Fibrin Stabilization (Factor XIII), Fibrin Structure and Thrombosis

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Abstract
Factor XIII (FXIII) is a zymogen that is converted into an active transglutaminase (FXIIIa) by the concerted action of thrombin and Ca2+. Its main task is to cross-link α-, and γ-chains of fibrin and α2-plasmin inhibitor to fibrin. By this way FXIIIa strengthens fibrin and protects it from the prompt elimination by fibrinolytic system. The changes of FXIII level in thrombotic diseases are hardly explored and there are contradictory results concerning the protective effect of Val34Leu polymorphism against arterial or venous thrombosis. The results suggest that the thrombo-protective effect of Leu34 allele prevails only in certain genetic and/or environmental constellations.

Structure and Function of Blood Coagulation Factor XIII

Blood coagulation factor XIII (FXIII) is a zymogen of tetrameric structure (A2B2) containing two potentially active A subunits (FXIII-A) and two inhibitory/carrier B subunits (FXIII-B). (The structure and function of FXIII has been reviewed extensively in references 1,2.) In the plasma FXIII is bound to a minor variant of the fibrinogen γ-chain, through its B subunit [3]. In the γ-chain a 20 amino acids sequence is substituted for the C-terminal 4 amino acids of the major variant γ(A). In addition to the plasma, FXIII is also present in platelets, monocytes/macrophages and in their bone marrow precursor cells. The primary site of FXIII-A synthesis are cells of bone marrow origin, while FXIII-B is synthesized by hepatocytes and the two subunits form a tetrameric complex in the plasma. Cellular FXIII lacks FXIII-B and exists as the dimer of FXIII-A (A2). The structure of the genes coding for FXIII-A and FXIII-B and the primary structure of both subunits have been revealed. The three dimensional structure of the A2 zymogen shows a compact symmetrical arrangement. FXIII-A consists of 5 domains: an activation peptide, a central core domain, two β barrels and a β sandwich domain.

Plasma FXIII is transformed into an active transglutaminase (FXIIIa) by the proteolytic action of thrombin and by Ca2+ in the final phase of the coagulation cascade.
Thrombin removes an activation peptide of 37 amino acid residues from the N-terminal end of FXIII-A, then in the presence of Ca²⁺ the inhibitory/carrier B subunits (FXIII-B) dissociate and FXIII-A assumes an enzymatically active configuration. The presence of fibrin greatly accelerates the activation process.

Transglutaminases (protein-glutamine: amine γ-glutamyltransferase; EC 2.3.2.13) catalyze an acyl transfer reaction (Fig 1). In the first step of the modified double displacement reaction a peptide-bound glutamine residue forms a thioacyl intermediate with the active site Cys-314 and ammonia is released. In the absence of an amine substrate hydrolysis of the thioacyl intermediate occurs and the peptide bound glutamine becomes deamidated. If a substrate primary amine is present the acyl group is transferred to the acyl acceptor primary amine and the amine becomes linked to the glutamyl residue through an isopeptide bond. If the substrate amine is provided by the ε-amino group of a peptide bound lysine residue an ε(γ-glutamyl)lysyl is formed and peptide chains become covalently cross-linked.

Inherited FXIII deficiency is rare (1:3,000,000), but if non-supplemented the patients usually suffer from severe hemorrhagic diathesis. Delayed umbilical bleeding after birth and the high frequency of intracranial bleeding are characteristic symptoms. FXIII deficient women cannot carry out pregnancy (reviewed in references 4-7) and FXIII deficiency is relatively frequently associated with impaired wound healing. More than 50 causative mutations have been described in the gene coding for FXIII-A [8,9] and a few cases with mutation in the FXIII-B gene have been also reported [9,10].

**FXIIIa-induced Cross-linking Reactions in the Fibrin Clot and Their Role in the Regulation of Fibrinolysis**

The main biochemical task of FXIII in the normal hemostasis is to cross-link fibrin chains and to covalently attach proteins important in the regulation of fibrinolysis to the newly formed fibrin network. Cross-linking by FXIIIa improves the mechanical strength, rigidity and elasticity of the clot and increases its resistance to fibrinolysis [11-16]. Inhibition of FXIIIa activity enhances fibrin degradation mediated by plasmin in vitro [17] and accelerates thrombolysis in animal models of venous and arterial thrombosis and in experimental pulmonary embolism [18-20].

