Mini-review

The role of factor XIIIVal34Leu in cardiovascular disease

H.P. KOHLER and P.J. GRANT

From the Unit of Molecular Vascular Medicine, Leeds General Infirmary, University of Leeds, Leeds, UK

Introduction

Blood coagulation factor XIII (also called fibrin-stabilizing factor) plays an important role in clot stabilization by crosslinking fibrin chains. Factor XIII (FXIII) was discovered over 70 years ago by Barkan et al., who observed the insolubility of fibrin clots in the presence of calcium. Most studies on FXIII have been carried out in patients with FXIII deficiency which results in a serious bleeding diathesis, defective wound healing and a high risk of miscarriage in the deficient female. Most of these subjects show no plasma FXIII activity because of a complete absence of the A-subunit in plasma, platelets and monocytes. Little is known about the role of FXIII in vascular diseases. Although Kloczko et al. showed increased levels of FXIII A-subunit antigen in patients with obliterative atherosclerosis of the lower limbs and in patients with diabetic angiopathy, small patient numbers make the interpretation of these data difficult. However, increased plasma concentration of cross-linked fibrin polymers in acute myocardial infarction has been described, assuming the presence of increased plasma FXIII activity in patients with coronary artery disease. Results from our laboratory add further support in the involvement of FXIII in cardiovascular diseases. Here we discuss some new insights regarding the role of FXIII in vascular disease, with emphasis on a common polymorphism in the A-subunit gene of FXIII (FXIIIVal34Leu) and its association with myocardial infarction, stroke and venous thromboembolism.

Structure and function of FXIII

Blood coagulation FXIII is a transglutaminase composed of two A- and two B-subunits circulating in plasma as a tetramer (A\(_2\)B\(_2\)). All A-subunit molecules in plasma are in complex with the B-subunit (carrier protein) at a concentration of approximately 21 \(\mu\)g/ml, whereas the B-subunit is present in both free and complexed form. The total B concentration is around 21 \(\mu\)g/ml, about half of which circulates free in plasma (10 \(\mu\)g/ml). In circulation, more than 90% of FXIII is bound to fibrinogen by a binding site on fibrinogen for the B-subunit. Through this interaction, fibrinogen serves as a carrier for the plasma zymogen.

FXIII-catalysed cross-linked fibrin starts to form. Activated factor XIII catalyses the covalent crosslinking from our laboratory add further support in the involvement of FXIII in cardiovascular diseases. 10,11 Here we discuss some new insights regarding the role of FXIII in vascular disease, with emphasis on a common polymorphism in the A-subunit gene of FXIII (FXIIIVal34Leu) and its association with myocardial infarction, stroke and venous thromboembolism.

FXIII A-subunit gene

The factor XIII A-subunit gene has been localized to chromosome 6, contains 15 exons separated by 14
introns and extends over 160 kb. The coding region is translated into a polypeptide of 731 amino acids. More than 20 mutations in the A-subunit gene of FXIII have been reported in patients with severe FXIII deficiency which lead to complete absence of the A-subunit. These point mutations or minor deletions lead to amino acid substitutions, stop codons or splicing defects. Several common sequence changes in the FXIII A-subunit gene not associated with FXIII deficiency have also been described (Figure 1).4,30

**FXIIIVal34Leu**

One of the polymorphisms commonly occurring in a normal population is a G→T point mutation in codon 34, exon 2 of the A-subunit gene which codes for a Valine→Leucine change (FXIIIVal34Leu) only three amino acids from the thrombin activation site. This polymorphism was originally described in a Finnish population of 600 controls in whom the allele frequency was 23%, similar to that in our study population and in small groups of Finnish, Russian, German and Japanese subjects. Because of the proximity of the amino acid change to the thrombin activation site, this polymorphism could influence FXIII activation and is therefore a candidate for a role in the pathogenesis of thrombotic disorders.

**FXIII Val34Leu in patients with coronary heart disease**

Our laboratory has recently made the observation that the coding polymorphism in the A-subunit gene of FXIII (FXIIIVal34Leu) is protective against myocardial infarction (MI) suggesting for the first time a role for FXIII in vascular diseases. FXIII genotype at codon 34 was determined in a case control study of 398 Caucasian patients with suspected coronary artery disease and 196 healthy controls. Patients were evaluated for a past history of MI by WHO criteria and had undergone angiography for investigation of coronary artery disease. The prevalence of FXIIIVal34Leu was significantly lower in subjects with MI than in those without (32 vs. 50%, \( p = 0.0009 \)) and than in controls (32 vs. 48%, \( p = 0.005 \)) indicating a major gene effect conferring protection against MI (Figure 2). The small number of patients with a past history of MI who possessed

