Genetic and Environmental Determinants of Fibrin Structure and Function
Relevance to Clinical Disease

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Abstract—The formation of a fibrin clot is one of the key events in atherothrombotic vascular disease. The structure of the fibrin clot and the genetic and environmental factors that modify it have effects on its biological function. Alterations in fibrin structure and function have implications for the clinical presentation of vascular disease. This review briefly describes the key features involved in the formation of a fibrin clot, its typical structure, and function. This is followed by a review of the current literature on genetic and environmental influences on fibrin structure/function and the relationship to clinical disease. (Arterioscler Thromb Vasc Biol. 2004;24:1-9.)

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D iseases associated with the development of thrombosis are a major cause of morbidity and mortality in the developed world. Atherothrombotic vascular disorders develop over many decades and involve the interaction of classic atherogenic risk factors such as diabetes, hyperlipidemia, and hypertension with abnormalities of the inflammatory and hemostatic systems. Arterial disease develops in a high-pressure, high-flow system, with lipid deposition and smooth muscle hyperplasia occurring to form an atherosclerotic plaque. Subsequently, the plaque becomes unstable and ruptures exposing a prothrombotic lipid core which extend to form relatively flexible αC-domains, each ending in a globular domain.2,3

In situations of tissue injury and inflammation, thrombin is generated after cleavage of prothrombin by the Xase complex. Thrombin subsequently binds to fibrinogen and cleaves the amino termini of the fibrinogen Aα and Bβ chains at region E. This results in the release of fibrinopeptides A and B from fibrinogen, producing fibrin and initiating fibrin clot formation. Release of fibrinopeptide A by thrombin is fast and exposes a polymerization site on the E region of fibrin.6,5 This combines with a complementary binding site on the γ chain in the D region of an adjacent fibrin molecule to form a double-stranded twisted protofibril of fibrin. Cleavage of fibrinopeptide B by thrombin is slower but also exposes a binding site in the E region. This has been proposed to bind to a complementary binding site on the β chain in the D region. The cleavage rate of fibrinopeptide B has been associated with the rate of lateral aggregation of the protofibrils and the thickness of the fibrin fibers.3

While a critical mass of fibrin is polymerizing, thrombin simultaneously activates factor XIII by a calcium-dependent mechanism. Factor XIII is composed of 2 A and 2 B subunits; 90% of the A subunit is bound to the fibrin clot. Once activated, factor XIIIa is involved in cross-linking of the fibrin clot by transglutaminase reactions between glutamine and lysine residues on fibrin. The first cross links are formed between γ chains of 2 neighboring fibrin molecules in the longitudinal orientation of the fibrils.6,7 This results in the formation of 2 isopeptide bonds between glutamine 398 or 399 and lysine 406 that connect the D regions of 2 fibrin...
molecules longitudinally. Cross-linking of the fibrin α-chains occurs more slowly than that of the γ chains. There are 4 glutamine residues potentially involved, and at least 15 lysine residues have been identified. This number of potential cross-linking sites allows for a highly complex and intricate network to be formed between neighboring αC domains in the fibrin clot. These α cross-links provide stability to the fibrin clot, and they seem to form a protective barrier preventing plasmin-degrading fibrin. The result of fibrin polymerization and FXIII-induced cross-linking is the formation of thick fibrin bundles and a complex branched network conferring strength, rigidity, and resistance to lysis to the fibrin clot.

The typical structure of a fibrin clot as visualized by electron microscopy is shown in Figure 2.

A relationship exists between fibrin clot structure and fibrinolysis such that clots composed of thick fibers, with a less tightly cross-linked, reduced number of branch points, and more permeable networks are lysed faster than clots containing thin fibers, with increased density and a more tightly knit cross-linked structure. Although individually, thin fibers are lysed quicker than thick fibers, it is the fibrin network configuration and the number of fibers per volume of clot that has a bigger impact on the fibrinolysis rate than fiber thickness alone. It is evident that there is considerable variation in the fibrin clot structure of different individuals, suggesting that both genetic and environmental factors have a role in determining the balance between stability and susceptibility of the clot to fibrinolysis.

