

## Fibrinogen and Fibrin in Hemostasis and Thrombosis

Sravya Kattula, James R. Byrnes, Alisa S. Wolberg

Despite its recognition as a key component of blood clots, the roles of fibrinogen and fibrin (collectively fibrin[ogen]) in hemostasis and thrombosis are insufficiently understood. Consequently, fibrin(ogen) remains an active focus of investigation at all levels of the research spectrum, including fundamental basic/discovery science, epidemiology, and clinical practice and applications. This article briefly reviews basic biology and biochemistry of fibrinogen and fibrin formation, structure, and stability and highlights recent studies published in *Arteriosclerosis, Thrombosis, and Vascular Biology* and elsewhere. These have enhanced our understanding of fibrin(ogen) and revealed new potential applications for fibrin detection in thrombosis.

### Fibrinogen Biology and Structure

The fibrinogen molecule is a 340-kDa homodimeric glycoprotein consisting of 2A $\alpha$ , 2B $\beta$ , and 2 $\gamma$  polypeptide chains linked by 29 disulfide bridges. Fibrinogen synthesis occurs primarily in hepatocytes (Figure 1). Assembly of the 6 chains takes place in a stepwise manner in which single chains assemble first into A $\alpha$ - $\gamma$  and B $\beta$ - $\gamma$  complexes, then into A $\alpha$ /B $\beta$ / $\gamma$  half-molecules, and finally into hexameric complexes (A $\alpha$ /B $\beta$ / $\gamma$ )<sub>2</sub>.<sup>1</sup> All 6 fibrinogen chains are assembled with their N termini located in a central E nodule and extend outward in a coiled-coil arrangement. The B $\beta$  and  $\gamma$  chains terminate in globular regions known as  $\beta$ C and  $\gamma$ C modules, respectively. These regions collectively comprise the so-called D nodule. The A $\alpha$  chains are the longest; at the end of the coiled-coil region, each chain extends into a highly flexible series of repeats followed by a globular  $\alpha$ C region. Using high-resolution atomic force microscopy, Protopopova et al<sup>2</sup> obtained striking images of fibrinogen that visualize each of these structural components.

In healthy individuals, fibrinogen circulates in plasma at high concentrations (2–5 mg/mL). However, fibrinogen is an acute phase protein, and during acute inflammation, plasma fibrinogen levels can exceed 7 mg/mL. The fibrinogen chains are encoded in 3 genes that are thought to have arisen through gene duplication. Mechanisms that regulate expression of the fibrinogen genes are still largely undetermined. Genome-wide association studies have identified single-nucleotide polymorphisms within the fibrinogen genes,<sup>3</sup> as well as loci

distinct from fibrinogen that implicate transcription factors (eg, hepatocyte nuclear factors 1 and 4 [TCF1 and HNF4], signal transducer and activator of transcription 3 [STAT3])<sup>3–5</sup> and inflammatory signaling pathways downstream of interleukin-6<sup>5</sup> in fibrinogen gene expression. In addition, microRNAs (miR) in the hsa-miR-29 family and hsa-miR-409-3p down-regulate fibrinogen expression in hepatoma cells in vitro,<sup>6</sup> revealing mechanisms that may fine-tune fibrinogen levels in response to environmental cues.

### Fibrin Formation, Structure, and Stability

During coagulation, fibrinogen is converted into insoluble fibrin (Figure 1). Fibrin formation involves thrombin-mediated proteolytic cleavage and removal of N-terminal fibrinopeptides from the A $\alpha$  and B $\beta$  chains. Insertion of these newly exposed  $\alpha$ - and  $\beta$ -knobs into a- and b-holes in the  $\gamma$ C and  $\beta$ C regions of the D nodule, respectively, on another fibrin monomer permits the half-staggered association of fibrin monomers into protofibrils. Subsequent aggregation of protofibrils into fibers yields a fibrin meshwork that is essential for blood clot stability. This process has been extensively reviewed.<sup>7–11</sup>

Clot formation, structure, and stability are strongly influenced by the conditions present during fibrin generation. These include the concentrations of procoagulants, anticoagulants, fibrin(ogen)-binding proteins, molecules,<sup>12–20</sup> and metal ions,<sup>21,22</sup> as well as contributions of blood and vascular cells, cell-derived microvesicles,<sup>23–30</sup> and presence of blood flow<sup>31,32</sup> (Figure 2). Many of these mechanisms have been reviewed.<sup>33,34</sup>

The contribution of thrombin concentration to fibrin formation and structure has received considerable attention. High thrombin concentrations produce dense networks of highly branched fibrin fibers, and these clots are relatively resistant to fibrinolysis. In contrast, low thrombin concentrations produce coarse networks of relatively unbranched fibrin fibers, and these clots are relatively susceptible to fibrinolysis.<sup>12,17,35,36</sup> Most studies have reported that compared with fibers formed from low thrombin concentrations, fibers generated by high thrombin concentrations are thinner. However, turbidimetry and microscopy studies of fully hydrated clots suggest that high thrombin concentrations decrease the average protofibril content per fiber but only slightly decrease the fiber size, leading to a generally less compact fiber.<sup>37</sup> Thus, the substantially thinner fibers observed at high thrombin concentrations in earlier studies may reflect fiber compaction or shrinkage that occurs during dehydration. Regardless, the association of fibrin clot structural parameters with clinical pathologies—dense networks of thinner/compact fibers with increased thrombotic risk<sup>38–40</sup> and coarse networks of thicker/less compact fibers with increased bleeding risk<sup>36,41</sup>—suggests that fibrin structure is a critical determinant of hemostasis and thrombosis.

From the Department of Pathology and Laboratory Medicine, McAllister Heart Institute, University of North Carolina, Chapel Hill.

Correspondence to Alisa S. Wolberg, PhD, Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, 819 Brinkhous-Bullitt Bldg, CB No. 7525, Chapel Hill, NC 27599. E-mail alisa\_wolberg@med.unc.edu

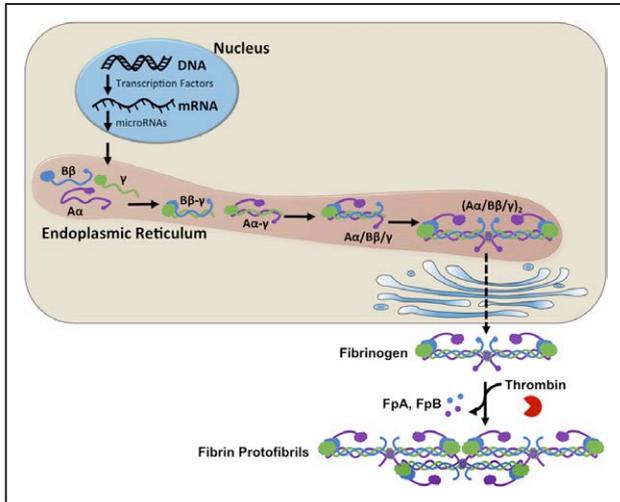
(*Arterioscler Thromb Vasc Biol*. 2017;37:e13–e21.

DOI: 10.1161/ATVBAHA.117.308564.)

© 2017 American Heart Association, Inc.

*Arterioscler Thromb Vasc Biol* is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.117.308564



**Figure 1.** Fibrinogen synthesis and expression. Fibrinogen synthesis is regulated by both transcriptional and translational mechanisms. After individual fibrinogen chains are translated, fibrinogen assembly occurs stepwise. Single chains assemble first into A $\alpha$ - $\gamma$  and B $\beta$ - $\gamma$  precursors, then into A $\alpha$ /B $\beta$ / $\gamma$  half-molecules, and finally into hexameric complexes (A $\alpha$ /B $\beta$ / $\gamma$ )<sub>2</sub>. Once fibrinogen is released into blood, it circulates until thrombin cleaves fibrinopeptides from the A $\alpha$  and B $\beta$  chains (FpA and FpB, respectively) to form fibrin monomers. These monomers then polymerize in a half-staggered arrangement to form fibrin protofibrils and ultimately the fibrin network at a site of tissue injury.