FXIIIa cross-links fibrin γ-, and α-chains and the major cross-linked products are γ-chain dimers and high Mr α-chain polymers (extensive up-to-date reviews on fibrin structure and its relation to the cross-linking process are provided in references 21-23). γ-chain dimer formation involves reciprocal intermolecular cross-linking between 406 lysine and γ 398/399 glutamine residues. It is an extremely quick process, requires only minute amount of FXIIIa, and immediately follows the removal of fibrinopeptide A from fibrin. γ-containing fibrin is cross-linked more slowly than the major γ(A)γ(A) form [3,23]. The multiple cross-linking of α-chains among several acyl donor and...
acyl acceptor sites proceeds more slowly than γ-chain dimer formation. Minor, slowly forming cross-linked products are γ-α chain heterodimers [24,25] and γ-chain trimers / tetramers [24-27].

α2-plasmin inhibitor (α2-PI) is an excellent acyl donor substrate for FXIIIa and it can be cross-linked to the α-chain of fibrin and fibrinogen [28-31]. There are two N-terminal isoforms of α2-PI in the plasma. From part of the secreted Met1 isoform a peptide of 12 amino acids is cleaved off by a plasma protease that transforms Met1-2-PI into Asn1-α2-PI [32-34]. Asn1-α2-PI is much more effective in the cross-linking reaction than Met1-α2-PI and the rate of binding of its major acyl donor site, Gln2, to fibrin α-chain is comparable to the rate of γ-chain dimerization [33,34]. At a much slower rate α2-PI-fibrin α-chain heterodimers are cross-linked to other α-chains and incorporated into the highly cross-linked α-chain polymers. α2-PI cross-linked to fibrin retains its full inhibitory capacity.

Both fibrin cross-linking and the cross-linking of α2-PI to fibrin have been suggested to play a major role in protecting fibrin from fibrinolysis. FXIII, in excess to its plasma concentration resulted in excessive cross-linking of α-chains and inhibited the degradation of fibrin by plasmin [35]. γ-chain dimerization might also contribute to the increased resistance of cross-linked fibrin to fibrinolysis [36]. Another mechanism, the down-regulation of the binding of plasminogen to fibrin by fibrin cross-linking might also render fibrinolysis less effective [37]. The significance of fibrin cross-linking in inhibiting fibrinolysis was also demonstrated in experimental pulmonary embolism [20].

Other experiments demonstrated that cross-linking of fibrin is less important [38,39] and the presence of α2-PI is essential for the anti-fibrinolytic effect exerted by FXIII. Inhibition of the binding of α2-PI to fibrin considerably accelerates the lysis of the clot [17,40,41]. α2-PI mutated at its active site also enhanced fibrin degradation [42]. Using an antibody that inhibited the formation of FXIIIa-fibrin complex, i.e. fibrin cross-linking, but did not influence the cross-linking of α2-PI to fibrin, McDonagh and Fukue showed that both mechanisms are important in the inhibition of fibrinolysis [43]. It was also shown that plasma FXIII is the major player in exerting these cross-linking reactions in the blood, the contribution of platelet FXIII is insignificant [44].

The data discussed above suggest the following hypothesis: the cross-linking of α2-PI to fibrin occurs at an early stage of fibrin formation and provides protection of newly formed fibrin against the activated fibrinolytic system, while the increased resistance to thrombolysis of matured thrombi could be the consequence of extensive cross-linking of fibrin α-chains, including those α-chains to which α2-PI had been attached.