Abnormal Clot Architecture and Atherothrombotic Vascular Disease

The fibrin clot network architecture is altered in several diseases associated with atherothrombosis, including MI and diabetes. After MI, fibrin clots have reduced permeability and a lower fiber mass-to-length ratio. There are independent associations between permeability and the extent and severity of coronary artery stenosis and also of fibrinolytic activity. Patients with severe coronary artery disease have more rigid clot structures and an elevated fiber mass-to-length ratio. It is of note that this is the case despite subjects being treated with aspirin, which has been shown to increase fibrin clot permeability and potentially enhance the response to fibrinolysis. The plasma from healthy male first-degree relatives of patients with premature coronary artery disease also form ex vivo fibrin clots with abnormal structure. These clots are less porous, have thicker fibers, and begin polymeriza-

![Figure 1. A diagrammatic representation of fibrinogen. E indicates central region; A, fibrinopeptides A; B, fibrinopeptides B; D, distal region; γ', a gamma chain variant found in 7% to 15% of circulating fibrinogen.](image)

![Figure 2. The structure of a fibrin clot seen on electron microscopy and the genetic and environmental factors affecting it, leading to a tighter, less lysable, prothrombotic clot. Scale bar indicates 5 μm.](image)
tion more quickly than matched controls.\textsuperscript{25} The differences are not attributable to classic risk factors or common polymorphisms. In subjects with type 1 diabetes mellitus, the fibrin clot is less porous but with no difference in fiber mass-to-length ratio.\textsuperscript{20} This is independent of fibrinogen concentration.

**Genetic and Environmental Factors Altering Fibrin Structure**

Because fibrin structure/function is one of the major final phenotypes arising from activation of the fluid phase of coagulation, it would be expected to be a synthesis of all the genetic and environmental interactions described in the coagulation cascade. However, despite the observation that individual coagulation factors have a relatively high degree of heritability,\textsuperscript{26} this does not appear to be the case for fibrin structure/function, in which the major influence appears to be environmental.\textsuperscript{27} Several factors influencing clot architecture have been identified (Figure \textbullet\textbullet\textbullet) within the coagulation system, including altered fibrinogen concentrations,\textsuperscript{28} genetic variations in fibrinogen\textsuperscript{15} and factor XII,\textsuperscript{29} and altered concentrations of thrombin and prothrombin.\textsuperscript{30} One of the challenges in this field is to increase our understanding of the mechanisms by which lifestyle changes (exercise, obesity, smoking) translate into increased cardiovascular risk through these pathways.

**Fibrinogen**

**Fibrinogen Concentration**

Elevated fibrinogen has consistently been demonstrated to be a major risk factor for thrombosis and cardiovascular disease.\textsuperscript{31,32} Fibrinogen is an acute phase protein and is increased in many conditions, including advancing age, female gender, smoking, diabetes mellitus, elevated low-density lipoprotein cholesterol and triglycerides, hypertension, inflammation, and infection.\textsuperscript{31} It is reduced by exercise/physical fitness, weight loss, and smoking cessation. Whether fibrinogen is just a marker of the inflammation related to vascular disease, or whether it is involved in actively mediating the vascular disease process is not entirely known, although recent studies using transgenic mice with hyperfibrinogenemia have suggested that it is a true modifier of vascular disease, augmenting fibrin deposition in certain organs and regulating fibrin turnover.\textsuperscript{31} Despite this uncertainty, it is clear that fibrinogen concentration per se has a profound effect on fibrin clot structure. For example, the rate of fibrinopeptide A cleavage increases with increasing fibrinogen concentration,\textsuperscript{34} and this is associated with a shorter lag phase and a more dense and tight fibrin network.\textsuperscript{35} Elevated fibrinogen levels also lead to the formation of thicker fibers\textsuperscript{28,36} and larger thrombi with tight and rigid network structures\textsuperscript{31} which reduces the ability of the clot to be deformed and fibrinolyzed.\textsuperscript{37} It also interferes with the binding of plasminogen to its receptor, which in turn reduces fibrinolysis.\textsuperscript{33} It is of interest that fibrates, \(B\) blockers, and angiotensin-converting enzyme inhibitors all lower fibrinogen.\textsuperscript{31} Their effect on fibrin clot structure and function is not known, although it would be expected that any intervention that lowers fibrinogen would tend to produce a less compact clot structure with thicker fibers.