![Figure 1. Structure of the factor XIII A-subunit polypeptide showing common sequence changes. The long arrows indicate position of the amino acid changes at codon 34, 564, 650, and 651. Restriction fragment length polymorphism (RFLP) is shown for codon 564, 650, 651 and single strand conformation polymorphism (SSCP) pattern for codon 34.](image-url)
FXIIIVal34Leu had significantly higher levels of plasminogen-activator-inhibitor 1 (PAI-1), and the PAI-1 4G/4G genotype was commoner when compared to those without MI. This association was not present in patients possessing the wild-type allele. These results indicate that this common polymorphism is protective against MI unless there is coexistent suppression of fibrinolysis due to elevated PAI-1 levels, and provide indirect evidence for a functional effect of this polymorphism, possibly through the formation of weaker fibrin structures. In further studies in this area, those subjects with FXIIIVal34Leu and MI also clustered other haemostatic risk factors (factor XII, tPA) and showed evidence of increased insulin resistance as measured by the HOMA method (unpublished data). Although these data clearly need confirming, they provide provocative evidence to indicate that interactions between insulin resistance and FXIIIVal34Leu lead to loss of the cardioprotection afforded by FXIIIVal34Leu alone.

Prevalence of FXIIIVal34Leu in different ethnic groups

Pima Indians from the Gila River Indian Community in Arizona, USA, have a low incidence of coronary artery disease despite the world's highest reported prevalence of non-insulin-dependent diabetes, a factor known to associate with a high risk of MI in Caucasians. In South Asians, there is also a high incidence of type II diabetes but a correspondingly high incidence of MI. The prevalence of FXIIIVal34Leu in these three different ethnic groups was inversely related to the prevalence of coronary heart disease in these populations (leucine allele frequency: 0.13 in Asians, 0.28 in Caucasians and 0.4 in Pima Indian, \(p < 0.0001\)). In other words, the leucine allele was more common in those subjects at low cardiovascular risk. Although it is possible that this is a random ethnic variation unrelated to disease, these findings do support the idea of a cardioprotective effect of FXIIIVal34Leu, and could contribute to the contrasting cardiovascular risk in these populations. The paper by Suzuki et al. presents haplotypic combinations of five polymorphisms (including codon 34) in Caucasians (Germans, Finnish, Russian) and Japanese using a numerical code. These haplotype data do not provide allele frequencies for each genotype; however, the Leu allele seems to be less common in Japanese than in the three Caucasian populations.

FXIIIVal34Leu in patients with cerebrovascular disease

In view of the possible protective role of FXIIIVal34Leu in the pathogenesis of MI, we hypothesized that possession of this polymorphism might also be protective against ischaemic stroke. In a case-control study of 612 patients with acute stroke, we were unable to demonstrate any association between FXIIIVal34Leu and ischaemic stroke characterized by CT scan. This can perhaps be explained by the heterogeneous nature of ischaemic stroke. In particular, the association between insulin resistance and stroke is less consistent than with heart disease. However in the small number of subjects with evidence of haemorrhagic stroke, there was an increased prevalence of FXIIIVal34Leu, in direct contrast to that observed in subjects with MI. The association with haemorrhagic stroke provides further evidence for a role for this polymorphism in vascular diseases and supports the rather compelling hypothesis that FXIIIVal34Leu is protective against thrombosis and involved in the pathogenesis of haemorrhagic vascular disorders.

FXIIIVal34Leu in venous thrombosis

Fibrin formation is particularly important in the development of venous thrombosis, indicating that FXIII might have a role in this process. To investigate factor XIIIVal34Leu and potential interactions with established genetic risk factors for venous thrombosis such as Factor V Leiden and Prothrombin G→A2021, in the pathogenesis of venous thromboembolism (VTE), patients with a definite history of VTE attending the anticoagulation clinic of the Leeds General Infirmary were compared to healthy controls. Of these patients, 55.3% had sustained a deep venous thrombosis (DVT) and 44.7% had a clinical diagnosis of pulmonary embolism. VTE patients showed an increased frequency of the FXIII Val/Val genotype (63% vs. 49%) and a lower
frequency of the Val/Leu genotype (31% vs. 42%) than controls ($p=0.007$). There was no evidence for an interaction between factor XIII Val34Leu genotype, FV Leiden and prothrombin G → A20210. When case subjects were divided into those with DVT only and cases of pulmonary embolism, both FXIII Val34Leu and Factor V Leiden were related to DVT, but there was no significant association with pulmonary embolism.

The results of this study indicate that possession of the genotype coding for FXIII Val34Leu is also protective against VTE, in a similar manner to that seen for MI. An interesting area for further study would be to identify interactions with other risk factors for VTE.