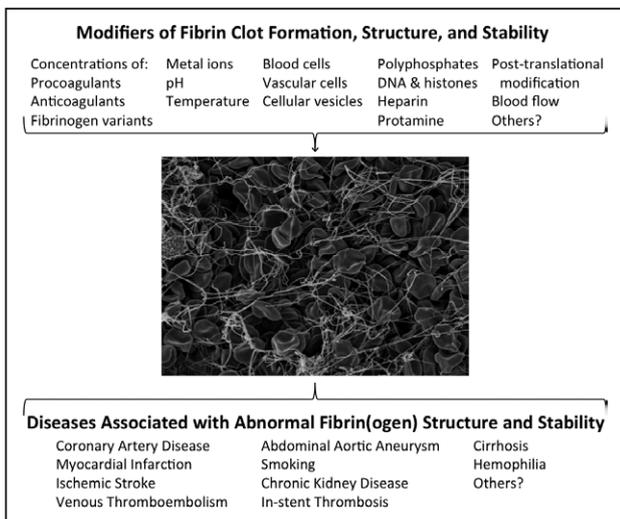
### Endogenous Mediators of Thrombin Generation and Fibrin

Multiple mechanisms mediate thrombin generation and consequently the thrombin concentration present during fibrin formation. First, the levels of pro- and anticoagulants present during coagulation strongly influence procoagulant activity. For example, elevated levels of prothrombin are associated with increased thrombin generation,<sup>35,42</sup> formation of dense fibrin networks,<sup>35</sup> and increased venous thrombus weight in mice.<sup>43</sup> These studies, designed to model the clinical situation in humans with the G20210A prothrombin mutation

associated with increased circulating prothrombin levels,<sup>44</sup> suggest that increased thrombin generation enhances venous thrombosis risk in part by promoting abnormal fibrin deposition and structure. Second, the location of thrombin generation impacts fibrin network formation. Effective assembly and activity of the prothrombinase complex (factors Xa, Va, and prothrombin) requires a lipid surface.<sup>45</sup> Localization of prothrombinase on a cell surface establishes a thrombin concentration gradient that influences both fibrin formation and network structure. In vitro experiments using in situ thrombin generation on fibroblasts and endothelial cells reveal a significantly denser fiber network proximal versus distal to the cell surface.<sup>29</sup> These structural differences give rise to substantially different fibrinolytic susceptibilities in different regions of the clot; fibrin located near the cell surface is significantly more resistant to lysis than fibrin located distal to the cell surface.<sup>29</sup> Third, blood flow (shear) present during fibrin formation influences local thrombin concentrations by (re)supplying procoagulant proteins and removing activated enzymes.<sup>46</sup> Flow also aligns fibrin fibers,<sup>31,32</sup> which may have profound effects on fibrin formation and mechanical and fibrinolytic stability.<sup>47</sup> Furthermore, the shear rate affects clot formation triggered on tissue factor- plus collagen-coated plates, resulting in different fibrin deposition in different regions of a thrombus.<sup>48</sup> Nanoindentation analysis to evaluate clot biophysical properties shows that this fibrin distribution pattern determines clot microelasticity, which may impact thrombus stability and risk of embolization.<sup>48</sup> Fourth, thrombin movement through the thrombus is substantially influenced by solute transport mechanisms mediated by cell packing density; this may also influence the amount of fibrin deposition in different regions of the clot.<sup>49</sup>

### Effects of Antithrombotic and Hemostatic Agents on Fibrin

Given the prominent role of thrombin concentration in determining fibrin network formation and structure, it is not surprising that antithrombotic agents that reduce thrombin activity reduce fibrin deposition and consequently thrombus formation. Because factor XI (FXI[a]) augments thrombin generation, in part by synergizing tissue factor-mediated procoagulant activity,<sup>50</sup> FXI inhibition strategies to reduce thrombosis have received considerable attention.<sup>51</sup> These approaches include conventional anti-FXI inhibitory antibodies, as well as technology in which antisense oligonucleotides (ASOs) result in the specific degradation of a target mRNA and corresponding reduction in target protein level. These studies reveal surprisingly specific effects of FXI inhibition in models of thrombosis and bleeding. Pharmacological FXI inhibition does not reduce local platelet adhesion in tissue factor and collagen-coated capillary tubes but reduces platelet activation and aggregation downstream of the growing thrombus.<sup>52</sup> Similarly, in an arteriovenous shunt model of thrombosis in nonhuman primates, neither anti-FXI antibodies nor ASOs alter platelet deposition on a collagen-rich segment of graft, but both decrease thrombus propagation (platelet accumulation and fibrin deposition) downstream of the collagen-rich region.<sup>53,54</sup> A promising phase I clinical trial demonstrated



**Figure 2.** Modifiers of fibrin(ogen) and association with disease. Clot formation, structure, and stability are influenced by conditions present during fibrin generation. Abnormal clot formation is observed in several diseases.

success of anti-FXI ASO treatment in humans undergoing elective total knee arthroplasty; ASO-mediated reduction of plasma FXI levels decreased symptomatic or asymptomatic venous thrombosis/thromboembolism (VTE) incidence, and the higher ASO dose tested was superior to enoxaparin.<sup>55</sup> In addition to its role in VTE, FXI also seems to contribute to atherogenesis in mice. Mice with deficiency in apolipoprotein E (*Apoe*<sup>-/-</sup>) spontaneously develop atherosclerotic lesions, but *Apoe*<sup>-/-</sup> mice with genetic FXI deficiency show reduced atherosclerosis progression.<sup>56</sup> Moreover, anti-FXI ASO treatment reduces thrombus formation and fibrin deposition in a model of plaque rupture in *Apoe*<sup>-/-</sup> mice.<sup>57</sup> Thus, FXI inhibition may also be effective for reducing arterial thrombosis in humans. Notably, FXI reduction has not been associated with increased bleeding in any of these studies, suggesting that FXI antagonism may be safer than current antithrombotics. However, given that bleeding occurs in a subset of patients with FXI deficiency<sup>58,59</sup> and the finding of altered structure and stability of plasma clots from these patients,<sup>60</sup> the safety of FXI inhibition should be carefully monitored in future trials. Regardless, these findings collectively support continued efforts to investigate and advance FXI inhibition strategies into the clinic.

Heparin and heparin-like compounds are used to prevent thrombosis, presumably because of their ability to reduce thrombin activity. However, heparin binds to the central E nodule of fibrin,<sup>61</sup> and both unfractionated heparin and low molecular weight heparin can also directly alter fibrin structure in an antithrombin-independent manner.<sup>62</sup> Observed changes include effects on fibrin fiber thickness, as well as porosity. These changes are not observed with the pentasaccharide, fondaparinux. Demonstration of these direct effects of unfractionated heparin and low molecular weight heparin on fibrin structure suggests tests that assess efficacy based solely on thrombin inhibition may not fully capture therapeutic effects of these drugs. Global assays that assess both thrombin generation and fibrin formation<sup>63</sup> may more closely reflect therapeutic effects of these drugs.

The common heparin reversal agent, protamine, also modulates fibrin network structure and stability. Protamine interacts directly with fibrinogen and is incorporated into clots, resulting in the production of thicker fibrin fibers in clots that are more susceptible to fibrinolysis.<sup>64</sup> Recently, Kalathottukaren et al<sup>64</sup> characterized a synthetic polycation they termed UHRA (universal heparin reversal agent) as an alternative to protamine. Universal heparin reversal agent can neutralize both heparin anticoagulant activity and polyphosphate procoagulant activity without the off-target effects on fibrin quality observed with protamine.<sup>64</sup> Further studies to evaluate the therapeutic potential of universal heparin reversal agent are anticipated.

### Effect of Cells and Cell Components on Fibrin Formation, Structure, and Stability

Clot quality is heavily influenced by cells and cell-derived components present at the injury site.<sup>23–30</sup> Recent studies have revealed previously unrecognized effects of red blood cells (RBCs) and neutrophil extracellular traps (NETs) on fibrin formation, structure, and stability. These may have substantial implications for understanding coagulation disorders.

RBCs are present in hemostatic and thrombotic clots, but their ability to influence clot formation or function has been unclear. RBCs can support thrombin generation<sup>65–67</sup> and thereby alter procoagulant activity at the site of clot formation. The presence of RBCs during clot formation also increases fibrin network heterogeneity, but whether RBCs increase<sup>28</sup> or decrease<sup>27</sup> fibrin fiber thickness is unclear. Once in the clot, RBCs alter clot viscoelastic properties<sup>28</sup> and, by reducing plasminogen activation, increase resistance of clots to fibrinolysis.<sup>27</sup> Effects of RBCs on fibrin structure are reduced in the presence of eptifibatid, suggesting that these effects are modulated in part by an interaction between fibrin(ogen) and a cell surface integrin.<sup>27</sup> This is consistent with observations suggesting that fibrin(ogen) binds to RBCs via a  $\beta_3$ -like molecule on the RBC surface,<sup>68</sup> although neither the binding site on fibrin(ogen) nor the putative RBC receptor has been identified. Studies of contracted whole blood clots show profound compression of resident RBCs into structures termed polyhydrocytes.<sup>25,69</sup> Adoption of this tight packing arrangement reduces clot permeability and may explain the resistance of older, compact clots to thrombolysis.<sup>25</sup>

NETs are composed of DNA, histones, and antimicrobial proteins and have surfaced as an intriguing link between inflammation and coagulation. NETs can be detected within venous thrombi,<sup>70</sup> and levels of cell-free DNA (CFDNA) in plasma are increased in patients with deep vein thrombosis, suggesting that NETs contribute to thrombosis pathogenesis.<sup>71</sup> However, specific effects of NETs and NET components on coagulation are complex. Briefly, NETs can interact with cells and coagulation factors and influence their activation and activity. NET components promote thrombin generation by activating the intrinsic pathway of coagulation and by inducing platelet-dependent mechanisms in toll-like receptors-2 and -4-dependent mechanisms.<sup>72–74</sup> Histones also enhance activated protein C generation by thrombin/thrombomodulin in vitro and in mice.<sup>75</sup> By altering these procoagulant and anticoagulant pathways, NETs may alter local thrombin levels and indirectly alter fibrin formation and quality. In vitro studies suggest that CFDNA promotes formation of densely packed networks of thick fibrin fibers.<sup>74</sup> This observation is interesting, given that dense fibrin networks are more typically associated with decreased fiber thickness/compaction. Thus, this finding suggests that CFDNA, like its highly charged cousin polyphosphate, modulates fibrin structure at least partly through a direct interaction with fibrin(ogen). In addition, clots formed in the presence of CFDNA exhibit delayed fibrinolysis via a mechanism that involves a CFDNA-dependent reduction in plasmin fibrinolytic activity.<sup>74</sup> These effects may have substantial clinical implications. For example, CFDNA levels are elevated in patients with sepsis, and the effects of CFDNA on fibrin structure and fibrinolysis are also observed in plasmas from sepsis patients.<sup>74,76</sup> The role of NETs and potential use of DNA-dissolving treatment (eg, DNase) remains an active area of investigation.