**Fibrinogen Genes**

The genes for the 3 polypeptide chains making up fibrinogen are located together in a cluster on the distal arm of chromosome 4, bands q23-q32.\textsuperscript{28} The \(A\) gene, which consists of 5 exons, is located at the center of the fibrinogen cluster.\textsuperscript{38} It produces a polypeptide chain that is 625 residues long. The gene for the \(\beta\) chain is located 13 kb downstream of that of \(A\); it contains 8 exons, which are transcribed in the opposite direction to those of the \(A\) and \(\gamma\) genes, and codes for a 461-residue polypeptide.\textsuperscript{39} The third and last gene of the fibrinogen cluster is located 10 kb upstream of the \(A\) gene and codes for a 411-residue polypeptide.

**Variations in the Splicing of Fibrinogen Gene Transcripts**

\(\gamma\)/\(\gamma'\) Fibrinogen

A major functional splice variant of fibrinogen is known as \(\gamma\)/\(\gamma'\) fibrinogen. It accounts for between 7\% and 15\% of the fibrinogen found in plasma.\textsuperscript{40} For the production of this variant, the \(\gamma\) transcript is alternatively spliced, leading to a negatively charged 16-residue extension at the \(\gamma\)-chain carboxyl terminus.\textsuperscript{41} Fibrinogen \(\gamma'\) contains binding sites for thrombin\textsuperscript{12} and FXIII B subunit.\textsuperscript{42} Binding of FXIII B subunit to fibrinogen \(\gamma'\) suggests that it acts as a carrier of FXIII, and by doing so it increases the local concentration of FXIII at the level of the fibrin clot, allowing for more cross-linking.\textsuperscript{43} Clots made with \(\gamma\)/\(\gamma'\) fibrinogen certainly have a more highly cross-linked and stable fibrin structure and are more resistant to lysis than those made from \(\gamma\)/\(\gamma\) fibrinogen.\textsuperscript{44} They also demonstrate reduced fiber diameter, increased branching, and reduced pore size (Table).\textsuperscript{45} This seems clinically relevant because similar fibrin structures have previously been related to an increased risk of thrombosis.\textsuperscript{13,21} and patients with coronary artery disease have higher \(\gamma\)/\(\gamma'\) fibrinogen levels, which is an effect that is independent of total fibrinogen levels.\textsuperscript{47}

\(A\alpha\gamma C\) Fibrinogen

The second major variation that occurs in splicing of the fibrinogen gene transcripts occurs in the \(A\) gene transcript, where alternative splicing introduces an additional sixth exon, leading to a massive 236-residue extension at the carboxyl terminus of the \(A\) chain. This extended \(A\) chain or \(A\alpha\gamma C\) occurs in \(\approx 1\%\) of total fibrinogen, and its molecular mass is increased by \(>50\%\) compared with the normal \(A\) chain.\textsuperscript{48} Little is known about the physiological regulation of splicing for either the \(A\alpha\gamma C\) or the \(\gamma\)' variants.

**Noncoding Polymorphisms of Fibrinogen Gene**

There are several polymorphisms that occur in the fibrinogen genes. Most of them are located in the nontranslated regions of the genes. These include a \(TaqI\) polymorphism in the 3' region of the \(A\) gene, a \(BclI\) variation in the 3' region of the \(B\) gene, and \(-148C/T\) and \(-435G/A\) polymorphisms in the 5' promoter region of the \(B\) gene.\textsuperscript{49} The \(BclI\) polymorphism
has been shown to associate with increased fibrinogen levels and is more common in subjects with severe coronary artery disease.\textsuperscript{50,51} The \(-455G/A\) substitution in the \(B\beta\) gene occurs in an IL-6–responsive HNF1 element and has been associated with increased fibrinogen levels in a manner that is environment-dependent.\textsuperscript{53,54} In vitro studies of the \(B\beta-455\) A/G polymorphism have indicated that the substitution alters nuclear protein binding profiles, reporter luciferase gene expression, and that it may explain up to \(\sim 11\%\) of the variation in fibrinogen levels.\textsuperscript{55} On a clinical basis, it has been shown that the \(B\beta-455\) A/G polymorphism is associated with the development of coronary artery disease in type 2 diabetic subjects, independently of fibrinogen levels.\textsuperscript{56} The same polymorphism has been related to the progression of coronary artery disease\textsuperscript{57,58} and the development of cerebral infaracts,\textsuperscript{59} but not in all studies (Table).\textsuperscript{60}