In addition to α2-PI, FXIIIa also cross-links further components of the fibrinolytic system to fibrin. The cross-linking of plasminogen [45,46] and thrombin-activable fibrinolytic inhibitor (procarboxypeptidase U; TAFI) [47] to fibrin is interesting, however the physiological significance of these reactions remains to be elucidated. The cross-linking of type-2 plasminogen activator inhibitor (PAI-2) has been well characterized [48-50] and it was shown that fibrin-linked PAI-2 remains fully active [50]. With the exception of pregnancy PAI-2 does not appear in the plasma, but activated monocyte incorporated in the thrombus might secrete it [49,50]. PAI-2 targeted to fibrin by cross-linking might be involved in the protection against urokinase-type plasminogen activator.

Cross-linking of fibrin and α2-PI to fibrin also occurs in the extravascular compartment, like in the tumor stroma or at the site of inflammation. Extravascular cross-linked fibrin deposits have been detected in guinea pig carcinomas, in lymph nodes with Hodgkin’s disease [51-53] and in rheumatoid synovial tissue [54,55]. Both plasma FXIII leaked out through the capillary wall and cellular FXIII derived from tumor associated and inflammatory macrophages [51,52,56] could contribute to the cross-linking process. The presence of FXIII, α2-PI and α2-PI-plasmin neoantigen over fibrin strands in lymph nodes with Hodgkin’s disease strongly suggests that fibrin-linked α2-PI provides an effective protection for fibrin against plasmin degradation in the tumor stroma, as well.

**Factor XIII Levels in Thrombotic Vascular Diseases**

The relationship between coronary sclerosis and plasma FXIII activity/antigen concentration has been addressed in a few studies. Unfortunately, activity measurements in these studies were carried out with the microplate incorporation assay [57,58] in which the zero order kinetic is not followed and, due to insufficient FXIII activation, the results of activity measurements are strongly influenced by Val34Leu polymorphism. Results obtained by this assay are difficult to interpret and to compare to FXIII antigen levels (see comments in reference [59]). Somewhat elevated FXIII-A and FXIII-B antigen levels (120% and 111% versus 100% and 106%) were demonstrated in patients with coronary sclerosis. However the difference in the FXIII-B, but not FXIII-A level disappeared after adjustment for age, sex, platelets, fibrinogen, cholesterol and triglyceride [60]. In another study elevated level of FXIII-B was found in male patients with coronary sclerosis and their first-degree relatives, the tetrameric FXIII concentration did not change [61]. In UK Asians coronary sclerosis failed to influence FXIII subunit.
levels [62]. In another study in which patients and controls were classified by coronary angiography neither of the FXIII subunits showed association with coronary sclerosis [63]. In two studies the history of myocardial infarction (MI) had no influence on FXIII levels [60,62], while in the Second Northwick Park Heart Study patients who developed MI had lower adjusted FXIII-A levels than controls at recruitment (129.2% versus 113.3%) [64]. In two early studies [65,66] on the changes of FXIII levels during the acute phase of MI a depression of FXIII activity, with a nadir on day 4, was demonstrated [66]. Surprisingly, no changes in FXIII-A and FXIII-B plasma levels were detected by electro-immunoassay.

FXIII-A and antigen levels increased in obliterative atherosclerosis of the lower limb [67] and also in diabetic angiopathy [68]. Stroke patients had significantly higher level of FXIII-B (125.1%) than controls (110.8%), while there was no significant difference in FXIII-A antigen [64]. FXIII-A concentration was lower in stroke patients who had died during a follow-up period of 54 months than in patients who were still alive after 54 months. In acute stroke a depression of FXIII concentration developed [65]. In a small-scale early study (n=12/19) significant decrease of FXIII-A activity and concentration of FXIII-A was observed in the acute phase of DVT that returned to normal within two weeks [69]. FXIII-A and FXIII-B levels were unaltered in the non-acute phase and subunit levels were not associated with significant risk for DVT [70].

**Fig 2.** The effect of factor XIII-A Leu34 allele on the risk for myocardial infarction. The respective references are in parentheses, the year of publication and the number of cases/controls are also indicated on the left side of the figure. Horizontal lines represent 95% confidence intervals, calculated odds ratios are shown above the lines.

*In the ARIC study a case cohort design was used for the nested genetic study and the risk for coronary heart disease (CHD) incidence was calculated.