### Alternatively Spliced Fibrinogen

Multiple alternatively spliced forms of fibrinogen can be detected in plasma. Of these, an alternatively spliced form

of the  $\gamma$  chain ( $\gamma'$ ) is the most prevalent and has received the most attention. The  $\gamma'$  chain has the final 4 amino acids of the native  $\gamma$  chain replaced with 20 amino acids that add substantial negative charge.<sup>77–79</sup> Molecules containing the  $\gamma'$  chain circulate as a heterodimer with the  $\gamma$ A chain (2A $\alpha$ , 2B $\beta$ , and  $\gamma$ A/ $\gamma'$ ) and comprise 8% to 15% of total fibrinogen in healthy individuals.<sup>79,80</sup>

Epidemiological studies have associated altered arterial and venous thrombosis risk with the level of circulating  $\gamma$ A/ $\gamma'$  fibrinogen. For example, elevated levels of  $\gamma$ A/ $\gamma'$  fibrinogen have been associated with increased incidence of coronary artery disease,<sup>81</sup> myocardial infarction,<sup>82</sup> and ischemic stroke,<sup>83</sup> leading to the hypothesis that  $\gamma$ A/ $\gamma'$  fibrinogen promotes arterial thrombosis. However, a recent prospective study showed that although  $\gamma$ A/ $\gamma'$  fibrinogen is associated with increased incidence of cardiovascular disease, peripheral arterial disease, and heart failure, this association is lost when the analysis is adjusted for total fibrinogen and C-reactive protein.<sup>84</sup> Thus, the association of elevated fibrinogen with these pathologies may be mediated, at least in part, by a coexisting inflammatory reaction. In contrast, reduced levels of  $\gamma$ A/ $\gamma'$  fibrinogen and decreased  $\gamma'$ /total fibrinogen ratio have been fairly consistently associated with increased risk of VTE<sup>85</sup> and thrombotic microangiopathy.<sup>86</sup> These findings suggest that  $\gamma$ A/ $\gamma'$  fibrinogen is protective against venous thrombosis and raise interesting questions about the operant mechanism.

In vitro studies investigating the relative role(s) of the fibrinogen isoforms during clot formation indicate that both  $\gamma$ A/ $\gamma$ A and  $\gamma$ A/ $\gamma'$  isoforms are incorporated into the fibrin network; however, the  $\gamma$ A/ $\gamma'$  isoform has unique properties that modify its role during clotting and subsequently the function of the fibrin clot. Studies have generally reported thinner fibrin fibers in clots containing the  $\gamma$ A/ $\gamma'$  isoform and associated this effect with increased resistance to fibrinolysis.<sup>87,88</sup> However, Domingues et al<sup>37</sup> detected reduced packing of  $\gamma$ A/ $\gamma'$  molecules in fully hydrated protofibrils, suggesting that reduced packing results in the appearance of decreased fiber diameter in dehydrated clots. Moreover, in contrast to clots formed by  $\gamma$ A/ $\gamma$ A fibrinogen, characteristics of fibers formed by  $\gamma$ A/ $\gamma'$  fibrinogen are relatively unaffected by the thrombin concentration.<sup>37</sup>

The  $\gamma$ A/ $\gamma'$  isoform supports high-affinity binding to thrombin exosite II<sup>89,90</sup> that led to its recognition as antithrombin I. Thrombin binding to the  $\gamma'$  chain competitively inhibits thrombin-mediated platelet activation,<sup>91</sup> reduces thrombin-mediated fibrinopeptide B cleavage,<sup>92</sup> and decreases factor VIII<sup>93</sup> and V<sup>94</sup> activation. In in vitro microfluidic models,  $\gamma$ A/ $\gamma'$  fibrinogen reduces clot growth primarily at venous, but not arterial, wall shear rates, suggesting that the impact of its antithrombin I activity depends in part on the location of the thrombotic event (vein or artery).<sup>94</sup> Studies with mice have documented antithrombotic effects of  $\gamma$ A/ $\gamma'$  in models of both venous and arterial thrombosis. Expression of the human  $\gamma$ A/ $\gamma'$  fibrinogen isoform in mice that are heterozygous for the factor V Leiden mutation reduces thrombus volume after electrolytic injury to the femoral vein.<sup>95</sup> In a model of FeCl<sub>3</sub>-induced carotid artery injury, healthy mice infused with unfractionated human fibrinogen have a shortened time to vessel occlusion, and this effect is recapitulated by infusion

of  $\gamma$ A/ $\gamma$ A, but not  $\gamma$ A/ $\gamma'$ , fibrinogen. Although  $\gamma$ A/ $\gamma'$ -infused mice are not protected against thrombus formation in this model, they do have lower levels of circulating plasma thrombin-antithrombin complexes compared with  $\gamma$ A/ $\gamma$ A-infused mice, consistent with increased thrombin-binding capacity of  $\gamma$ A/ $\gamma'$  fibrin(ogen).<sup>96</sup> Collectively, these studies suggest that  $\gamma$ A/ $\gamma'$  has generally antithrombotic roles during coagulation, and its expression may serve to downregulate inflammation-induced prothrombotic activity.

## Fibrin Crosslinking

Covalent crosslinking of fibrin chains is a critical determinant of fibrin stability. Crosslinking is mediated predominantly by transglutaminase factor XIII (FXIII) found in plasma and platelets. Plasma FXIII is a 320-kDa heterotetrameric zymogen (FXIII-A<sub>2</sub>B<sub>2</sub>) composed of 2 catalytic subunits (FXIII-A<sub>2</sub>) tightly associated (K<sub>d</sub> ≈ 10<sup>-10</sup> mol/L)<sup>97</sup> with 2 noncatalytic subunits (FXIII-B<sub>2</sub>). FXIII-A<sub>2</sub>B<sub>2</sub> circulates at ≈ 70 nmol/L (14–28 μg/mL)<sup>98</sup> in complex with fibrinogen. Although early data suggested that FXIII-A<sub>2</sub>B<sub>2</sub> preferentially binds the alternatively spliced fibrinogen  $\gamma'$  chain, more recent studies have localized binding to  $\gamma$ -chain residues 390 to 396 with additional contributions from the A $\alpha$ -chain.<sup>99–101</sup>

Catalytically active FXIII (FXIIIa) introduces  $\epsilon$ -N-( $\gamma$ -glutamyl)-lysyl crosslinks between glutamine and lysine residues on fibrin  $\gamma$ - and  $\alpha$ -chains, yielding  $\gamma$ - $\gamma$  dimers and high molecular weight species ( $\gamma$ -multimers,  $\alpha$ -polymers, and  $\alpha$  $\gamma$ -hybrids). FXIII can also crosslink other plasma proteins (eg,  $\alpha_2$ -antiplasmin and fibronectin) to fibrin. Covalent crosslinking of  $\alpha_2$ -antiplasmin to fibrin prevents expulsion of  $\alpha_2$ -antiplasmin from the clot during clot compression or contraction<sup>102</sup> and is essential for clot stability. For example, in a mouse model of middle cerebral artery occlusion in which plasma clots formed ex vivo are placed into  $\alpha_2$ -antiplasmin-deficient mice, clots made from  $\alpha_2$ -antiplasmin-sufficient plasma are more resistant to dissolution than clots made from  $\alpha_2$ -antiplasmin-deficient plasma.<sup>103</sup> The importance of FXIIIa-mediated crosslinking for clot stability has been reviewed.<sup>104–106</sup>

Recently, we discovered that FXIIIa-mediated fibrin crosslinking also promotes RBC retention in clots, exposing a newly recognized role for this activity during VTE.<sup>99,107</sup> Briefly, compared with FXIII-sufficient mice (F13a<sup>+/+</sup>), FXIII-deficient (F13a<sup>-/-</sup>) mice produce thrombi that have reduced RBC retention and consequently are smaller.<sup>99</sup> This effect of FXIII on RBC retention in clots is mediated specifically by fibrin  $\alpha$ -chain crosslinking.<sup>107</sup>

The timing of fibrin crosslinking also seems to be integral to RBC retention in clots. Mice that have reduced binding of FXIII to fibrinogen and delayed FXIII activation and fibrin crosslinking (Fib $\gamma$ <sup>390–396A</sup>) show significantly decreased RBC retention and thrombus size, similar to that seen in F13a<sup>-/-</sup> mice.<sup>99</sup> Interestingly, the FXIII Val34Leu polymorphism that exhibits accelerated FXIII activation paradoxically conveys moderate protection against VTE by modulating clot structure in a fibrinogen concentration-dependent manner.<sup>108</sup> These interesting and apparent paradoxical findings raise important and clinically relevant questions on the role of FXIII activation

kinetics and fibrin crosslinking in thrombosis. Duval et al<sup>109</sup> tested the contribution of the Val34Leu polymorphism to thrombus formation in mice. Despite observing increased FXIII activation and crosslinking in vitro and in vivo, *F13a*<sup>-/-</sup> mice infused with recombinant FXIII-Leu34 showed no difference in thrombus size compared with FXIII-Val34-infused mice in the FeCl<sub>3</sub> model of femoral vein thrombosis.<sup>109</sup> These data suggest that the Leu34 variant does not alter thrombus size; however, because FeCl<sub>3</sub> injury induces rapid formation of platelet-rich thrombi, effects of the Val34Leu polymorphism on the slow process of RBC- and fibrin-rich venous thrombus formation remain unknown. Further investigations on the contribution of the FXIII-Leu34 polymorphism to fibrin formation and thrombosis are warranted.

