi M, Peyvandi F, Afrasiabi A, *et al.* Bleeding symptoms in heterozygous carriers of inherited coagulation disorders in southern Iran. Blood Coagul Fibrinolysis 2011 [Epub ahead of print].
This study was suggested since bleeding symptoms in heterozygous subjects were reported and few data on heterozygotes are available in the literature.

16 Ichinose A. Physiopathology and regulation of factor XIII. Thromb Haemost 2001; 86:57–65.

17 Köhler HP, Ichinose A, Seitz R, *et al.* Diagnosis and classification of factor XIII deficiencies. J Thromb Haemost 2011. doi: 10.1111/j.1538-7836.2011.04315.x.
The recommenation of ISTH SSC on the diagnosis and classification of FXIII deficiencies, and supplementary material with methodological aspects and commercially available reagent kits.

18 Morange P, Trigui N, Frere C, *et al.* Molecular characterization of a novel mutation in the factor XIII a subunit gene associated with a severe defect: importance of prophylactic substitution. Blood Coagul Fibrinolysis 2009; 20:605–606.

19 Ivaskevicius V, Biswas A, Bevans C, *et al.* Identification of eight novel coagulation factor XIII subunit A mutations: implied consequences for structure and function. Haematologica 2010; 95:956–962.
Novel FXIII-A mutations; their structural consequences were explored by molecular modeling.

20 Anwar R, Langlois S. The Arg703Trp missense mutation in F13A1 is a de novo event. Br J Haematol 2009; 146:118–120.

21 Louhichi N, Medhaffar M, Hadjsalem I, *et al.* Congenital factor XIII deficiency caused by two mutations in eight Tunisian families: molecular confirmation of a founder effect. Ann Hematol 2010; 89:499–504.
The mutation is due to an ancient founder effect. The first well established founder effect among FXIII-A deficiencies.

22 El Mahmoudi H, Amor MB, Gouider E, *et al.* Small insertion (c.869insC) within F13A gene is dominant in Tunisian patients with inherited FXIII deficiency due to ancient founder effect. Haemophilia 2009; 15:1176–1179.

23 Ivaskevicius V, Biswas A, Loreth R, *et al.* Mutations affecting disulphide bonds contribute to a fairly common prevalence of F13B gene defects: results of a genetic study in 14 families with factor XIII B deficiency. Haemophilia 2010; 16:675–682.
Description of 12 novel FXIII-B mutations, one in homozygous form.

24 Ichinose A, Souri M. As many as 12 cases with haemorrhagic acquired factor XIII deficiency due to its inhibitors were recently found in Japan. Thromb Haemost 2011; 105:10–11.
Eleven new cases of FXIII deficiencies due to autoantibodies against FXIII-A and one due to an autoantibody against FXIII-B.

25 Luo Y, Zhang G, Zuo W, *et al.* Acquired factor XIII inhibitor in monoclonal gammopathy of undetermined significance: characterization and cross-linked fibrin ultrastructure. Ann Hematol 2010; 89:833–834.
Report on a neutralizing autoantibody against FXIII-A.

26 Luo YY, Zhang GS. Acquired factor XIII inhibitor: clinical features, treatment, fibrin structure and epitope determination. Haemophilia 2011 [Epub ahead of print].
A case report on a FXIII-A deficiency due to a neutralizing autoantibody.

27 Ishida F, Okubo K, Ito T, *et al.* Spontaneous regression of the inhibitor against the coagulation factor XIII A subunit in acquired factor XIII deficiency. Thromb Haemost 2010; 104:1284–1285.

28 Ajzner E, Schlammadinger A, Kerenyi A, *et al.* Severe bleeding complications caused by an autoantibody against the B subunit of plasma factor XIII: a novel form of acquired factor XIII deficiency. Blood 2009; 113:723–725.

29 Lawrie AS, Green L, Mackie IJ, *et al.* Factor XIII: an under diagnosed deficiency – are we using the right assays? J Thromb Haemost 2010; 8:2478–2482.
Adaptation of ammonia release FXIII assays to automated coagulometers. Demonstration of the importance of blank deduction in the low range of FXIII activity.

30 Ajzner E, Muszbek L. Kinetic spectrophotometric factor XIII activity assays: the subtraction of plasma blank is not omissible [corrected]. J Thromb Haemost 2004; 2:2075–2077.

31 Todd T, Perry DJ. A review of long-term prophylaxis in the rare inherited coagulation factor deficiencies. Haemophilia 2010; 16:569–583.
This study reports guidelines for the treatment of patients affected with FXIII deficiency.

32 Castaman G. Prophylaxis of bleeding episodes and surgical interventions in patients with rare inherited coagulation disorders. Blood Transfus 2008; 6 (Suppl 2):s39–s44.

33 Bolton-Maggs PH, Perry DJ, Chalmers EA, *et al.* The rare coagulation disorders: review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. Haemophilia 2004; 10:593–628.

34 Lusher J, Pipe SW, Alexander S, Nugent D. Prophylactic therapy with Fibrogammin P is associated with a decreased incidence of bleeding episodes: a retrospective study. Haemophilia 2010; 16:316–321.

35 Gootenberg JE. Factor concentrates for the treatment of factor XIII deficiency. Curr Opin Hematol 1998; 5:372–375.

36 Hsieh L, Nugent D. Factor XIII deficiency. Haemophilia 2008; 14:1190–1200.

37 Dreyfus M, Barrois D, Borg JY, *et al.* Successful long-term replacement therapy with FXIII concentrate (Fibrogammin((R)) P) for severe congenital factor XIII deficiency: a prospective multicentre study. J Thromb Haemost 2011 [Epub ahead of print].
This is the first prospective cohort study reporting a systematic longitudinal follow-up of patients with severe FXIII deficiency, receiving FXIII replacement therapy with FXIII concentrate.

38 Lovejoy AE, Reynolds TC, Visich JE, *et al.* Safety and pharmacokinetics of recombinant factor XIII-A2 administration in patients with congenital factor XIII deficiency. Blood 2006; 108:57–62.

39 Inbal A, Oldenburg J, Carcao M, *et al.* Recombinant factor XIII, safe and novel treatment for congenital factor XIII deficiency (ASH Annual Meeting Abstracts). Blood 2010; 116:20.
This study reports information about efficacy and safety of a novel recombinant FXIII.