The Role of Factor XIII Val34Leu Polymorphism in Thrombotic Disease

Die Rolle des Faktor XIII Val34Leu Polymorphismus bei thrombotischen Erkrankungen

Silke Ehrenforth[1], M. von Depka[2]

Summary: Activated blood coagulation factor (F) XIII (FXIIIa), a transglutaminase comprised of two A and two B subunits in a tetrameric structure (A_2B_2) of 320 kd, has a central role in the haemostatic system by cross-linking fibrin monomers in the final step of blood coagulation, thus stabilizing the fibrin clot and increasing its resistance to fibrinolysis. In addition, FXIIIa is implicated in the cross-linking of several other proteins, such as a-2-antiplasmin, fibronectin, and collagen. The impact of genetic variations of FXIII in thrombotic disorders has not been studied until recently, when a common polymorphism was described as a new candidate genetic factor influencing the risk of thrombotic diseases. This polymorphism results from a G to T transition in codon 34 of exon 2 of the catalytic FXIII A-subunit gene, leading to the substitution of leucine for valine (FXIIIVal34Leu) close to the thrombin activation site. Genotype at this polymorphism is closely related to FXIII fibrin cross-linking activity, and FXIIILeu is associated with increased thrombin activation of FXIII with associated changes in fibrin structure. Initially, FXIII Val34Leu was shown to be significantly less common in British patients with a history of myocardial infarction than in controls, suggesting for the first time a new role for FXIII in a polygenic thrombotic disease. In addition to its proposed protective effect against thrombotic heart diseases, the Leu34 allele has also been correlated with protection against venous thromboembolism and thrombotic cerebral artery occlusion, whereas it seems to confer an increased risk for intracerebral haemorrhage. Because this genetic variation is associated with a higher activity of the enzyme, the mechanism accounting for the putative anti-thrombotic effect of FXIII Val34Leu is not well understood. However, it has been hypothesized that increased rates of FXIII activation could lead to ineffective cross-linking, or that the kinetics of the cross-linking reactions may be disrupted because of the effects of FXIIIa on other proteins. Previous studies have demonstrated that the FXIII Val34Leu polymorphism is highly prevalent in several Caucasian populations, with reported Leu34 allele frequencies of around 0.25, whereas it is less prevalent in populations of African and Asian origin. The known significant ethnic heterogeneity linked to the FXIII Val34Leu polymorphism is of relevance when analyzing its role in vascular diseases. In summary, published studies indicate that blood coagulation FXIII is involved in the multifactorial pathogenesis of vascular diseases and suggest a contribution of FXIII Val34Leu in determining the risk of myocardial infarction, stroke and venous thromboembolism.

Keywords: blood coagulation factor XIII; Factor XIII Val34Leu polymorphism; Genetics; Risk factors; Cardiovascular disease; Venous Thromboembolism.

Zusammenfassung: Der Blutgerinnungsfaktor (F) XIII (fibrinstabilisierender Faktor), welcher ein Molekulargewicht von 320 kDa besitzt, liegt im Plasma als tetramerer Komplex vor, bestehend aus je zwei Untereinheiten A (katalytisch wirksam) und B (Carrierfunktion) (A_2B_2). Das Zymogen A_2B_2 wird durch Thrombin zur aktiven Transglutaminase aktiviert (FXIIIa), welche über die Ausbildung von intermolekularen Amidbindungen die End-zu-End-polymerisierten Fibrinmonomere kovalent miteinander vernetzt, so daß ein stabiles Fibringerinnsel entsteht. Neben dem Hauptsubstrat Fibrin sind verschiedene andere Proteine identifiziert worden, die durch FXIIIa quervernetzt werden können, wie beispielsweise a-2-Antiplasmin, Fibronektin und Kollagen. Über die mechanische Stabilisierung des gebildeten Fibringerinnsels und Verstärkung dessen fibrinolytischer Resistenz durch den Einbau von Fibrinolyseinhibitoren in das Gerinnsel, kommt dem FXIIIa eine zentrale Rolle im komplexen hämostatischen System zu. Trotz seiner vielfältigen Funktionen erlang die Bedeutung von FXIII Genvarianten im Rahmen thrombotischer Erkrankungen erst kürzlich ein zunehmendes Interesse, nachdem ein häufig vorkommender FXIII Polymorphismus als potentieller thrombo-protektiver Parameter beschrieben wurde. Dieser Polymorphism resultiert aus einer G zu T Transition im Codon 34 des Exon 2 der katalytischen A-Region des FXIII-Gens, welcher zu einem Austausch von Leucin gegen Valin (FXIIIVal34Leu) nahe der Thrombinschnittstelle führt. Der prozentuale Anteil der Mutationsträger an der gesunden Bevölkerung variiert zwischen 32 % und 52 %, wobei für die

[1]Department of Internal Medicine, University Hospital, Frankfurt am Main, Germany
[2]Department of Internal Medicine, Medical School, Hannover
Corresponding Author: Dr. Silke Ehrenforth, Johann-Wolfgang-Goethe-Universität, Zentrum für Innere Medizin, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany. Fax: +49 69-6301-6738