### Clot Contraction

An essential function during coagulation is the platelet-mediated consolidation of clots in a process known as clot contraction (or retraction). This process involves fibrin(ogen) binding to platelet integrin receptor  $\alpha_{\text{IIb}}\beta_3$  and is influenced by both platelet and fibrin(ogen) concentrations.<sup>110</sup> Although recognized as a fundamental process during coagulation, clot contraction has received little attention, particularly in a clinical setting. This gap is noteworthy given findings that associate platelet aggregation and clot contraction with decreased clot permeability and increased resistance to fibrinolysis, 2 parameters thought to impact thrombosis risk.<sup>25,111</sup> Tutwiler et al<sup>69</sup> evaluated the kinetics of clot contraction in blood samples collected from patients with recent acute ischemic stroke and correlated parameters with hemostatic and hematologic laboratory characteristics. Surprisingly, compared with clots from healthy individuals, whole blood clots from patients with recent ischemic stroke exhibit reduced clot contraction.<sup>69</sup> However, because samples were collected after symptom onset, these changes may reflect a consequence, rather than cause, of the thrombotic event. Ischemic stroke patients had quantitative and qualitative defects in circulating platelets (decreased platelet count, shape change, and P-selectin exposure in unstimulated platelets and decreased fibrinogen-binding capacity of activated platelets),<sup>69</sup> suggesting that the ischemic event may consume platelets and induce a refractory phenotype in circulating platelets that are not incorporated into the thrombus. Prospective analysis of blood samples before stroke onset is necessary to determine if altered contraction promotes occlusive thrombus formation.

### Abnormal Fibrinogen and Fibrin Structure in Thrombosis

Production of clots with abnormal structure and stability has been demonstrated in plasma samples from patients with increased cardiovascular disease risk.<sup>38-40</sup> After percutaneous coronary intervention, patients who develop in-stent thrombosis demonstrate abnormal plasma clot characteristics (eg, permeability, turbidity, and lysis time) compared with patients who did not develop in-stent thrombosis.<sup>112</sup> Similarly, compared with healthy controls, plasma clots from patients with abdominal aortic aneurysm have more densely packed fibrin networks with smaller pores and were more resistant to lysis.<sup>113</sup>

Moreover, effects are aneurysm size dependent; patients with larger aneurysms have more densely packed fibers compared with patients with smaller aneurysms.<sup>113</sup> In both in-stent thrombosis and abdominal aortic aneurysm patients, these effects on clot properties are independent of total fibrinogen levels but may be related to effects of other plasma proteins on fibrin formation.<sup>112,113</sup> It remains unclear whether these fibrin clot abnormalities are only a biomarker for an operant pathophysiologic mechanism, or whether abnormal fibrin clot structure is causative in the disease.

### Post-Translational Modifications of Fibrinogen

Although genetic mutations in fibrinogen (congenital dysfibrinogenemia) have been associated with abnormal fibrin clot formation and bleeding and thrombosis,<sup>114</sup> acquired fibrinogen abnormalities are likely far more prevalent. The high concentration of fibrinogen in circulation makes it a frequent target of enzymes and activities that modify its structure and function. These post-translational modifications include nitration, homocysteinylation, and glycation and are reviewed elsewhere.<sup>115</sup>

Importantly, fibrinogen modifications have been observed in plasma clots from individuals with increased cardiovascular risk. For example, cigarette smoking is associated with abnormal fibrinogen levels and is a major risk factor for cardiovascular disease.<sup>116</sup> A multiethnic cohort of current, former, and nonsmokers observed that current chronic smokers with a longer number of pack-years have fibrinogen levels higher than either former smokers or nonsmokers,<sup>117</sup> suggesting that smoke exposure causes an acquired, but reversible, hyperfibrinogenemia that increases cardiovascular risk. Interestingly, even acute exposure to cigarette smoke is associated with the production of plasma clots with thinner fibrin fibers and increased platelet aggregation,<sup>118</sup> suggesting that cigarette smoke exposure also induces immediate (post-translational) functional changes in fibrin formation and structure that promote thrombogenicity.<sup>118</sup> Although the specific mechanisms were not determined, the authors speculated that free radicals (reactive oxygen species) present in cigarette smoke modify fibrinogen in circulation. Consistent with that premise, other studies have specifically associated oxidative modification of fibrinogen with cardiovascular disease and increased thrombotic risk.<sup>119,120</sup> Compared with age-, sex-, and risk factor-matched controls, patients with postacute myocardial infarction have elevated plasma markers of oxidative stress, including increased fibrinogen carbonylation. Moreover, fibrinogen isolated from these plasma samples demonstrates abnormal clotting characteristics, including the production of clots with thinner fibrin fibers.<sup>120</sup> Fibrinogen carbonyl content correlates negatively with fibrin clot turbidity (a proxy for fibrin network structure) and positively with extent of fibrin  $\beta$ -chain remaining during fibrinolysis (a proxy for resistance to fibrinolysis).

Cirrhosis is also associated with elevated thrombosis risk, and fibrinogen isolated from patients with cirrhosis demonstrates carbohydrate modifications and increased carbonyl content.<sup>119,121</sup> Compared with controls, both plasma clots and clots made from purified fibrinogen from cirrhotic patients demonstrate abnormal clotting characteristics, including decreased

clot permeability and shorter clot lysis times.<sup>119</sup> Somewhat paradoxically, patients included in this study<sup>119</sup> reported bleeding, mostly variceal, rather than thrombosis. However, the more frequent association of the observed clot characteristics with thrombotic risk suggests that abnormal clot structure ultimately contributes to thrombosis in these patients.

Patients with chronic kidney disease also have increased risk of thrombotic events. Fibrinogen purified from patients with chronic kidney disease on hemodialysis shows evidence of glycosylation and guanidinylation.<sup>122</sup> Compared with fibrin clots from healthy controls, clots made with guanidinylated fibrinogen have significantly thinner, or perhaps more compact, fibrin fibers. Notably, formation of denser fibrin networks was independently associated with mortality risk in the hemodialysis patients.<sup>122</sup>

Collectively, these findings implicate fibrin(ogen) modification in thrombosis associated with multiple pathologies. Additional studies are needed to demonstrate direct pathological contributions of each of these modifications to fibrin(ogen) function in vivo.

### Fibrin(ogen) Detection as a Diagnostic Tool

VTE diagnosis includes imaging technologies such as Doppler ultrasound or computed tomography to detect deep vein thrombosis or pulmonary embolism, respectively.<sup>123,124</sup> These technologies show vascular abnormalities and flow disturbances around the thrombus but do not reveal information about thrombus composition. Development of technologies that can detect thrombus composition may have clinical use. Notably, whereas early thrombi have substantial crosslinked fibrin content, this fibrin is replaced with collagen during thrombus resolution.<sup>125</sup> Because fibrin-rich thrombi are more susceptible to fibrinolysis than collagen-rich thrombi,<sup>126</sup> distinguishing early, fibrin-rich thrombi from older, collagen-rich thrombi may aid in identifying thrombi that are susceptible to fibrin-degrading thrombolytic therapy. Currently, assessment of thrombus age is highly subjective and only poorly able to identify patients who may respond to thrombolytic treatment.<sup>126</sup> However, 2 recent studies of thrombosis detection in rodents have advanced methods to detect intravascular thrombi and reveal information about thrombus fibrin content. Blasi et al<sup>127</sup> demonstrated the ability of a fibrin-binding probe,<sup>64</sup> Cu-FBP8, to detect both venous and arterial thrombi in a single whole-body positron emission tomographic scan. Probe uptake correlated positively with fibrin content in both arterial and venous clots, distinguishing young (high probe uptake) from old (low probe uptake) thrombi.<sup>127</sup> This positron emission tomography–based imaging method enables imaging of multiple thrombi in one examination and may be a non-invasive and sensitive approach to assess changes in thrombus composition over time. Similarly, the spatial and temporal uptake of a gadolinium-based fibrin-specific magnetic resonance imaging contrast agent, EP-2104R, also correlates positively with time-dependent changes in thrombus fibrin content.<sup>128</sup> Furthermore, thrombi that exhibit high EP-2104R uptake are more susceptible to tissue-type plasminogen activator–mediated dissolution, suggesting that EP-2104R can be used to identify thrombi that are susceptible to thrombolytic therapy.<sup>128</sup> Additional studies are warranted to determine

whether these methods can be used to identify human patients with greatest potential benefit of thrombolytic therapy.