## Coding Polymorphisms of Fibrinogen Gene

### \(A\alpha\) Thr312Ala Polymorphism

In addition to the noncoding variations, there are 2 coding polymorphisms in the fibrinogen cluster that introduce an amino acid change in the mature protein. One of these occurs in the \(A\alpha\) gene and leads to a substitution of threonine with alanine at residue 312 of the \(A\alpha\) chain.\textsuperscript{61} Thr312Ala is located in an area of the molecule that is important for the interaction of fibrinogen with factor XIII. Two of the factor XIII cross-linking sites, \(A\alpha\)Gln328 and \(A\alpha\)Lys303, are located in this area and are important for the cross-linking to another \(A\alpha\) chain and \(\alpha\)2 antiplasmin, respectively. The substitution of Thr312 with Ala leads to increased factor XIII-dependent \(A\alpha\) chain cross-linking and stiffness of the clot. The ultrastructure of the clot demonstrates larger average fibrin fiber diameters for Ala312 clots, but with a similar number of branch points as Thr312 (Table).\textsuperscript{62}

Clinical studies have reported that the Ala allele of the Thr312Ala polymorphism predisposes to embolization in arterial and venous systems. There is an association of the Ala allele with poststroke mortality in subjects with atrial fibrillation.\textsuperscript{63} It has also been associated with pulmonary embolism in subjects with deep vein thrombosis.\textsuperscript{64} It may be possible that stiffer clots as observed in vitro are more brittle and tend to fragment more easily.\textsuperscript{62}

### \(B\beta\) Arg448Lys Polymorphism

The second coding polymorphism occurs in the \(B\beta\) chain, where arginine 448 is substituted with lysine.\textsuperscript{61} This amino acid change is located in the C-terminal domain of the \(B\beta\) polypeptide, where it could have an effect on the configuration of this domain. Preliminary data have shown that possession of the Lys448 allele is associated with lower clot permeability and a tighter, finer structure than possession of the Arg448 allele,\textsuperscript{65} although another study did not confirm this (Table).\textsuperscript{66} Clinically, this polymorphism has been associated with macrovascular disease\textsuperscript{50,67} in some but not all studies.

## Genetic and Environmental Interaction on Fibrinogen Levels

Overall, the genetic influence on the variation of fibrinogen and fibrin is relatively large. Twin studies have demonstrated that the percentage of variation of fibrinogen attributable to genetic factors is \(\sim 40\%\) to \(50\%.\textsuperscript{26,27}\) A similar, although slightly lower, degree of heritability of fibrinogen of \(34\%\) has been found in family studies.\textsuperscript{68} Even a phenotype that is more complex, such as the ultrastructure of the fibrin clot, is susceptible to genetic variation.\textsuperscript{27} Although overall heritability of both fibrinogen and fibrin is considerable, the contribution of individual genetic polymorphisms on intermediate phenotypes such as protein structure and function of the
plasma level of coagulation proteins appears to be relatively small. This may be one reason why associations between the clinical disease phenotype and single genetic polymorphisms in coagulation factors are inconsistent.

**Factor XIII**
Additional genetic regulation of fibrin structure and function occurs through variations in proteins other than fibrinogen that are involved in the process of fibrin clot formation and that interact with the variations occurring in the fibrinogen genes described. An example is a polymorphism in the factor XIII A subunit (Val34Leu), which codes for an amino acid change close to the thrombin cleavage site at position 37.