kaukasischen Populationen eine durchschnittliche Leu34 Allelfrequenz von 0,25 angegeben wird. Pathogenetisch weist der Val34Leu Genotyp eine enge Verbindung mit der Fibrin-vernetzenden FXIII-Aktivität auf, wobei FXIIILeu mit einer erhöhten thrombininduzierten FXIII-Aktivierung sowie einer veränderten Fibrinstruktur assoziiert ist. In einer initialen Studie wurde bei britischen Patienten mit Myokardinfarkt eine gegenüber Kontrollpersonen signifikant geringere FXIII Val34Leu Prävalenz beobachtet, was zum ersten Mal auf eine Bedeutung des FXIII im Rahmen der Thrombogenese hinwies. Neben dem daraufhin postulierten protektiven Effekt hinsichtlich kardiovaskulärer Erkrankungen, wurde für den FXIII Val34Leu Genotyp anschließend auch eine protektive Wirkung gegenüber venösen Thrombosen und atherothrombotisch bedingten Schlaganfällen sowie andererseits ein erhöhtes Risiko für intrazerebrale Blutungen beschrieben. Der dem anti-thrombotischen Effekt zugrunde liegende Wirkungsmechanismus bleibt bis dato jedoch ungeklärt. Hierbei wurde u.a. hyothetisiert, dass die mit dem Val34Leu einhergehende erhöhte FXIII Aktivierung zu einer ineffektiven Quervernetzung der Fibrinmonomere führt oder aber, dass die entsprechende Reaktionskinetik über die Wirkung von FXIIIa auf andere Proteine maßgeblich gestört wird. Zusammenfassend ist festzuhalten, dass zahlreiche Studien auf eine neu Rolle des Gerinnungsfaktors XIII in der multifaktoriellen Pathogenese thrombotischer Gefäßverschlüsse hinweisen und dem prevalenten FXIII Val34Leu Polymorphismus möglicherweise eine klinisch relevante Bedeutung bei der Risikoabschätzung bezüglich Myokardinfarkt, ischämischer Apoplexie und venös-thrombotischer Ereignisse zukommt.

Schlüsselwörter: Gerinnungsfaktor XIII; Faktor XIII Val34Leu Polymorphismus; Cardiovaskuläre Erkrankungen; Venöse Thrombose/Genetik; Risikofaktoren.

Over the last years, several genetic defects of proteins regulating blood coagulation have been established as risk factors predisposing for vascular thrombosis, most notably resistance to activated protein C due to the factor (F) V G1691A mutation (FV Leiden), the prothrombin gene G20210A variant, and protein C, S, and antithrombin deficiency. More recently, there is a growing interest in the role of FXIII in thrombotic vascular diseases, after several studies have provided evidence that a common variation of the FXIII gene might be protective against arterial and venous thrombosis.

Structure and Function of Factor XIII

Blood coagulation FXIII is a protransglutaminase found in plasma as a 320 kd tetramer of two catalytic A-subunits which circulate in plasma in an inactive form bound to two B-subunits (A_2B_2), which serve a carrier function for the A subunit in plasma [1, 2; see also Fig.1]. Transformation of the pro-enzyme FXIII into an active transglutaminase (FXIIIa) during the terminal phase of the coagulation cascade is induced by the proteolytic action of thrombin, which cleaves the peptide bond between Arg37 and Gly38 of the A subunit [1, 3], resulting in the release of an amino-terminal activation peptide. This is followed by the dissociation of the A-subunit dimer from the B-subunit in a reaction facilitated by fibrin and calcium ions [4, 5]. Activated FXIII (FXIIIa) plays an important role in the final stage of the blood coagulation pathway by catalyzing the covalent cross-linking of two g-glutamyl with two e-lysine residues of the g-chain of fibrin followed by polymerisation of the a-chain [6-8]. The resulting formation of crosslinked fibrin from (soluble) fibrin monomers is pivotal to the development of a stable thrombus with increased resistance to chemical, mechanical, and proteolytic insults. In addition, several other proteins in the coagulation and fibrinolytic system are substrates for activated FXIII, such as a2-antiplasmin [9, 10], thrombin-activable fibrinolysis inhibitor (TAFI) [11], von Willebrand factor [12], thrombospondin [13], and fibronectin [14, 15]. Cross-linking of these substrates into the clot further contributes to the mechanical strength and resistance to proteolysis of fibrin. By these mechanisms, FXIIIa is essential for maintaining hemostasis, and deficiency of FXIII, a rare autosomal recessive disorder, is characterized by bleeding diathesis and defective wound healing.

The Factor XIII Gene Val34Leu Polymorphism

The FXIII A-subunit gene is localized on chromosome 6, spans more than 160kb and contains 15 exons separated by 14 introns [16]. Whereas several mutations of the FXIII A-subunit gene have been related to FXIII deficiency [10, 17–20], the role of FXIII gene variants in thrombotic diseases has not been studied until recently, when a common FXIII polymorphsim was suggested to decrease the risk of myocardial infarction [21, 22] and thrombotic cerebral artery occlusion [23]. This polymorphism results from a G to T transition in exon 2 of the FXIII A-subunit gene, coding for a valine (Val) to leucine (Leu) change at amino acid position 34 (FXIII Val34Leu) near the cleavage site of the FXIII activation peptide [17]. This genetic variation is very common in Caucasian populations, with reported Val34Leu carrier frequencies ranging from approximately 32 % to 52 % (averages about 44 %), and Leu allele frequency of around 0.25 (range 0.17–0.29), respectively [21–35]. However, information upon its distribution in different ethnic groups is scarce. In a recent study reported by *Attié-Castro* et al. [34], FXIII Val34Leu was detected in 44.3 % of Caucasians (Brazilians of European descent and Portuguese), in 28.9 % of Blacks (Brazilians, and Africans from Cameroon, Zaire, and Angola), in 2.5 % of Asians