### Summary

This review has highlighted both established and newer findings on fibrin(ogen) expression and function that demonstrate its central role in clot formation during hemostasis and thrombosis. Additional studies beyond the scope of this review have exposed intriguing roles for fibrin(ogen) in inflammation, infection, neurological disease, cancer, and other pathologies. Collectively, these discoveries have uncovered critical links between disease pathways and rationalize the significant association of many diseases with increased bleeding and thrombosis risk. Identification of these pathways may yield new therapeutic targets with enhanced specificity and safety. Consequently, efforts to advance both basic research in fibrin(ogen) genetics, biology, biochemistry, and biophysics and translational applications for fibrin(ogen) detection and altering fibrin(ogen) function are likely to have broad impact on health and disease.

### Acknowledgments

We thank Joan D. Beckman and Chase B. Brandner for reading the article.

### Sources of Funding

This research is supported by funding from the National Institutes of Health (R01HL126974 to A.S. Wolberg and HL069768 to the University of North Carolina at Chapel Hill/S. Kattula) and a National Science Foundation Graduate Research Fellowship (DGE-1144081 to J.R. Byrnes).

### Disclosures

None.

### References

- Redman CM, Xia H. Fibrinogen biosynthesis. Assembly, intracellular degradation, and association with lipid synthesis and secretion. *Ann NY Acad Sci*. 2001;936:480–495.
- Protopopova AD, Barinov NA, Zavyalova EG, Kopylov AM, Sergienko VI, Klinov DV. Visualization of fibrinogen  $\alpha$ C regions and their arrangement during fibrin network formation by high-resolution AFM. *J Thromb Haemost*. 2015;13:570–579. doi: 10.1111/jth.12785.
- Huffman JE, de Vries PS, Morrison AC, et al. Rare and low-frequency variants and their association with plasma levels of fibrinogen, FVII, FVIII, and vWF. *Blood*. 2015;126:e19–e29. doi: 10.1182/blood-2015-02-624551.
- Soria JM, Almasy L, Souto JC, Buil A, Lathrop M, Blangero J, Fontcuberta J. A genome search for genetic determinants that influence plasma fibrinogen levels. *Arterioscler Thromb Vasc Biol*. 2005;25:1287–1292. doi: 10.1161/01.ATV.0000161927.38739.6f.
- de Vries PS, Chasman DI, Sabater-Lleal M, et al. A meta-analysis of 120 246 individuals identifies 18 new loci for fibrinogen concentration. *Hum Mol Genet*. 2016;25:358–370. doi: 10.1093/hmg/ddv454.
- Fort A, Borel C, Migliavacca E, Antonarakis SE, Fish RJ, Neerman-Arbez M. Regulation of fibrinogen production by microRNAs. *Blood*. 2010;116:2608–2615. doi: 10.1182/blood-2010-02-268011.
- Budzynski AZ, Olexa SA, Pandya BV. Fibrin polymerization sites in fibrinogen and fibrin fragments. *Ann NY Acad Sci*. 1983;408:301–314.
- Lord ST. Molecular mechanisms affecting fibrin structure and stability. *Arterioscler Thromb Vasc Biol*. 2011;31:494–499. doi: 10.1161/ATVBAHA.110.213389.
- Okumura N, Terasawa F, Haneishi A, Fujihara N, Hirota-Kawadobora M, Yamauchi K, Ota H, Lord ST. B:b interactions are essential for polymerization of variant fibrinogens with impaired holes 'a'. *J Thromb Haemost*. 2007;5:2352–2359. doi: 10.1111/j.1538-7836.2007.02793.x.

10. Spraggon G, Everse SJ, Doolittle RF. Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. *Nature*. 1997;389:455–462. doi: 10.1038/38947.
11. Yang Z, Mochalkin I, Doolittle RF. A model of fibrin formation based on crystal structures of fibrinogen and fibrin fragments complexed with synthetic peptides. *Proc Natl Acad Sci USA*. 2000;97:14156–14161. doi: 10.1073/pnas.97.26.14156.
12. Blombäck B, Carlsson K, Fatah K, Hessel B, Procyk R. Fibrin in human plasma: gel architectures governed by rate and nature of fibrinogen activation. *Thromb Res*. 1994;75:521–538.
13. Francis CW, Marder VJ. Increased resistance to plasmin degradation of fibrin with highly crosslinked alpha-polymer chains formed at high factor XIII concentrations. *Blood*. 1988;71:1361–1365.
14. Glover CJ, McIntire LV, Brown CH III, Natelson EA. Rheological properties of fibrin clots. Effects of fibrinogen concentration, Factor XIII deficiency, and Factor XIII inhibition. *J Lab Clin Med*. 1975;86:644–656.
15. Machlus KR, Cardenas JC, Church FC, Wolberg AS. Causal relationship between hyperfibrinogenemia, thrombosis, and resistance to thrombolysis in mice. *Blood*. 2011;117:4953–4963. doi: 10.1182/blood-2010-11-316885.
16. Nair CH, Shah GA, Dhall DP. Effect of temperature, pH and ionic strength and composition on fibrin network structure and its development. *Thromb Res*. 1986;42:809–816.
17. Ryan EA, Mockros LF, Weisel JW, Lorand L. Structural origins of fibrin clot rheology. *Biophys J*. 1999;77:2813–2826. doi: 10.1016/S0006-3495(99)77113-4.
18. Smith SA, Morrissey JH. Polyphosphate enhances fibrin clot structure. *Blood*. 2008;112:2810–2816. doi: 10.1182/blood-2008-03-145755.
19. Whyte CS, Chernysh IN, Domingues MM, Connell S, Weisel JW, Ariens RA, Mutch NJ. Polyphosphate delays fibrin polymerisation and alters the mechanical properties of the fibrin network. *Thromb Haemost*. 2016;116:897–903. doi: 10.1160/TH16-01-0062.
20. Smith SA, Morrissey JH. 2013 scientific sessions Sol Sherry distinguished lecture in thrombosis: polyphosphate: a novel modulator of hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol*. 2015;35:1298–1305. doi: 10.1161/ATVBAHA.115.301927.
21. Henderson SJ, Xia J, Wu H, Stafford AR, Leslie BA, Fredenburgh JC, Weitz DA, Weitz JI. Zinc promotes clot stability by accelerating clot formation and modifying fibrin structure. *Thromb Haemost*. 2016;115:533–542. doi: 10.1160/TH15-06-0462.
22. Wolberg AS, Gabriel DA, Hoffman M. Analyzing fibrin clot structure using a microplate reader. *Blood Coagul Fibrinolysis*. 2002;13:533–539.
23. Aleman MM, Gardiner C, Harrison P, Wolberg AS. Differential contributions of monocyte- and platelet-derived microparticles towards thrombin generation and fibrin formation and stability. *J Thromb Haemost*. 2011;9:2251–2261. doi: 10.1111/j.1538-7836.2011.04488.x.