**FXIII Val34Leu affects cross-linking activity**

The finding that FXIII Val34Leu appears to be protective against MI and VTE, whilst increasing the risk of haemorrhagic stroke, raises the question of the functional effects of FXIII Val34Leu on clot formation. In preliminary studies of FXIII activity determined by a microtitre incorporation assay using fibrinogen (adsorbed to microtitre wells) and biotinylcadaverine as substrate, similar to a method described by Song et al., there was a highly significant stepwise increase in FXIII activity with possession of the leucine allele (Figure 3). The close proximity of the mutation to the thrombin activation site may account for this finding, and this is an area under current study. There was no difference between FXIII A-subunit and B-subunit antigen levels (ELISA method) between the groups. The apparent paradox of FXIII Val34Leu being protective against MI whilst being associated with increased rates of fibrin cross-linking remains. However, a number of possibilities could explain this observation and could be consistent with the clinical findings. It is possible that increased rates of FXIII activation actually lead to ineffective fibrin crosslinking or alternatively to deficient cross-linking of other natural substrates for FXIII, such as α2-antiplasmin, von Willebrand factor, collagen, FVII, fibronectin, thrombospondin or vitronectin. Alternatively, fibrin is an important component of the atheromatous plaque, and taken in isolation the results relating to MI might indicate that increased cross-linking associated with FXIII Val34Leu leads to plaque stabilization and a lower risk of MI. Unfortunately whilst this might be an attractive hypothesis, it does not explain the protective effect in VTE, in which atheroma formation is not a feature.

Perhaps the most plausible explanation lies in the increased sensitivity of FXIII Val34Leu to thrombin which could upset the equilibrium between FXIII activation and cleavage of the substrate, fibrinogen to fibrin. Preliminary data from our laboratories indicate that much lower levels of thrombin are required to activate FXIII Val34Leu than wild-type FXIII. These lower levels of thrombin appear to be inadequate to cleave fibrinogen. This needs further investigation, but levels of thrombin that activate FXIII Val34Leu without producing activated FXIII substrate (fibrin) could lead to FXIII degradation without clot formation. This idea could explain all the clinical associations with FXIII Val34Leu, but should be regarded with caution until the results are confirmed.

**Conclusion**

The genetics of FXIII in relation to cardiovascular disease is a novel field, as clinical studies in the past have concentrated on FXIII genetics in relation to FXIII A-subunit deficiency. As outlined, recent work from the Unit of Molecular Vascular Medicine in Leeds has indicated that a common point mutation in the A-subunit gene of FXIII which leads to an amino acid change (FXIII Val34Leu) close to the thrombin activation site is protective against MI and deep vein thrombosis, showing for the first time a role for FXIII in thrombotic disorders. In addition, the Leu allele is associated with haemorrhagic stroke. These results suggest that this mutation is protective against thrombosis and associated with an increased risk of haemorrhagic vascular diseases.

These clinical observations, together with our functional studies on the cross-linking activity of FXIII Val34Leu, indicate that this common polymorphism interferes with the enzymic function of FXIII in a manner yet to be defined. The formation of tight and rigid fibrin networks in vitro has been associated with MI in men under the age of 45 years, and it...
has been suggested that FXIII could have a role in this process. Activated FXIII cross-links fibrin to increase the mechanical strength of a fibrin clot. Furthermore, FXIII also incorporates plasmin inhibitor (a2-antiplasmin) into the fibrin network, which increases resistance to fibrinolysis. However, the role of FXIIIVal34Leu in determining fibrin gel porosity and fibrin fibre thickness remains to be investigated. The biochemical mechanism of the antithrombotic nature of FXIIIVal34Leu is currently under investigation. In addition, the relationship between FXIII antigen levels (A- and B-subunit) in patients with coronary artery disease, stroke and venous thrombosis remains to be investigated.

There is also a growing interest in the role of FXIII in wound healing, Crohn’s disease, cancer and tissue repair. The potential role of FXIIIVal34Leu in these disorders also requires further investigation.

Further studies are required to identify the effects of FXIIIVal34Leu on the pathophysiology of the coagulation processes and its role in the pathogenesis of thrombotic disorders. However, these findings do raise the possibility of novel therapeutic interactions in thrombotic disorders by interfering with the process leading to fibrin cross-linking. This is an area that is currently under investigation in our laboratories.

Acknowledgements

The work described in this review has been supported by grants from the British Heart Foundation, The Medical Research Council, The Stroke Association, The Swiss Foundation for Medical-Biological Grants and the Leeds General Infirmary Special Trustees.

References