**FXIII Val34Leu Polymorphism**
The substitution of valine with leucine is a relatively conservative amino acid change, but it occurs in an area that plays a critical role in the interaction between thrombin and factor XIII, so that cleavage of the FXIII activation peptide by thrombin is enhanced 2- to 3-fold in the Leu34 variant (Table). This enhanced activation rate of factor XIII Leu34 has been reported to influence fibrin structure and function in a manner that is dependent on the concentration of fibrinogen. Fibrin clots formed in the presence of FXIIIleu34 form quicker and have thinner fibers, smaller pores, and reduced permeability compared with the Val34 variant. It appears that early cross-linking of fibrin by FXIIIleu34, which is activated at the time of fibrinopeptide A release, inhibits lateral aggregation of the fibrin fibers, whereas delayed cross-linking by FXIII34Val allows for more lateral aggregation before the cross-linking occurs. At high concentrations of fibrinogen, plasma samples homozygous for the Leu34 allele form clots with increased permeability and looser structures than do clots formed from plasma samples homozygous for the Val allele. Therefore, a protective effect of the Leu34 allele should emerge only in the presence of increased fibrinogen concentrations.

Clinically, possession of the FXIIIleu34 allele has been found to be lower in some (but not all) studies of patients with MI and cerebral infarction. Bearing in mind that fibrinogen concentrations are often increased in cardiovascular disease, it is possible that environmental factors alter fibrinogen concentrations and consequently the structure of the clot formed in the presence of the Leu34 allele to give a protective effect.

**Factor XIII B Polymorphisms**
Polymorphisms of the FXIII B subunit are uncommon, although His95Arg is relatively common and appears to reduce the risk of MI in women. However, its effect on fibrin structure and function is unknown (Table).

**Prothrombin and Thrombin**
Elevated levels of prothrombin have been associated with a risk of arterial and venous thrombosis, and a mutation in the 3'-untranslated region of the prothrombin coding gene is associated with increased prothrombin levels. Increased prothrombin results in increased thrombin generation, which affects fibrin clot structure. Clots produced in conditions of low thrombin concentration are composed of thicker fibers and are more porous, whereas those formed at higher thrombin concentrations have thinner and more tightly cross-linked fibers. It has not been determined whether the altered clot structure produced at different thrombin concentrations in vivo has clinical relevance, but many previous studies have shown that thin-fibered tightly cross-linked clots have increased resistance to fibrinolysis.

**Other Factors Influencing Clot Architecture**
Aside from the major genetic and environmental influences described, there are other clinically relevant environmental factors that influence fibrin clot structure and function. Many have a well-established link to an increased risk of atherothrombotic vascular disease.

**Cross-Linked Proteins and Circulating Salts**
Activated factor XIII cross-links several other proteins to the α chain of the fibrin molecule, including α2-antiplasmin, the major physiological inhibitor of plasmin, TAFI, and plasminogen activator inhibitor-2. Cross-linking of these proteins makes the clot less susceptible to lysis. FXIIIa cross-links fibronectin, which alters the mechanical properties of the clot by increasing fiber thickness and clot permeability. It also promotes migration and adherence of cells into the clot, presumably aiding the wound healing process. Collagen is cross-linked to fibrin, which may stabilize the extracellular matrix forming at tissue injury sites. Actin, myosin, and vinculin when cross-linked cause clot retraction and stabilization of the platelet cytoskeleton. The effect of different salts on clot structure has also been investigated. Chloride appears to be the most important physiological modulator of fibrin polymerization, because chloride ions bind to fibrin and prevent the lateral aggregation of protofibrils, resulting in thinner fibers that are more curved. This effect is dependent on ambient pH.

**Glucose and Treatment**
Subjects with diabetes and insulin resistance are more likely to have atherothrombotic cardiovascular disease. Fibrinogen concentrations are higher in diabetes, which will contribute to a more prothrombotic fibrin clot structure as discussed. Studies in type I diabetic subjects have shown that they have tighter, less permeable fibrin clots with normal fiber thickness than do healthy controls, and this was independent of fibrinogen concentration. In vitro, adding glucose to plasma leads to the formation of clots with a tighter, less permeable fibrin network. Recent work in our laboratory suggests that ambient glucose levels independently affect fibrin clot architecture and that increasing glycated hemoglobin (HbA1c) is independently associated with the formation of tightly cross-linked, thin-fibered fibrin clots (Dunn, unpublished data). This may be caused by concomitant glycosylation of the proteins involved in fibrin clot formation. It is of
interest that 4 to 6 months of insulin treatment with continuous subcutaneous insulin infusion in patients with longstanding type 1 diabetes led to an increase in fibrin gel porosity independent of improved glycemic control or insulin levels, but this appeared to be related to total cholesterol and plasma fibrinogen levels.93 Other drugs used to treat diabetes have effects on fibrin structure and function. Gliclazide increases fibrin fiber thickness but reduces permeability, overall rendering the clot more susceptible to fibrinolysis.94 Clots formed in the presence of dimethylbiguanide (metformin) lyse more quickly19 and have been shown to interfere with thrombin-induced FXIII activation, inhibit fibrinopeptide A and B release, and thus reduce FXIII cross-linking activity, resulting in thinner fibers but a reduced pore size.95