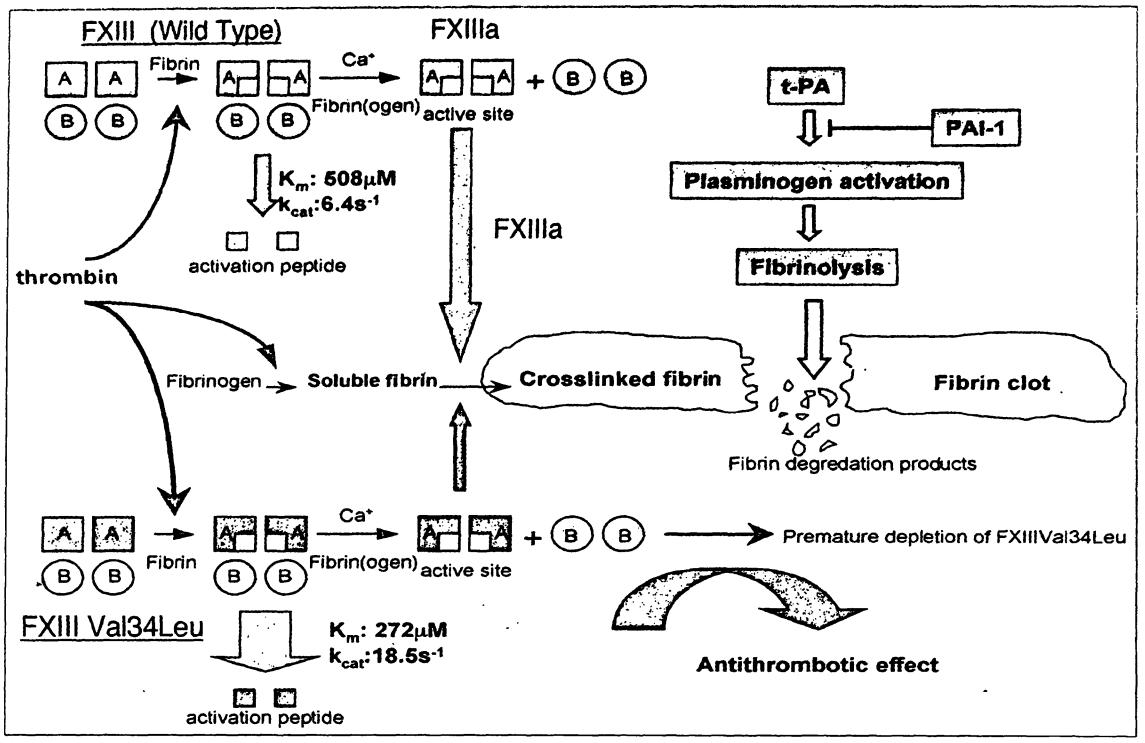

Figure 1 FXIII pathway and possible effects on clot formation by genotype.
After activation by thrombin, a small activation peptide is cleaved from the A-subunit. In a further step, the FXIII tetramer dissociates by separating the B subunits (carrier protein) from both A subunits. The active site of FXIII is now ready for the crosslinking reaction. The rate of FXIII activtion and therefore cleavage of the activation peptide is influenced by FXIII genotype with an increase in kcat but a decrease in Km in possession of the Leu allele. These changes lead to premature depletion of FXIII-Val34Leu and a decrease in crosslinking reaction. Fibrinogen is activated by thrombin to form soluble fibrin. In a further step, soluble fibrin is cross-linked by FXIII to form a stable, insoluble fibrin network. Plasminogen activation by tissue plasminogen activator (t-PA) and therefore initiation of fibrinolysis is inhibited by plasminogen activator inhibitor-1 (PAI-1), a strong marker of insulin resistance.

Reprint from *Kohler HP.* Role of blood coagulation factor XIII in vascular diseas. Swiss Med Wkl 2001; 131:31–34 [51] with kind permission of *H.P. Kohler* and EMH Schweizerischer Ärzteverlag AG.

(Japanese descendents), and in 51.2 % of Amerindians. These data demonstrate that FXIII Val34Leu exhibits a significant ethnic heterogeneity, a finding that is relevant for studies analyzing the role of this polymorphism in vascular diseases.

The Factor XIII Val34Leu Polymorphism in Arterial Occlusive Disease

Because of the high allele frequency and the proximity of the amino acid change to the thrombin activation site, FXIII Val34Leu was a candidate factor involved in the pathogenesis of thrombotic events. In a first case-control study of Caucasian patients with angiographically proven coronary artery disease and a history of myocardial infarction, the mutant Leu34 allele was reported to be significantly less common in cases than in controls, suggesting for the first time a new role for FXIII in polygenic thrombotic vascular disease [21]. Subsequently, the proposed cardioprotective effect of FXIII Val34Leu, which has been shown to be particularly strong in homozygous carriers, while heterozygosity conferred moderate protection, has been confirmed in other studies [22, 36, 37]. It is also interesting to note, that the frequency of Val34Leu carriership in different ethnic groups has been shown to be inversely related to the prevalence of coronary artery disease in different ethnic groups [25], leading to the hypothesis that this polymorphism might contribute to the contrasting cardiovascular risk in different populations. It has been suggested that possession of the mutant Leu allele might favour the formation of weaker fibrin structures, thereby conferring a protective effect against arterial thrombosis [21] or predisposing to hemorrhagic stroke [26]. Two published studies evaluated the association between FXIII Val34Leu and a history of stroke [23, 26]. The first study revealed no significantly different frequencies of the Val34Leu mutation among British patients with thrombotic cerebral infarction as compared to (not individually matched) healthy controls, though there was a marginally significant increase in Leu34 in subjects with a diagnosis of

primary intracerebral hemorrhage [26]. By contrast, a subsequent large multi-centre case-control study of French patients showed a significantly negative association of FXIIIVal34Leu with atherothrombotic stroke (OR 0.58, 95 %CI 0.44–0.75), suggesting that the Leu34 allele also has a protective effect against thrombotic cerebral artery occlusion [23]. Further prospective studies are therefore wanted to clarify precisely the role of FXIII Val34Leu in cerebrovascular disease.