**Val34Leu Polymorphism in the A Subunit of Factor XIII and its Relationship to the Risk for Thrombotic Vascular Diseases**

In the last couple of years the Val34Leu polymorphism in the activation peptide of FXIII-A [71], has stirred considerable interest, because of its suspected thrombo-protective effect (discussed in details below). The frequency of Leu34 allele in the Caucasian population varies within the range of 24.5-28.8% [72-81]. In blacks [77,81] and Asian Indians [79] the allele frequency is lower and in the Japanese population this polymorphism is extremely rare [81,82]. In certain South American Indian tribes the frequency of Leu34 allele is even higher, up to 40%, than among Caucasians [79,81].

It has been demonstrated with cellular [83] as well as with plasma [72,84] FXIII that from the Leu34 variant the release of activation peptide by thrombin proceeds at a 2.5-fold higher rate than from the Val34 variant. Val34Leu polymorphism does not influence the transglutaminase activity of FXIIIa [72,83,84]. The higher rate of proteolytic truncation of Leu34 FXIII-A resulted in earlier activation of FXIII and consequently, accelerated the cross-linking of fibrin γ, and α-chains [72,83,84] and the cross-linking of α2-PI to fibrin [85]. Ariens et al. demonstrated that the Val34Leu polymorphism also influences the structure of fibrin, probably through the alteration of fibrin cross-linking kinetic [84,86]. (For further details see a most recent review in reference 87).
Factor XIII in Coronary and Peripheral Artery Diseases

Studies concerning the effect of Leu34 carrierhip on the risk for MI is summarized in Fig. 2. In the initial case-control study it was demonstrated by Kohler et al. in a Caucasian patient population FXIII-A Val34Leu polymorphism provided a protective effect against MI [73]. Three other FXIII-A common polymorphisms, Pro564Leu, Va1650Ile and G1u651Gln were found not to be associated with protection against coronary artery disease [80]. The original observation on the protective effect of FXIII-A 34Leu allele was confirmed by smaller studies from Finland [74,62], from Brazil [88] and from Northern Italy [89]. In the Brazilian study Leu34 carrierhip also attenuated the increased risk caused by metabolic risk factors and smoking.

However, contradictory results also started to accumulate. Studies from Southern France [76] and Spain [90,91], as well as a huge Italian study involving 1210 cases [92] did not find any effect of the Val34Leu polymorphism on the risk of MI. In a Spanish population the homozygous Leu/Leu genotype even represented a risk for MI. The Atherosclerosis Risk in Communities (ARIC) Study [93] and a study involving the genetically isolated New-found-land population [94] suggest that the absence of protection by the Leu34 allele is not restricted to Mediterranean communities. In the latter study the occurrence of combined carriers of FXIII-A Leu34 and prothrombin 20210A alleles was 12-fold higher in MI patients than in control subjects. Leu34 allele did not provide protection against MI for young women [95] and in MI patients below the age of 45 years the presence of Leu34 allele strongly reduced the efficiency of thrombolytic therapy (OR: 5.11, CI:1.28-21.12) [96].

In spite of the contradictory results Val34Leu polymorphism might be a relatively weak protective factor the effectiveness of which could be augmented by certain genetic and/or environmental constellations. Two examples for such interactions are presented below. In young women with obesity the Leu34 allele provided significant protection against MI, while in non-obese subject it did not [95]. In women on postmenopausal estrogen therapy the presence of the FXIII-A Leu34 allele was associated with a reduced risk of MI and its combination with an otherwise ineffective polymorphism His95Arg in FXIII-B reduced the risk even further [97].

Considering the relatively high number of studies investigating the effect of Val34Leu polymorphism on MI, it is surprising that only a few study has addressed its relationship with the development of occlusive vascular disease. The Leu34 allele does not seem to be protective against the development of coronary sclerosis [61,98] and peripheral artery disease [99], but maybe linked to a later onset of the latter disease.