Lipids and Treatment
It has been shown that total cholesterol may determine fibrin clot structure,93 and fibrin gel porosity has been associated with lipoproteins in young patients sustaining MI.13 It is of interest that statins appear to reduce thrombin formation and inhibit FXIII activation, reducing the formation of a stable clot.96 This appears to be independent of the effect of cholesterol-lowering and may be related to an anti-inflammatory effect of this class of drugs. They do not, however, alter fibrinogen concentrations.97

Homocysteine
A high-plasma homocysteine is a risk factor for atherothrombotic vascular disease.94 In vitro, clots formed in the presence of homocysteine have thicker, shorter fibers with a more compact structure.99 Clots formed with fibrinogen from homocysteinemic plasma are more resistant to lysis.100 In addition, homocysteine binds to circulating fibronectin and hinders fibrin/fibronectin binding.101 This may reduce the amount of fibronectin in the clot, impairing wound healing.

Inflammatory Markers
Inflammatory markers are associated with an increased risk of vascular disease. Complement activation has been shown to induce alterations in fibrin structure, including the formation of thinner fibers with increased tensile strength arranged into tight networks that are resistant to fibrinolysis.102 These changes in turn appear to promote further activation of the complement system.102 The effect of C-reactive protein and cytokines on fibrin clot structure does not appear to have been investigated, although C-reactive protein has been shown to induce plasminogen activator inhibitor-1 release in vitro, which would inhibit fibrinolysis.103

Lifestyle Factors—Diet and Smoking
Work in this area has been limited. A westernized diet is known to contribute to the development of cardiovascular disease, possibly because of its high-fat and low-fiber content. A study has shown that the water-soluble dietary fiber pectin given as a supplement to hypercholesterolemic subjects alters the fibrin network favorably, making it more permeable and less rigid.104 This is independent of fibrinogen concentration but accompanied by a decrease in total cholesterol and lipoprotein A. Red wine,105 fish oil, and olive oil106 consumption have been shown to lower fibrinogen. Further studies would be needed to determine whether these changes have any therapeutic relevance to thrombotic disease. No studies have been performed on the effect of smoking on fibrin clot architecture, but smoking increases fibrinogen levels,107 and this is likely to influence fibrin characteristics.

Conclusion
Atherothrombotic vascular disease has reached epidemic proportions in the developed world and represents the major cause of morbidity and mortality in westernized populations. The final phenotypes of acute coronary syndromes, including MI, cerebrovascular disease, and acute limb ischemia are most commonly related to the development of a platelet-rich fibrin mesh on a damaged vessel wall, leading to occlusive thrombus formation. The fibrin clot plays a pivotal role in the pathophysiology of vascular disease, and it is becoming clear that the structure/function of the fibrin clot is complex with genetic and environmental determinants. However, the information known about this is relatively limited and further research is required to understand the factors that modify fibrin structure/function and the clinical implications of these alterations. More work also needs to be performed looking at the relationship of fibrin clot structure to its stability and how this relates to atherogenesis. The development of FXIII inhibitors by computational chemistry should also help to elucidate this further. We can now appreciate that alterations in gene expression and coding function, splice variants, and posttranslational modifications of protein products all influence fibrin structure/function. The extent to which classical risk factors for cardiovascular disease interact with these processes and the mechanisms that might underpin such associations have not been evaluated and represent a great challenge in this field. A fuller knowledge of the role of the proteome in generating the fibrin phenotype and the links with classical risk factors may provide major opportunities for the development of novel fibrin-directed therapeutics to work alongside the well-established antiplatelet agents. This raises the possibility of combined antiplatelet and fibrin-modifying agents to prevent and treat the atherothrombotic syndromes.

References
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