Factor XIII Val34Leu in Venous Thrombosis

Fibrin cross-linking by FXIII is also important in the development of deep venous thrombosis (DVT). Accordingly, there have been several studies analyzing the possible association of the FXIII Val34Leu polymorphism with DVT, providing rather controversial data [27, 29, 31–33, 35, 38–41; see also Table 1]. Initially, *Catto* et al. showed a significantly lower frequency of FXIII Val34Leu among relatively old patients (median age 62 years) with venous thromboembolism (VTE) than in healthy controls (OR 0.56, 95 %CI 0.38 to 0.82), indicating for the first time that the Leu34 allele confers also protection against VT [27]. However, a subgroup analysis revealed that this protective effect is restricted to DVT, whereas no significant association between FXIII Val34Leu and the occurrence of pulmonary embolism (PE) could be demonstrated in this study [27]. Since no separate information were given for the particular subgroup of patients with PE, it remains an open question whether the lack of association between FXIII Val34Leu and PE reported by *Catto* et al. might be affected by selection of patients (e.g. coexistance of cadiovascular risk factors). In a second report, no association of the heterozygous Val34Leu change with a history of DVT was found in a group of younger patients (mean age 41 years), but the homozygous Leu34 carrier status was strongly associated with a protective effect (OR, 0.16; CI, 0.05 to 0.5) [29]. Further evidence for a mildly protective effect against the development of VT associated with FXIII Val34Leu carriership has been provided by two large case-control studies, the Leiden Thrombophilia Study (LETS) [38] and the Paris Thrombosis Study (PATHROS) [40], whereas in a Hungarian study, the Leu34 allele frequencies among young patients with a first thrombotic episode before the age of 45 years (mean age at first onset 27.7 years) and healthy controls did not differ significantly, neither in the age-group between 30 and 45 years, nor in those younger than 30 years [31]. The discrepancies between the published data may reflect differences in criteria used to select the groups and in the statistical power of the studies, among others. In a meta-analysis of five case control studies [27, 29, 32, 38, 40] performed by *Alhenc-Gelas* [40], the OR (95 %CI) associated with the presence of the Leu allele in the whole population (1,340 cases, 2,211 controls) was 0.80 (0.69–0.94), while the ORs (95 % CI) associated with the Val/Leu and Leu/Leu genotypes were 0.86

Table 1 Factor XIII Val34Leu and the Risk of Venous Thromboembolism

Study	Patients (n)	Controls (n)	Carrier Frequency % Patients vs Controls		OR (95 %CI)		
			V/L	L/L	V/L	L/L	V/L+L/L
Catto 1999 United Kingdom	226	224	31 vs 42	12 vs 22			0.63 (0.38–0.82)
Franco 1999 Brazil	189	187	37 vs 31.6	1.6 vs 9.6	1.1 (0.7–1.7)	0.16 (0.05–0.5)	0.9 (0.6–1.4)
Rosendaal 1999 LETS, The Netherlands	474	474	34.8 vs 36.7	4.2 vs 5.7	0.91 (0.69–1.19)	0.71 (0.39–1.29)	
Corral 2000 Spain	97	97	35.1 vs 28.9	2.1 vs 3.1	–	–	
Alhenc-Gelas 2000 France	354	1229	32.4 vs 35.6	3.9 vs 5.2	0.81(0.63–1.05)	0.69 (0.38–1.26)	
Meta-analysis according to *Alhenc-Gelas* 2000	1340	2211			0.86 (0.74–0.99)	0.58 (0.41–0.82)	0.8 (0.69–0.94)
Ehrenforth 2000 Germany	430	430	33 vs 38.6	4.9 vs 7	0.78 (0.61–1.03)	0.68 (0.32–1.46)	0.76 (0.58–0.95)
Balogh 2000 Hungary	273	288	40.7 vs 38.5	6.6 vs 6.6		1.04 (0.53–2.06)	1.09 (0.78–1.52)
Margaglione 2000 Italy	427	1049	30.5 vs 28.8	4.7 vs 6.6	1.1 (0.7–1.2)	1.4 (0.9–2.3)	1.0 (0.8–1.3)

(0.74–0.99) and 0.58 (0.41–0.82), respectively, suggesting that this polymorphism may contribute to determining the risk of VTE.

Another interesting study was reported by *Franco* et al. who evaluated the effect of FXIII Val34Leu on the risk of VTE conferred by the FV Leiden mutation in a retrospective blinded study, by determining its prevalence in 352 FVL carriers, who were first-degree relatives of 132 thrombotic porositi carrying the FVL mutation [42]. The overall annual incidence of a first episode of VTE in FVL relatives was 0.31 % in Leu34 carriers and 0.44 % in non-carriers, yielding a relative risk (RR) for thrombotic episodes of 0.7 (95 %CI: 0.3–1.5). The relative risk for VTE in the age group of 15–30 years was 1.0 (95 % CI 0.3–3.2), suggesting that, in younger FVL carriers, FXIII Val34Leu does not confer protection against venous thrombosis. Further age-specific RR for VTE in FVL relatives were (for Val34Leu carriers and non-carriers, respectively): 0.4 (95 % CI 0.05–3.0) in the age group of 30–45 years, 0.6 (95 %CI 0.1–2.9) in the group aged 45–60 years and 0.5 (95 % CI 0.06–4.5) in subjects older than 60 years. Thus, a trend towards a decreased risk for VTE related to FXIII Val34Leu was observed in FVL carriers older than 30 years. However, prospective studies will reveal if routine screening for FXIII Val34Leu in FVL carriers, in order to identify carriers that will remain asymptomatic over life, is justified.