24. Campbell RA, Overmyer KA, Selzman CH, Sheridan BC, Wolberg AS. Contributions of extravascular and intravascular cells to fibrin network formation, structure, and stability. *Blood*. 2009;114:4886–4896. doi: 10.1182/blood-2009-06-228940.
25. Cines DB, Lebedeva T, Nagaswami C, Hayes V, Masefski W, Litvinov RI, Rauova L, Lowery TJ, Weisel JW. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood*. 2014;123:1596–1603. doi: 10.1182/blood-2013-08-523860.
26. Zubairova LD, Nabiullina RM, Nagaswami C, Zuev YF, Mustafin IG, Litvinov RI, Weisel JW. Circulating microparticles alter formation, structure, and properties of fibrin clots. *Sci Rep*. 2015;5:17611. doi: 10.1038/srep17611.
27. Wohner N, Sótónyi P, Machovich R, Szabó L, Tenekedjiev K, Silva MM, Longstaff C, Kolev K. Lytic resistance of fibrin containing red blood cells. *Arterioscler Thromb Vasc Biol*. 2011;31:2306–2313. doi: 10.1161/ATVBAHA.111.229088.
28. Gersh KC, Nagaswami C, Weisel JW. Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes. *Thromb Haemost*. 2009;102:1169–1175. doi: 10.1160/TH09-03-0199.
29. Campbell RA, Overmyer KA, Bagnell CR, Wolberg AS. Cellular procoagulant activity dictates clot structure and stability as a function of distance from the cell surface. *Arterioscler Thromb Vasc Biol*. 2008;28:2247–2254. doi: 10.1161/ATVBAHA.108.176008.
30. Jerome WG, Handt S, Hantgan RR. Endothelial cells organize fibrin clots into structures that are more resistant to lysis. *Microsc Microanal*. 2005;11:268–277. doi: 10.1017/S134192760505052X.
31. Campbell RA, Aleman M, Gray LD, Falvo MR, Wolberg AS. Flow profoundly influences fibrin network structure: implications for fibrin formation and clot stability in haemostasis. *Thromb Haemost*. 2010;104:1281–1284. doi: 10.1160/TH10-07-0442.
32. Gersh KC, Edmondson KE, Weisel JW. Flow rate and fibrin fiber alignment. *J Thromb Haemost*. 2010;8:2826–2828. doi: 10.1111/j.1538-7836.2010.04118.x.
33. Weisel JW. Structure of fibrin: impact on clot stability. *J Thromb Haemost*. 2007;5(suppl 1):116–124. doi: 10.1111/j.1538-7836.2007.02504.x.
34. Wolberg AS. Thrombin generation and fibrin clot structure. *Blood Rev*. 2007;21:131–142. doi: 10.1016/j.blre.2006.11.001.
35. Wolberg AS, Monroe DM, Roberts HR, Hoffman M. Elevated prothrombin results in clots with an altered fiber structure: a possible mechanism of the increased thrombotic risk. *Blood*. 2003;101:3008–3013. doi: 10.1182/blood-2002-08-2527.
36. Wolberg AS, Allen GA, Monroe DM, Hedner U, Roberts HR, Hoffman M. High dose factor VIIa improves clot structure and stability in a model of haemophilia B. *Br J Haematol*. 2005;131:645–655. doi: 10.1111/j.1365-2141.2005.05820.x.
37. Domingues MM, Macrae FL, Duval C, McPherson HR, Bridge KI, Ajjan RA, Ridger VC, Connell SD, Philippou H, Ariens RA. Thrombin and fibrinogen  $\gamma'$  impact clot structure by marked effects on intrafibrillar structure and protofibril packing. *Blood*. 2016;127:487–495. doi: 10.1182/blood-2015-06-652214.
38. Undas A, Zawilska K, Ciesla-Dul M, Lehmann-Kopydłowska A, Skubiszak A, Ciepluch K, Tracz W. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. *Blood*. 2009;114:4272–4278. doi: 10.1182/blood-2009-05-222380.
39. Mills JD, Ariens RA, Mansfield MW, Grant PJ. Altered fibrin clot structure in the healthy relatives of patients with premature coronary artery disease. *Circulation*. 2002;106:1938–1942.
40. Zabczyk M, Plens K, Wojtowicz W, Undas A. Prothrombotic fibrin clot phenotype is associated with recurrent pulmonary embolism after discontinuation of anticoagulant therapy. *Arterioscler Thromb Vasc Biol*. 2016;37:1–9.
41. He S, Blombäck M, Jacobsson Ekman G, Hedner U. The role of recombinant factor VIIa (FVIIa) in fibrin structure in the absence of FVIII/FIX. *J Thromb Haemost*. 2003;1:1215–1219.
42. Allen GA, Wolberg AS, Oliver JA, Hoffman M, Roberts HR, Monroe DM. Impact of procoagulant concentration on rate, peak and total thrombin generation in a model system. *J Thromb Haemost*. 2004;2:402–413.
43. Aleman MM, Walton BL, Byrnes JR, Wang JG, Heisler MJ, Machlus KR, Cooley BC, Wolberg AS. Elevated prothrombin promotes venous, but not arterial, thrombosis in mice. *Arterioscler Thromb Vasc Biol*. 2013;33:1829–1836. doi: 10.1161/ATVBAHA.113.301607.
44. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88:3698–3703.
45. Kane WH, Davie EW. Blood coagulation factors V and VIII: structural and functional similarities and their relationship to hemorrhagic and thrombotic disorders. *Blood*. 1988;71:539–555.
46. Brass LF, Diamond SL. Transport physics and biorheology in the setting of hemostasis and thrombosis. *J Thromb Haemost*. 2016;14:906–917. doi: 10.1111/jth.13280.
47. Varjú I, Sótónyi P, Machovich R, Szabó L, Tenekedjiev K, Silva MM, Longstaff C, Kolev K. Hindered dissolution of fibrin formed under mechanical stress. *J Thromb Haemost*. 2011;9:979–986. doi: 10.1111/j.1538-7836.2011.04203.x.
48. Swieringa F, Baaten CC, Verdoold R, Mastenbroek TG, Rijnveld N, van der Laan KO, Bree EJ, Collins PW, Lancé MD, Henskens YM, Cosemans JM, Heemskerk JW, van der Meijden PE. Platelet control of fibrin distribution and microelasticity in thrombus formation under flow. *Arterioscler Thromb Vasc Biol*. 2016;36:692–699. doi: 10.1161/ATVBAHA.115.306537.
49. Stalker TJ, Welsh JD, Tomaiuolo M, Wu J, Colace TV, Diamond SL, Brass LF. A systems approach to hemostasis: 3. Thrombus consolidation regulates intrathrombus solute transport and local thrombin activity. *Blood*. 2014;124:1824–1831. doi: 10.1182/blood-2014-01-550319.
50. Leiderman K, Chang WC, Ovanesov M, Fogelson AL. Synergy between tissue factor and exogenous factor XIa in initiating coagulation. *Arterioscler Thromb Vasc Biol*. 2016;36:2334–2345. doi: 10.1161/ATVBAHA.116.308186.
51. Gailani D, Gruber A. Factor XI as a therapeutic target. *Arterioscler Thromb Vasc Biol*. 2016;36:1316–1322. doi: 10.1161/ATVBAHA.116.306925.
52. Zilberman-Rudenko J, Itakura A, Wiesenekker CP, Vetter R, Maas C, Gailani D, Tucker EI, Gruber A, Gerdes C, McCarty OJ. Coagulation factor XI promotes distal platelet activation and single platelet consumption in the bloodstream under shear flow. *Arterioscler Thromb Vasc Biol*. 2016;36:510–517. doi: 10.1161/ATVBAHA.115.307034.