Factor XIII and Stroke

Results of studies concerning the effect of FXIII-A Val34Leu polymorphism on the risk of stroke are summarized in Table 1. No association of Leu34 allele with ischemic stroke was demonstrated in the initial study on this subject [100]. Similar results were obtained in two smaller studies from Spain [90,101]. A protective effect of the Leu34 allele was, however demonstrated in two larger studies [75,89]. In addition, the protective effect of Leu34 carrierhip out-weighted the harmful effect of smoking. In the largest study, carried out so far, Endler et al. analyzed 718 patients with ischemic stroke and 369 controls for Val34Leu polymorphism [102] and reached the conclusion that the association of FXIII Val34Leu polymorphism with a decreased risk of ischemic stroke is highly unlikely. It is interesting that in young women carrying two copies of the Leu allele Reiner et al. reported a nearly 4-fold increased of ischemic stroke [95]. However, the latter study involved only 36 cases.

FXIII and Deep Vein Thrombosis

The eight studies dealing with the association of Val34Leu polymorphism and deep vein thrombosis (DVT) have been most recently reviewed by van Hylckama Vlieg et al. [70]. A statistically significant protective effect of the Leu34 allele against DVT was demonstrated in two studies [103,104], in one study only the homozygous genotype was protective [77], and in five studies no statistically significant association between Leu34 carriers or Leu34 homozygous genotype and the risk for DVT was found [70,72,90,105,106]. By meta-analysis of the results published, so far, excluding a study on cancer patients, the pooled risk estimates calculated for the Leu34 carriers and

Table 1. Factor XIII Val34Leu polymorphism and the risk for cerebrovascular diseases. References are give in parenthesis, year of publication is also shown. IS, ischaemic stroke; CVD, cerebrovascular disease; OR, odds ration; NS, not significant

<table>
<thead>
<tr>
<th>Reference</th>
<th>n (cases/controls)</th>
<th>Diagnosis</th>
<th>Finding</th>
<th>Country</th>
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<tbody>
<tr>
<td>[100] 1998</td>
<td>529/435</td>
<td>IS</td>
<td>Leu34 carrier frequency: 46.5%/41.7% (NS)</td>
<td>UK</td>
</tr>
<tr>
<td>[90] 2000</td>
<td>104/104</td>
<td>CVD</td>
<td>Leu34 allele frequency: 22.0%/18.0% (NS)</td>
<td>Spain</td>
</tr>
<tr>
<td>[75] 2000</td>
<td>456/456</td>
<td>IS</td>
<td>OR (Leu34 allele): 0.58 (0.44-0.75)</td>
<td>France</td>
</tr>
<tr>
<td>[101] 2001</td>
<td>116/467</td>
<td>CVD</td>
<td>OR (Leu34 allele): 1.23 (0.85-1.76)</td>
<td>Spain</td>
</tr>
<tr>
<td>[89] 2001</td>
<td>240/200</td>
<td>CVD</td>
<td>OR (Leu34 allele): 0.66 (0.47-0.90)</td>
<td>Italy</td>
</tr>
<tr>
<td>[95] 2002</td>
<td>36/345</td>
<td>IS</td>
<td>OR (Leu34 allele): 1.63 (0.74-2.06)</td>
<td>Holland</td>
</tr>
<tr>
<td>[102] 2003</td>
<td>718/369</td>
<td>IS</td>
<td>Leu34 allele frequency: 27.6%/28.3% (NS)</td>
<td>Austria</td>
</tr>
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for the Leu34 homozygotes (OR=0.9, CI: 0.8-1.0) and OR=0.8, CI: 0.6-1.0, respectively) demonstrated a slight overall protective effect of the Val34Leu polymorphism. No protective effect of Val34Leu polymorphism against DVT was demonstrated in cancer patients (OR: 1.0, CI: 0.55-2.01) [107].

Such a slight overall effect of Val34Leu polymorphism on the risk of DVT, does not justify the inclusion of the determination of FXIII-A Val34Leu polymorphism into a general thrombophilia profile [108]. However, here again, constellation with different genetic and environmental factors might influence the actual effect and research on finding such constellations may provide important information.

References


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