Taken together, the here mentioned clinical observations indicate that FXIII Val34Leu confers a protective effect against venous thromboembolism, albeit seemingly weak in heterozygous Leu34 carriers. However, vascular thrombosis represents a multifactorial disorder, and as such, the risk for the disease may vary in different human populations as a result of different combinations of genetic and acquired risk factors, Val34Leu being only one of them. It is possible that the only moderate or even lacking association between the FXIII Val34Leu polymorphism and VT observed in some of the above mentioned studies may be due to gene-gene or gene-environment interactions with respective risk factors not taken into account. In other words, the protective effect against VT episodes conferred by FXIII Val34Leu might be negated by coexistent hemostatic abnormalities or overshadowed by the concurrent presence of other thrombogenic risk factors, as it has been hypothesized e.g. for features of the insulin-resistance syndrome, diminished fibrinolysis related to high levels of the fibrinolytic inhibitor PAI-1 (plasminogen activator inhibitor-1), and the presence of the PAI-1 promoter 4G/4G genotype, respectively [21, 43, 44]. Furthermore, the findings reported in the above mentioned studies on FXIII Val34Leu in VT are in part difficult to assess since some of the study groups are only scantily characterized, e.g. no detailed information on clinical settings or characteristics of the thrombotic tendency were given. The impact of certain variant alleles of the genes encoding proteins regulating blood coagulation, however, may differ with respect to various clinical manifestations of venous thrombophilia, including age at onset as well as type and site of the thrombotic event. Until now, most research of the role of FXIII Val34Leu in venous thrombotic diseases has been done with patients having deep leg VT.

This fact and the partial discrepancy of the above results tempted us to investigate the distribution of the FXIII Val34Leu genotype in a group of patients with thrombotic manifestations at unusual sites (e.g. visceral or cerebral veins) and isolated pulmonary embolism, respectively, in which the etiology remains unexplained in a significant number of patients [33, 39, 45]. We therefore determined the prevalence of FXIIIVal34Leu in 342 Caucasian patients who suffered a first idiopathic and objectively confirmed isolated deep leg VT, or DVT combined with PE, or isolated PE (lacking clinical and/or radiological signs of DVT or STP) before the age of 45 years [45]. None of the patients had deficiencies of antithrombin, protein C/S or APA, nor malignancies, autoimmune or cardiovascular disorders. The 46 % prevalence of the Val34Leu polymorphism found among 430 apparently healthy controls was similar to that previously reported for other Caucasian populations. As compared to the age- and gender matched controls, the FXIII variant was equally prevalent in patients with isolated deep leg VT (Val/Leu 38.3 %, Leu/Leu 6.8 %) and only slightly less frequent in combined VTE (Val/Leu 35 %, Leu/Leu 5 %). Our findings observed in patients with a history of juvenile DVT or VTE are in agreement with recent data suggesting that the factor XIII variant decreases thrombotic risk mainly in older individuals [27, 29, 42].

In contrast, the Val34Leu variant was significantly under-represented (Val/Leu 25 %, Leu/Leu 2.5 %) among young patients with primary PE as compared to matched controls ($p<0.005$), giving an OR of 0.49 (95 %CI 0.24–0.77) [45]. These data indicate that the mutant Leu allele confers a protective effect against the early development of primary PE, or vice versa, that thrombi in carriers of the Val/Val genotype are more likely to embolize to the lungs. It might be possible that FXIII genotype related differences in fibrin cross-linking may affect the clinical presentation in terms of embolization, that the thrombus structure in Leu34 carriers is more adherent to the vessel wall and thus less prone to embolise, respectively. Alternatively, ineffective TAFI incorporation by FXIIIa into the fibrin clot could enhance fibrinolytic activity, leading to a less stable clot in the venous system, which embolises more easiliy into the pulmonary vessels [50]. Interestingly, we also found a significantly lower FXIII Val34Leu carrier frequency (32 %) among patients with an objectively confirmed history of juvenile vein thrombosis at unusual sites (upper extremity VT, cerebral VT or abdominal VT) than in healthy controls [39]. Taken together, our findings provide first, further evidence that FXIII Val34Leu is involved in the multifactorial pathogenesis of venous thromboembolic disorders and secondly, underline the view that the aeti-

ology of primary PE, deep leg VT and VT at unusual sites markedly differs from each other. Accordingly, the impact of FXIII Val34Leu seems to be significantly different with respect to various clinical manifestations of venous thrombophilia, which might have influenced the discrepant observations previously reported. In other words, the lacking protection against thrombotic events observed in previous studies might be restricted to patients with a history of deep leg VT. The role of the Val34Leu variant as a candidate factor influencing the risk of venous occlusive disease should therefore be further determined in more specified study groups, separately in patients with deep leg VT vs VT at unusal sites vs primary PE, respectively.