53. Crosby JR, Marzec U, Revenko AS, Zhao C, Gao D, Matafonov A, Gailani D, MacLeod AR, Tucker EI, Gruber A, Hanson SR, Monia BP. Antithrombotic effect of antisense factor XI oligonucleotide treatment in primates. *Arterioscler Thromb Vasc Biol*. 2013;33:1670–1678. doi: 10.1161/ATVBAHA.113.301282.
54. Tucker EI, Marzec UM, White TC, Hurst S, Rugonyi S, McCarty OJ, Gailani D, Gruber A, Hanson SR. Prevention of vascular graft occlusion and thrombus-associated thrombin generation by inhibition of factor XI. *Blood*. 2009;113:936–944. doi: 10.1182/blood-2008-06-163675.
55. Büller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, Segers A, Verhamme P, Weitz JI; FXI-ASO TKA Investigators. Factor XI antisense oligonucleotide for prevention of venous thrombosis. *N Engl J Med*. 2015;372:232–240. doi: 10.1056/NEJMoa1405760.
56. Shnerb Ganor R, Harats D, Schiby G, Gailani D, Levkovitz H, Avivi C, Tamarin I, Shaish A, Salomon O. Factor XI deficiency protects against atherogenesis in apolipoprotein E/factor XI double knockout mice. *Arterioscler Thromb Vasc Biol*. 2016;36:475–481. doi: 10.1161/ATVBAHA.115.306954.
57. van Montfort ML, Kuijpers MJ, Knaup VL, Bhanot S, Monia BP, Roelofs JJ, Heemskerk JW, Meijers JC. Factor XI regulates pathological thrombus formation on acutely ruptured atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2014;34:1668–1673. doi: 10.1161/ATVBAHA.114.303209.
58. Seligsohn U. Factor XI deficiency in humans. *J Thromb Haemost*. 2009;7(suppl 1):84–87. doi: 10.1111/j.1538-7836.2009.03395.x.
59. Asakai R, Chung DW, Davie EW, Seligsohn U. Factor XI deficiency in Ashkenazi Jews in Israel. *N Engl J Med*. 1991;325:153–158. doi: 10.1056/NEJM199107183250303.
60. Zucker M, Seligsohn U, Salomon O, Wolberg AS. Abnormal plasma clot structure and stability distinguish bleeding risk in patients with severe factor XI deficiency. *J Thromb Haemost*. 2014;12:1121–1130. doi: 10.1111/jth.12600.
61. Yakovlev S, Gorlatov S, Ingham K, Medved L. Interaction of fibrin(ogen) with heparin: further characterization and localization of the heparin-binding site. *Biochemistry*. 2003;42:7709–7716. doi: 10.1021/bi0344073.
62. Yeromonahos C, Marlu R, Polack B, Caton F. Antithrombin-independent effects of heparins on fibrin clot nanostructure. *Arterioscler Thromb Vasc Biol*. 2012;32:1320–1324. doi: 10.1161/ATVBAHA.112.245308.
63. Brummel-Ziedins KE, Wolberg AS. Global assays of hemostasis. *Curr Opin Hematol*. 2014;21:395–403. doi: 10.1097/MOH.0000000000000074.
64. Kalathottukaren MT, Abraham L, Kapopara PR, et al. Alteration of blood clotting and lung damage by protamine are avoided using the heparin and polyphosphate inhibitor UHRA [published online ahead of print December 29, 2016]. *Blood*. doi: 10.1182/blood-2016-10-747915. <http://www.bloodjournal.org/content/early/2016/12/28/blood-2016-10-747915?sso-checked=true>.
65. Whelihan MF, Zachary V, Orfeo T, Mann KG. Prothrombin activation in blood coagulation: the erythrocyte contribution to thrombin generation. *Blood*. 2012;120:3837–3845. doi: 10.1182/blood-2012-05-427856.
66. Peyrou V, Lormeau JC, Héroult JP, Gaich C, Pflieger AM, Herbert JM. Contribution of erythrocytes to thrombin generation in whole blood. *Thromb Haemost*. 1999;81:400–406.
67. Horne MK III, Cullinane AM, Merryman PK, Hoddeson EK. The effect of red blood cells on thrombin generation. *Br J Haematol*. 2006;133:403–408. doi: 10.1111/j.1365-2141.2006.06047.x.
68. Carvalho FA, Connell S, Miltenberger-Miltenyi G, Pereira SV, Tavares A, Ariens RA, Santos NC. Atomic force microscopy-based molecular recognition of a fibrinogen receptor on human erythrocytes. *ACS Nano*. 2010;4:4609–4620. doi: 10.1021/nn1009648.
69. Tutwiler V, Peshkova AD, Andrianova IA, Khasanova DR, Weisel JW, Litvinov RI. Contraction of blood clots is impaired in acute ischemic stroke. *Arterioscler Thromb Vasc Biol*. 2017;37:271–279. doi: 10.1161/ATVBAHA.116.308622.
70. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, Wroblewski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci USA*. 2010;107:15880–15885. doi: 10.1073/pnas.1005743107.
71. Diaz JA, Fuchs TA, Jackson TO, et al. Plasma DNA is elevated in patients with deep vein thrombosis. *J Vasc Surg Venous Lymphat Disord*. 2013;1:341–348.
72. Nouboussie DF, Whelihan MF, Yu YB, et al. In vitro activation of coagulation by human neutrophil DNA and histone proteins but not neutrophil extracellular traps [published online ahead of print December 5, 2016]. *Blood*. doi: 10.1182/blood-2016-06-722298. <http://www.bloodjournal.org/cgi/pmidlookup?view=long&pmid=27919911>.
73. Gould TJ, Vu TT, Swystun LL, Dwivedi DJ, Mai SH, Weitz JI, Liaw PC. Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms. *Arterioscler Thromb Vasc Biol*. 2014;34:1977–1984. doi: 10.1161/ATVBAHA.114.304114.
74. Gould TJ, Vu TT, Stafford AR, Dwivedi DJ, Kim PY, Fox-Robichaud AE, Weitz JI, Liaw PC. Cell-free DNA modulates clot structure and impairs fibrinolysis in sepsis. *Arterioscler Thromb Vasc Biol*. 2015;35:2544–2553. doi: 10.1161/ATVBAHA.115.306035.
75. Kowalska MA, Zhao G, Zhai L, David G III, Marcus S, Krishnaswamy S, Poncz M. Modulation of protein C activation by histones, platelet factor 4, and heparinoids: new insights into activated protein C formation. *Arterioscler Thromb Vasc Biol*. 2014;34:120–126. doi: 10.1161/ATVBAHA.113.302236.
76. Dwivedi DJ, Tolt LJ, Swystun LL, Pogue J, Liaw KL, Weitz JI, Cook DJ, Fox-Robichaud AE, Liaw PC; Canadian Critical Care Translational Biology Group. Prognostic utility and characterization of cell-free DNA in patients with severe sepsis. *Crit Care*. 2012;16:R151. doi: 10.1186/cc11466.
77. Chung DW, Davie EW. gamma and gamma' chains of human fibrinogen are produced by alternative mRNA processing. *Biochemistry*. 1984;23:4232–4236.
78. Fornace AJ Jr, Cummings DE, Comeau CM, Kant JA, Crabtree GR. Structure of the human gamma-fibrinogen gene. Alternate mRNA splicing near the 3' end of the gene produces gamma A and gamma B forms of gamma-fibrinogen. *J Biol Chem*. 1984;259:12826–12830.
79. Wolfenstein-Todel C, Mosesson MW. Human plasma fibrinogen heterogeneity: evidence for an extended carboxyl-terminal sequence in a normal gamma chain variant (gamma'). *Proc Natl Acad Sci USA*. 1980;77:5069–5073.
80. Francis CW, Marder VJ, Martin SE. Demonstration of a large molecular weight variant of the gamma chain of normal human plasma fibrinogen. *J Biol Chem*. 1980;255:5599–5604.
81. Lovely RS, Falls LA, Al-Mondhiry HA, Chambers CE, Sexton GJ, Ni H, Farrell DH. Association of gammaA/gamma' fibrinogen levels and coronary artery disease. *Thromb Haemost*. 2002;88:26–31.
82. Mannila MN, Lovely RS, Kazmierczak SC, Eriksson P, Samnegård A, Farrell DH, Hamsten A, Silveira A. Elevated plasma fibrinogen gamma' concentration is associated with myocardial infarction: effects of variation in fibrinogen genes and environmental factors. *J Thromb Haemost*. 2007;5:766–773. doi: 10.1111/j.1538-7836.2007.02406.x.
83. Cheung EY, Uitte de Willige S, Vos HL, Leebeek FW, Dippel DW, Bertina RM, de Maat MP. Fibrinogen gamma' in ischemic stroke: a case-control study. *Stroke*. 2008;39:1033–1035. doi: 10.1161/STROKEAHA.107.495499.
84. Appiah D, Schreiner PJ, MacLehose RF, Folsom AR. Association of plasma gamma' fibrinogen with incident cardiovascular disease: The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol*. 2015;35:2700–2706. doi: 10.1161/ATVBAHA.115.306284.
85. Uitte de Willige S, de Visser MC, Houwing-Duistermaat JJ, Rosendaal FR, Vos HL, Bertina RM. Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen gamma' levels. *Blood*. 2005;106:4176–4183.
86. Mosesson MW, Hernandez I, Raife TJ, Medved L, Yakovlev S, Simpson-Haidaris PJ, Uitte DE, Willige S, Bertina RM. Plasma fibrinogen gamma' chain content in the thrombotic microangiopathy syndrome. *J Thromb Haemost*. 2007;5:62–69. doi: 10.1111/j.1538-7836.2006.02270.x.
87. Collet JP, Nagaswami C, Farrell DH, Montalescot G, Weisel JW. Influence of gamma' fibrinogen splice variant on fibrin physical properties and fibrinolysis rate. *Arterioscler Thromb Vasc Biol*. 2004;24:382–386. doi: 10.1161/01.ATV.0000109748.77727.3e.
88. Allan P, Uitte de Willige S, Abou-Saleh RH, Connell SD, Ariens RA. Evidence that fibrinogen gamma' directly interferes with profibrin growth: implications for fibrin structure and clot stiffness. *J Thromb Haemost*. 2012;10:1072–1080. doi: 10.1111/j.1538-7836.2012.04717.x.
89. Fredenburgh JC, Stafford AR, Leslie BA, Weitz JI. Bivalent binding to gammaA/gamma'-fibrin engages both exosites of thrombin and protects it from inhibition by the antithrombin-heparin complex. *J Biol Chem*. 2008;283:2470–2477. doi: 10.1074/jbc.M707710200.
90. Meh DA, Siebenlist KR, Brennan SO, Holyst T, Mosesson MW. The amino acid sequence in fibrin responsible for high affinity thrombin binding. *Thromb Haemost*. 2001;85:470–474.
91. Lovely RS, Rein CM, White TC, Jouihan SA, Boshkov LK, Bakke AC, McCarty OJ, Farrell DH. gammaA/gamma' fibrinogen inhibits thrombin-induced platelet aggregation. *Thromb Haemost*. 2008;100:837–846.

