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Intrinsic Pathway of Coagulation and Thrombosis
Insights From Animal Models

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Abstract—Activation of the intrinsic pathway of coagulation contributes to the pathogenesis of arterial and venous thrombosis. Critical insights into the involvement of intrinsic pathway factors have been derived from the study of gene-specific knockout animals and targeted inhibitors. Importantly, preclinical studies have indicated that targeting components of this pathway, including FXI (factor XI), FXII, and PKK (prekallikrein), reduces thrombosis with no significant effect on protective hemostatic pathways. This review highlights the advances made from studying the intrinsic pathway using gene-specific knockout animals and inhibitors in models of arterial and venous thrombosis. Development of inhibitors of activated FXI and FXII may reduce thrombosis with minimal increases in bleeding compared with current anticoagulant drugs.

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The vertebrate coagulation system is essential for the maintenance of a closed high-pressure circulatory system.[1] Appropriate activation of coagulation in response to vascular injury is required for effective hemostasis that facilitates the cessation of bleeding. Primary hemostasis is initiated by accumulation and activation of platelets at the site of vascular injury.[2] During secondary hemostasis, activation of coagulation reinforces the platelet plug through deposition of an insoluble fibrin network.[2] Aberrant activation of coagulation can, however, lead to the formation of intravascular clots that underpin pathological thrombotic disorders, including myocardial infarction, stroke, and venous thromboembolism.[3]

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There are 2 primary pathways for the initiation of coagulation that converge at FX (factor X; Figure). In the extrinsic pathway exposure of subendothelial TF (tissue factor) complexed with activated FVII, known as the extrinsic Xase, catalyzes the generation of FXa (activated FX).[4] In the intrinsic pathway initially described by Davie and Ratnoff[5] and MacFarlane,[6] a waterfall-based model involving the sequential activation of FXII, FXI, and FIX leads to formation of the intrinsic Xase, a complex of FVIIIa (activated factor VIII) and FIXa (activated factor IX), that also catalyzes the generation of FXa. FXa in complex with its cofactor FVa forms the prothrombinase complex that catalyzes the cleavage of prothrombin (FII) to thrombin (FIIa). Thrombin as the terminal coagulation protease catalyzes the formation of insoluble fibrin through cleavage of soluble fibrinogen monomers and activation of the transglutaminase FXIII (Figure). In addition, thrombin is a potent activator of platelets through cleavage of cell surface protease-activated receptors.

In this review, we discuss the involvement of intrinsic pathway activation in the pathogenesis of thrombosis with a particular focus on insights gained from animal models. We further elaborate on the development of intrinsic pathway inhibitors and their potential utility as antithrombotic agents.

Regulation of Intrinsic Pathway Activation

FXII can readily be activated by exposure to negatively charged molecules and surfaces. Early studies revealed that incubation of FXII with negatively charged molecules, including dextran sulfate and silica, was sufficient to induce autoactivation.[7–9] More recently, a number of physiological surfaces have been identified that mediate autoactivation of FXII, including RNA, DNA, and polyphosphate.[10–13] It is interesting to consider, however, if other sources of negative surface, such as the plasma membrane of activated platelets, could also provide an abundant surface for FXII autoactivation.[14]

In addition to serving as a critical component of the intrinsic pathway, FXII is also a central component of the contact system that includes PKK (prekallikrein) and HK (high molecular weight kininogen). Activation of the intrinsic pathway can be enhanced by the actions of both PKK and

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Nonstandard Abbreviations and Acronyms	
αKK	α-kallikrein
ASO	antisense oligonucleotides
FXa	activated FX
FXI	factor XI
HK	high molecular weight kininogen
IL	interleukin
IVC	inferior vena cava
PKK	prekallikrein
siRNA	small interfering RNA
TF	tissue factor

HK. During the process of contact activation, initially generated FXIIa can activate PKK forming αKK (α-kallikrein) that can itself activate FXII establishing a positive feedback loop. Critically, αKK mediated activation of FXII is ≈30× more efficient than autoactivation of FXII on a negative surface.[7] The reciprocal activation of FXII and PKK is further amplified through the cofactor activity of HK.[15,16] Interestingly, single chain FXII zymogen that has not undergone limited proteolysis, possesses low proteolytic activity and can activate PKK in a process that is enhanced by polyphosphate.[17,18]