The Putative Protective Effect Conferred by Factor XIII Val34Leu

The proposed protective effect against vascular thrombosis conferred by the FXIII Val34Leu polymorphism is yet not well understood and indeed puzzling. In view of the close proximity of the Val34Leu change to the thrombin cleavage site, however, it seems reasonable to suppose that this polymorphism alters the activation rate of FXIII by thrombin and hence its fibrin-cross-linking activity or, alternatively, affects cross-linking of other coagulation proteins [24, 26, 28, 46]. Indeed, analysis of FXIII A-subunit proteolysis demonstrated that FXIII 34Leu was activated by thrombin more rapidly and by lower doses than 34Val [47, 48]. In addition, the Val34Leu variant has shown to increase the specific transglutaminase activity of FXIIIa, with Leu homozygotes having the higher activities compared with Val homozygotes, and Leu heterozygotes having intermediate activities [24, 28, 46]. By contrast, the FXIIIVal34Leu polymorphism has only little or even no effect on protein concentrations as measured by enzyme-linked immunosorbent assay (ELISA) technique [46, 49, 50]. In principle, faster activation and higher enzyme activity of FXIII Leu34 would be expected to result in enhanced fibrin cross-linking and increased fibrinolytic resistance of the fibrin clot, and thus could hardly be associated with its presumed protective effect against thrombotic events. It has been hypothesized, however, that increased rates of FXIII activation could lead to ineffective cross-linking or that the kinetics of the cross-linking reactions may be considerably disrupted as a result of the effects of FXIIIa on other proteins [46]. Indeed, by using turbidity and permeability measurements to analyse fibrin clots prepared from plasma of different subjects, it has recently been demonstrated that the Val34Leu polymorphism affects the function of FXIII by altering the molecular structure of the cross-linked fibrin clot to one with reduced mass/length ratio of the fibres and altered permeation characteristics [47]. Electromicroscopical analysis of the clot showed much thinner fibrin strands and a more fragile fibrin net (a finer meshwork) in carriers of the mutant Leu allele compared to the wild type group [47]. Furthermore, Factor XIII V34L was hypothesized to be susceptible to wasteful conversion of zymogen to activated enzyme [48]. Premature depletion of the mutant protein from circulation may also contribute to the protective effects against cardiovascular disease [48]. The potential consequences of increased thrombin activation of FXIII as well as the increase in its specific activity and the structural differences of fibrin cross-linked by FXIII 34Leu on clinical outcome, however, remain to be elucidated.

Factor XIII Assays

As mentioned above, the Val34Leu polymorphism has a strong effect on FXIII activation rates. This functional effect can be detected with FXIII activity assays, whereas the polymorphism has little effect on antigen levels measured by ELISA. However, as yet, only limited data are available about FXIII levels in subjects with vascular diseases and about the ability of different FXIII assays to detect (routinely) functional differences related to the Val34Leu polymorphism in vivo. Very recently a commercially available colorimetric assay based on incorporation of 5-(biotinamido) pentylamine (BAPA) into fibrin or fibrinogen has been developed which has been demonstrated to be very specific and sensitive to FXIII Val34Leu [50]. By using the incorporation assay, significant lower FXIII activities in wild-type subjects compared to mutants (mean ± 2S.D., Val/Val 64.9 ± 37.3 % vs. Val/Leu 99.5 ± 33.6 % and Leu/Leu 158.2 ± 37.7 %) have been shown [50]. The findings of a statistically highly significant stepwise increase in FXIII activity with possession of the Leu allele confirm previous observations [46] and indicate that the newly optimised incorporation might be a future tool in the assessment of thrombotic risk. However, as yet, measurements of FXIII levels themselves do not allow a definite conclusion to be drawn on the presence or absence of the Val34Leu mutation, thus DNA analysis has to be employed to determine the FXIII genotype.

Genotyping Methods

In most studies published to date, determination of the FXIII A-subunit genotypes (FXIIIVal34Leu) was performed either by polymerase chain reaction (PCR) and single-stranded conformational polymorphism (SSCP) technique, original or slightly modified according to *Kohler* et al. [21, 26, 27, 47]; or by PCR-restriction fragment length polymorphism genotyping (PCR/RFLP) using a mis-match primer method, as originally described by *Kangsadalampai* et al. [24, 29, 31–34]. In addition, solid-phase minisequencing [22] or allele specific PCR methods have been applied [30]. The allele specific PCR technique in particular represents a simple method for a rapid detection of the FXIII A-subunit genotype, which is useful for screening of the FXIII Val34Leu in large series of individuals, to assess more easily the role of this polymor-

phism in thrombotic disorders, respectively. In the first step, all individuals can be screened for the T allele (Leu34), while a second step must be performed to distinguish between homozygous and heterozygous carriers of this allele by screening for the absence or presence of the G allele (Val34).

Conclusion

According to published in vitro studies and clinical observations, it seems reasonable to view FXIII as one of the factors contributing to the multifactorial interactions involved in the pathogenesis of vascular diseases, and evidence for a protective effect against myocardial infarction, stroke and deep venous thrombosis conferred by the FXIII Val34Leu polymorphism has been demonstrated in several studies. In view of the high prevalence of the Leu allele in the general population, the attributed protection conferred by FXIII Val34Leu against vascular thrombosis is high, even if the magnitude of such an effect was modest in some subgroups of patients. Analysis of FXIIIVal34Leu genotype might therefore help to estimate the individual risk for both primary and recurrent thrombosis, although prospective studies are needed to evaluate precisely the use of its routine laboratory screening in thrombotic diseases. Furthermore, the exact mechanisms by which this polymorphism exerts its proposed anti-thrombotic effect remains to be elucidated, and several studies are currently being carried out to gain an understanding of the true in vivo effects of FXIII Val34Leu on clot formation and stabilisation.