92. Cooper AV, Standeven KF, Ariëns RA. Fibrinogen gamma-chain splice variant gamma' alters fibrin formation and structure. *Blood*. 2003;102:535–540. doi: 10.1182/blood-2002-10-3150.
93. Lovely RS, Boshkov LK, Marzec UM, Hanson SR, Farrell DH. Fibrinogen gamma' chain carboxy terminal peptide selectively inhibits the intrinsic coagulation pathway. *Br J Haematol*. 2007;139:494–503. doi: 10.1111/j.1365-2141.2007.06825.x.
94. Muthard RW, Welsh JD, Brass LF, Diamond SL. Fibrin,  $\gamma'$ -fibrinogen, and transclot pressure gradient control hemostatic clot growth during human blood flow over a collagen/tissue factor wound. *Arterioscler Thromb Vasc Biol*. 2015;35:645–654. doi: 10.1161/ATVBAHA.114.305054.
95. Mosesson MW, Cooley BC, Hernandez I, Diorio JP, Weiler H. Thrombosis risk modification in transgenic mice containing the human fibrinogen thrombin-binding gamma' chain sequence. *J Thromb Haemost*. 2009;7:102–110. doi: 10.1111/j.1538-7836.2008.03213.x.
96. Walton BL, Getz TM, Bergmeier W, Lin FC, Uitte de Willige S, Wolberg AS. The fibrinogen  $\gamma A/\gamma'$  isoform does not promote acute arterial thrombosis in mice. *J Thromb Haemost*. 2014;12:680–689. doi: 10.1111/jth.12534.
97. Katona E, Péntzes K, Csapó A, Fazakas F, Udvardy ML, Bagoly Z, Orosz ZZ, Muszbek L. Interaction of factor XIII subunits. *Blood*. 2014;123:1757–1763. doi: 10.1182/blood-2013-10-533596.
98. Fickenscher K, Aab A, Stüber W. A photometric assay for blood coagulation factor XIII. *Thromb Haemost*. 1991;65:535–540.
99. Aleman MM, Byrnes JR, Wang JG, Tran R, Lam WA, Di Paola J, Mackman N, Degen JL, Flick MJ, Wolberg AS. Factor XIII activity mediates red blood cell retention in venous thrombi. *J Clin Invest*. 2014;124:3590–3600. doi: 10.1172/JCI75386.
100. Byrnes JR, Wilson C, Boutelle AM, Brandner CB, Flick MJ, Philippou H, Wolberg AS. The interaction between fibrinogen and zymogen FXIII-A2B2 is mediated by fibrinogen residues  $\gamma$ 390-396 and the FXIII-B subunits. *Blood*. 2016;128:1969–1978. doi: 10.1182/blood-2016-04-712323.
101. Smith KA, Adamson PJ, Pease RJ, Brown JM, Balmforth AJ, Cordell PA, Ariëns RA, Philippou H, Grant PJ. Interactions between factor XIII and the alphaC region of fibrinogen. *Blood*. 2011;117:3460–3468. doi: 10.1182/blood-2010-10-313601.
102. Rijken DC, Abdul S, Malfliet JJ, Leebeek FW, Uitte de Willige S. Compaction of fibrin clots reveals the antifibrinolytic effect of factor XIII. *J Thromb Haemost*. 2016;14:1453–1461. doi: 10.1111/jth.13354.
103. Reed GL, Houg AK, Wang D. Microvascular thrombosis, fibrinolysis, ischemic injury, and death after cerebral thromboembolism are affected by levels of circulating  $\alpha$ 2-antiplasmin. *Arterioscler Thromb Vasc Biol*. 2014;34:2586–2593. doi: 10.1161/ATVBAHA.114.304530.
104. Lisman T, de Moerloose P. The role of fibrinogen and factor XIII in hemostasis, and the identification and treatment of associated disorders. *Semin Thromb Hemost*. 2016;42:331–332. doi: 10.1055/s-0036-1579656.
105. Schroeder V, Kohler HP. Factor XIII: structure and function. *Semin Thromb Hemost*. 2016;42:422–428. doi: 10.1055/s-0036-1571341.
106. Komáromi I, Bagoly Z, Muszbek L. Factor XIII: novel structural and functional aspects. *J Thromb Haemost*. 2011;9:9–20. doi: 10.1111/j.1538-7836.2010.04070.x.
107. Byrnes JR, Duval C, Wang Y, Hansen CE, Ahn B, Mooberry MJ, Clark MA, Johnsen JM, Lord ST, Lam WA, Meijers JC, Ni H, Ariëns RA, Wolberg AS. Factor XIIIa-dependent retention of red blood cells in clots is mediated by fibrin  $\alpha$ -chain crosslinking. *Blood*. 2015;126:1940–1948. doi: 10.1182/blood-2015-06-652263.
108. Lim BC, Ariëns RA, Carter AM, Weisel JW, Grant PJ. Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. *Lancet*. 2003;361:1424–1431. doi: 10.1016/S0140-6736(03)13135-2.
109. Duval C, Ali M, Chaudhry WW, Ridger VC, Ariëns RA, Philippou H. Factor XIII A-subunit V34L variant affects thrombus cross-linking in a Murine model of thrombosis. *Arterioscler Thromb Vasc Biol*. 2016;36:308–316. doi: 10.1161/ATVBAHA.115.306695.
110. Tutwiler V, Litvinov RI, Lozhkin AP, Peshkova AD, Lebedeva T, Ataullakhanov FI, Spiller KL, Cines DB, Weisel JW. Kinetics and mechanics of clot contraction are governed by the molecular and cellular composition of the blood. *Blood*. 2016;127:149–159. doi: 10.1182/blood-2015-05-647560.
111. Collet JP, Montalescot G, Lesty C, Soria J, Mishal Z, Thomas D, Soria C. Disaggregation of *in vitro* preformed platelet-rich clots by abciximab increases fibrin exposure and promotes fibrinolysis. *Arterioscler Thromb Vasc Biol*. 2001;21:142–148.
112. Undas A, Zalewski J, Krochin M, Siudak Z, Sadowski M, Pregowski J, Dudek D, Janion M, Witkowski A, Zmudka K. Altered plasma fibrin clot properties are associated with in-stent thrombosis. *Arterioscler Thromb Vasc Biol*. 2010;30:276–282. doi: 10.1161/ATVBAHA.109.194936.
113. Scott DJ, Prasad P, Philippou H, Rashid ST, Sohrabi S, Whalley D, Kordowicz A, Tang Q, West RM, Johnson A, Woods J, Ajjan RA, Ariëns RA. Clot architecture is altered in abdominal aortic aneurysms and correlates with aneurysm size. *Arterioscler Thromb Vasc Biol*. 2011;31:3004–3010. doi: 10.1161/ATVBAHA.111.236786.
114. Casini A, Neerman-Arbez M, Ariëns RA, de Moerloose P. Dysfibrinogenemia: from molecular anomalies to clinical manifestations and management. *J Thromb Haemost*. 2015;13:909–919. doi: 10.1111/jth.12916.
115. Hoffman M. Alterations of fibrinogen structure in human disease. *Cardiovasc Hematol Agents Med Chem*. 2008;6:206–211.
116. Kannel WB, D'Agostino RB, Belanger AJ. Fibrinogen, cigarette smoking, and risk of cardiovascular disease: insights from the Framingham Study. *Am Heart J*. 1987;113:1006–1010.
117. McEvoy JW, Nasir K, DeFilippis AP, Lima JA, Bluemke DA, Hundley WG, Barr RG, Budoff MJ, Szklo M, Navas-Acien A, Polak JF, Blumenthal RS, Post WS, Blaha MJ. Relationship of cigarette smoking with inflammation and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2015;35:1002–1010. doi: 10.1161/ATVBAHA.114.304960.
118. Barua RS, Sy F, Srikanth S, Huang G, Javed U, Buhari C, Margosan D, Ambrose JA. Effects of cigarette smoke exposure on clot dynamics and fibrin structure: an ex vivo investigation. *Arterioscler Thromb Vasc Biol*. 2010;30:75–79. doi: 10.1161/ATVBAHA.109.195024.
119. Hugenholtz GC, Macrae F, Adelmeijer J, Dulfer S, Porte RJ, Lisman T, Ariëns RA. Procoagulant changes in fibrin clot structure in patients with cirrhosis are associated with oxidative modifications of fibrinogen. *J Thromb Haemost*. 2016;14:1054–1066. doi: 10.1111/jth.13278.
120. Becatti M, Marcucci R, Bruschi G, Taddei N, Bani D, Gori AM, Giusti B, Gensini GF, Abbate R, Fiorillo C. Oxidative modification of fibrinogen is associated with altered function and structure in the subacute phase of myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2014;34:1355–1361. doi: 10.1161/ATVBAHA.114.303785.
121. Martinez J, MacDonald KA, Palascak JE. The role of sialic acid in the dysfibrinogenemia associated with liver disease: distribution of sialic acid on the constituent chains. *Blood*. 1983;61:1196–1202.
122. Schuett K, Savvaidis A, Maxeiner S, et al. Clot structure: a potent mortality risk factor in patients on hemodialysis [published online ahead of print January 5, 2017]. *J Am Soc Nephrol*. doi: 10.1681/ASN.2016030336. <http://jasn.asnjournals.org/cgi/lookup?view=long&pmid=28057772>.
123. Kahn SR, Lim W, Dunn AS, et al. Prevention of VTE in nonsurgical patients: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141(suppl 2):e195S–226S.
124. Wolberg AS, Rosendaal FR, Weitz JI, Jaffer IH, Agnelli G, Baglin T, Mackman N. Venous thrombosis. *Nat Rev Dis Primers*. 2015;1:15006. doi: 10.1038/nrdp.2015.6.
125. Wakefield TW, Myers DD, Henke PK. Mechanisms of venous thrombosis and resolution. *Arterioscler Thromb Vasc Biol*. 2008;28:387–391. doi: 10.1161/ATVBAHA.108.162289.
126. Patterson BO, Hinchliffe R, Loftus IM, Thompson MM, Holt PJ. Indications for catheter-directed thrombolysis in the management of acute proximal deep venous thrombosis. *Arterioscler Thromb Vasc Biol*. 2010;30:669–674. doi: 10.1161/ATVBAHA.109.200766.
127. Blasi F, Oliveira BL, Rietz TA, Rotile NJ, Naha PC, Cormode DP, Izquierdo-Garcia D, Catana C, Caravan P. Multisite thrombus imaging and fibrin content estimation with a single whole-body PET scan in rats. *Arterioscler Thromb Vasc Biol*. 2015;35:2114–2121. doi: 10.1161/ATVBAHA.115.306055.
128. Andia ME, Saha P, Jenkins J, Modarai B, Wiethoff AJ, Phinikaridou A, Grover SP, Patel AS, Schaeffter T, Smith A, Botnar RM. Fibrin-targeted magnetic resonance imaging allows *in vivo* quantification of thrombus fibrin content and identifies thrombi amenable for thrombolysis. *Arterioscler Thromb Vasc Biol*. 2014;34:1193–1198. doi: 10.1161/ATVBAHA.113.302931.

# Arteriosclerosis, Thrombosis, and Vascular Biology



JOURNAL OF THE AMERICAN HEART ASSOCIATION

## **Fibrinogen and Fibrin in Hemostasis and Thrombosis** Sravya Kattula, James R. Byrnes and Alisa S. Wolberg

*Arterioscler Thromb Vasc Biol.* 2017;37:e13-e21

doi: 10.1161/ATVBAHA.117.308564

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272  
Greenville Avenue, Dallas, TX 75231

Copyright © 2017 American Heart Association, Inc. All rights reserved.

Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the  
World Wide Web at:

<http://atvb.ahajournals.org/content/37/3/e13>

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

**Reprints:** Information about reprints can be found online at:  
<http://www.lww.com/reprints>

**Subscriptions:** Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:  
<http://atvb.ahajournals.org/subscriptions/>