References

1. Schwartz ML, Pizzo SV, Hill RL, McKee PA. Human factor XIII from plasma and platelets: molecular weights, subunit structures, proteolytic activation, and cross-linking of fibrinogen and fibrin. J Biol Chem 1973;248:1395-1407.
2. Ichinose A. The physiology and biochemistry of factor XIII. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, eds. Haemostasis and Thrombosis. 3rd ed. Edinburgh, Scotland: Churchill Livingstone; 1994:531-46.
3. Hornyak TJ, Bishop PD, Shafer JA. Alpha-Thrombin-catalyzed activation of human platelet factor XIII: relationship between proteolysis and factor XIIIa activity. Biochemistry 1989 28:7326-32.
4. Lorand L, Gray AJ, Brown K, Credo RB, Curtis CG. Dissociation of the subunit structure of fibrin stabilizing factor during activation of the zymogen. Biochem Biophys Res Comm 1974;56:914-22.
5. Curtis CG, Brown KL, Credo RB, Domanik RA, Gray A, Stenberg P, Lorand L. Calcium-dependent unmasking of active center cysteine during activation of fibrin stabilizing factor. Biochemistry 1974;13:3774-80.
6. Lorand L, Losowsky MS, Miloszewski KJ. Human factor XIII: fibirn-stabilizing factor. Prog Hemost Thromb 1980;5:245-90.
7. Greenberg CS, Birckbichler PJ, Rice RH. Transglutaminases: multifunctional crosslinking enzymes that stabilize tissues. FASEB J 1991;5:3071-7.
8. Aeschlimann D, Paulsson M. Transglutaminases: protein crosslinking enzymes in tissue and body fluids.Thromb Haemost 1994;71:402-15.
9. Sakata Y, Aoki N. Cross-linking of a2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J Clin Invest 1980;65:290-7.
10. Kimura S, Aoki N. Cross-linking site in fibrinogen for a2 -plasmin inhibitor. J Biol Chem 1986;261:15591–5.
11. Valnickova Z, Enghild JJ. Human procarboxypeptidase U, or thrombin-activable-fibrinolysis-inhibitor, is a substrate for transglutaminases. Evidence for transglutaminase-catalyzed cross-linking to fibrin. J Biol Chem 1998;273:27220–4.
12. Hada M, Kaminski M, Bockenstedt P, McDonagh J. Covalent cross-linking of von Willebrand factor to fibrin. Blood 1986; 68:95-101.
13. Bale MD, Westrick LG, Mosher DF. Incorporation of thrombospondin into fibrin clots. J Biol Chem 1985;260:7502-8.
14. Mosher DF, Schad PE, Kleinmann HK. Cross-linking of fibronectin to collagen by blood coagulation factor XIIIa. J Clin Invest 1979;64:781–7.
15. Procyk R, Blombäck B. Factor XIII-induced cross-linking in solutions of fibrinogen and fibronectin. Biochim Biophys Acta 1988; 967:304-13.
16. Ichinose A, Davie EW. Characterization of the gene for the A subunit of human factor XIII (plasma transglutaminase), a blood coagulation factor. Proc Natl Acad Sci USA 1988;85:5829-33.
17.Mikkola H, Syrjälä M, Rasi V, Vahtera E, Hamalainen E, Peltonen L, Palotie A. Deficiency in the A-subunit of coagulation factor XIII: two novel point mutations demonstrate different effects on transcript levels. Blood 1994;84:517-25.
18. Mikkola H, Yee VC, Syrjala M, Seitz R, Egbring R, Petrini P, Ljung R, Ingerslev J, Teller DC, Peltonen L, Palotie A. Four novel mutations in deficiency of coagulation factor XIII: consequences to expression and structure of the A-subunit. Blood 1996; 87:141-51.
19. Anwar R, Stewart AD, Miloszewski KJA, Losowsky MS. Molecular basis of inherited factor XIII deficiency: identification of multiple mutations provides insights into protein function. Brit J Haematol 1995;91:728-35.
20. Suzuki K, Henke J, Iwata M, Henke L, Tsuji H, Fukunaga T, Ishimoto G, Szekelyi M, Ito S. Novel polymorphisms and haplotypes in the human coagulation factor XIII A-subunit gene. Hum Genet 1996;98:393-5.
21. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. Thromb Haemost 1998;79:8-13.
22. Wartiovaara U, Perola M, Mikkola H, Totterman K, Savolainen V, Penttila A, Grant PJ, Tikkanen MJ, Vartiainen E, Karhunen PJ, Peltonen L, Palotie A. Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. Arteriosclerosis 1999;142:295–300.
23. Elbaz A, Poirier O, Canaple S, Chédru F, Cambien F, Amarenco P. The association between the Val34Leu polymorphism in the factor XIII gene and brain infarction. Blood 2000; 95:586-91.
24. Kangsadalampai S, Board PG. The Val34Leu polymorphism in the A-subunit of coagulation factor XIII contributes to the large normal range in activity and demonstrates that the activation peptide plays a role in catalytic activity. Blood 1998; 92:2766-70.
25. McCormack LJ, Kain L, Catto AJ, Kohler HP, Stickland MH, Grant PJ. Prevalence of FXIII V34L in populations with different cardiovascular risk. Thromb Haemost 1998;80:523-4.
26. Catto AJ, Kohler HP, Bannan S, Stickland M, Carter A, Grant PJ. Factor XIII Val 34 Leu: a novel association with primary intracerebral hemorrhage. Stroke 1998; 29:813-6.
27. Catto AJ, Kohler HP, Coore J, Mansfield MW, Stickland MH, Grant PJ. Association of a common polymorphism in the factor XIII gene with venous thrombosis. Blood 1999;93:906-8.
28. Anwar R, Gallivan L, Edmonds SD, Markham AF. Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. Blood 1999;93:897-905.
29. Franco RF, Reitsma PH, Laurenco D, Maffei FH, Morelli V, Tavella MH, Araujo AG, Piccinato CE, Zaglo MA. Factor XIIIVal34Leu is a genetic risk factor involved in the aetiology of venous thrombosis. Thromb Haemost 1999;81:676–9.
30. Henry M, Morange PE, Canavy M, Alessi C, Juhan-Vague. Rapid detection of factor XIII Val34Leu by allelespecific PCR. Thromb Haemost 1999;81:463.
31. Balogh I, Szôke G, Karpati L, Wartiovaara U, Katona E, Komaromi I, Haramura G, Pfliegler G, Mikkola H, Muszbek L. Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. Blood 2000;96:2479-86.
32. Corral J, Gonzales-Conejero R, Iniesta J, Rivera J. The FXIII Val34Leu polymorphism in venous and arterial thromboembolism. Hematologica 2000; 85:293-7.

33. Ehrenforth S, Krause M, Herrmann FH, Wulff K, Zwinge B, Scharrer I. Pathogenesis of primary pulmonary embolism: impact of the FXIIIVal34Leu polymorphism. Haemostasis 2000; 30 (1-2):36 [abstract].
34. Attié-Castro FA, Zago MA, Lavinha J , Elion J, Rodriguez-Delfin L, Guerreiro JF, Franço RF. Ethnic Heterogeneity of the Factor XIII Val34Leu Polymorphism. Thromb Haemost 2000;84: 601–3.
35. Renner W, Koppel H, Hoffmann C, Schallmoser K, Stanger O, Toplak H, Wascher TC, Pilger E. Prothrombin G20210A, Factor V Leiden, and Factor XIII Val34Leu. Common Mutations of Blood Coagulation Factors and Deep Vein Thrombosis in Austria. Thromb Res 2000; 99:35-9.
36. Kohler HP, Futers TS, Grant PJ. Prevalence of three common polymorphisms in the A-subunit gene of FXIII in patients with coronary artery disease. Association with FXIII activity and antigen levels. Thromb Haemost 1999; 81:511–5.
37. Franco RF, Pazin-Filho A, Tavella MH, Simoes MV, Marin-Neto JA, Zago MA. Factor XIII Val34Leu and the risk of myocardial infarction. Haematologica 2000; 85:67–71.
38. Rosendaal FR, Grant PJ, Ariens RAS, Poort SR, Bertina RF. Factor XIII Val34Leu, factor XIII antigen and activity levels and risk of venous thrombosis. Thromb Haemost 1999; 82:508-9 (Suppl.):1599 [abstract].
39. Ehrenforth S, Zwinge B, Krause M, Wulff K, Herrmann FH, von Depka M, Scharrer I. Analysis of factor XIII-a Val34Leu in patients with unusual manifestations of venous thrombosis. Annals of Hematol 2001a; 80 (suppl. I):14 [abstract].
40. Alhenc-Gelas M, Reny JL, Aubry ML, Aiach M, Emmerich J. The Val34Leu mutation and the risk of venous thrombosis. Thromb Haemost 2000;84:1117-8.
41. Margaglione M, Bossone A, Brancaccio V, Ciampa A, Di Minno G. Factor XIII Val34Leu polymorphism and risk of deep vein thrombosis. Thromb Haemost 2000; 84:1118-9.
42. Franco RF, Middeldorp S, Meinardi JR, van Pampus ECM, Reitsma PH. Factor XIII Val34Leu and the risk of venous thromboembolism in factor V Leiden carriers. Brit J Haematol 2000;111:118-21.
43. Kohler HP, Grant PJ. Clustering of FXIIIVal34Leu with haemostatic risk factors. Thromb Haemost 1998;80:862.
44. Kohler HP, Mansfield MW, Clark PS, Grant PJ. Interaction between insulin resistance and factor XIII Val34Leu in patients with coronary artery disease. Thromb Haemost 1999;82:1202-3.
45. Ehrenforth S, Zwinge B, Krause M, Wulff K, Herrmann FH, Aygören-Pürsün E, Scharrer I. Association of factor XIII gene Val34Leu polymorphism with pulmonary embolism. Annals of Hematol 2001; 80 (suppl. I):14 [abstract].
46. Kohler HP, Ariëns RAS, Whitaker P, Grant PJ. A common coding polymorphism in the FXIII A-subunit gene (FXIIIVAL34LEU) affects cross-linking activity. Thromb Haemost 1998;80:704.
47. Ariëns RAS, Phillipou H, Nagaswami C, Weisel JW, Lane DA, Grant PJ. The Factor XIII V34L polymorphism accelerates thrombin activation of Factor XIII and affects cross-linked fibrin structure. Blood 2000:96:988-5.
48. Trumbo TA, Maurer MC. Examining thrombin hydrolysis of the factor XIII activation peptide segment leads to a proposal for explaining the cardioprotective effects observed with the factor XIIIVal34Leu mutation. J Biol Chem 2000; 275:20627–31.
49. Ariëns RAS, Kohler HP, Mansfield MW, Grant PJ. Subunit antigen and activity levels of blood coagulation factor XIII in healthy individuals. Relation to sex, age, smoking, and hypertension. Arterioscler Thromb Vasc Biol 1999;19:2012–6.
50. Wilmer M, Rudin K, Kolde HJ, Poetzsch B, Lenz W, Moessmer G, Meili E, Egbring R, Gempeler-Messina P, Gempeler M, Bastian S, Kohler HP. Evaluation of a Sensitive Colorimetric FXIII Incorporation Assay. Effects of FXIII Val34Leu, Plasma Fibrinogen Concentration and Congenital FXIII Deficiency. Thrombosis Research 2001;102:81-91.
51. Kohler HP. Role of blood coagulation factor XIII in vascular diseas. Swiss Med Wkl 2001;131:31-4.