The intrinsic pathway can also be activated by components of the extrinsic and common pathways. Cross-activation is thought to be important for the sustained activation of coagulation as activity of the TF;FVIIa complex is inhibited by tissue factor pathway inhibitor.[19] The work of Josso and others described the capacity of the TF;FVIIa complex to efficiently activate FIX.[20,21] Consistent with the importance of this pathway in the activation of the clotting cascade, a mutation in FIX that limits activation by TF;FVIIa, but not FXIa, is associated with a mild form of hemophilia.[22] More recently, it has been shown that the TF;FVIIa and TF;FVIIa;FXa complexes

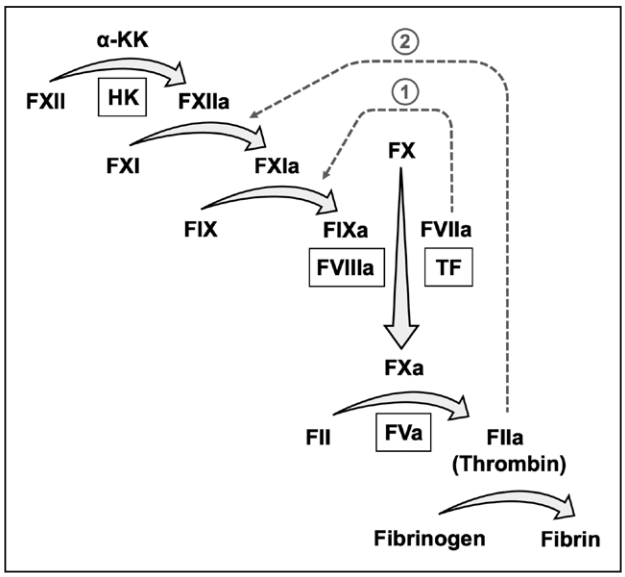

Figure. Model of the intrinsic pathway. A schematic overview of the intrinsic and extrinsic pathways of coagulation. Dotted lines represent proposed amplificatory pathways that involve crosstalk between components of the extrinsic or common pathway and the intrinsic pathway. αKK indicates α-kallikrein; F, factor; HK, high molecular weight kininogen; and TF, tissue factor.

can also activate FVIII.[23,24] Through this mechanism, the extrinsic Xase, TF;FVIIa, and product FXa can directly promote formation of the active intrinsic Xase, FVIIIa;FIXa. The work of Gailani and others has revealed that the terminal coagulation protease thrombin can also feedback activate FXI leading to a marked amplification of thrombin generation.[25–27] Interestingly, polyphosphate strongly enhances thrombin-mediated activation of FXI.[28] It has, however, been reported that the major physiological target of thrombin, fibrinogen, may limit the ability of thrombin to activate FXI in plasma-based systems.[29,30] Although described extensively in vitro, it remains to be determined if TF:FVIIa-mediated activation of FIX, TF;FVIIa;FXa mediated activation of FVIII, and thrombin-mediated activation of FXI play important physiological roles in vivo.

Arterial Thrombosis in Animal Models

Studies of different gene-specific knockout mice have provided considerable insight into the contribution of intrinsic pathway activation to arterial thrombosis (Table 1). Deletion of FIX in mice was found to reduce arterial thrombus formation in the carotid artery ferric chloride model but was associated with markedly increased bleeding consistent with the phenotype of patients with hemophilia B.[32] FXI-deficient mice were protected from arterial thrombosis in the carotid artery ferric chloride model without altering tail bleeding times.[32,34] Interestingly, FXI deficiency did not confer protection from arterial thrombosis in a mouse ear laser injury model.[34] Loss of FXII provided protection against thrombosis in both mesenteric artery and carotid artery ferric chloride models of thrombosis.[33,37–39] In addition, rats deficient for FXII are also protected against thrombosis in the carotid artery ferric chloride model.[38] Importantly, FXII knockouts do not have prolonged bleeding times in the tail transection or cuticle bleeding models.[37,38]

Components of the contact system that contribute to the activation of FXII also have phenotypes in models of arterial thrombosis. Deletion of the *Kng1* gene, required for plasma borne HK, was associated with reduced thrombus formation in the carotid artery Rose Bengal light-dye injury model of thrombosis.[42] Similarly, PKK-deficient mice had reduced thrombus formation in both the carotid artery ferric chloride and Rose Bengal light-dye injury models.[40,41] Although PKK serves as an important activator of FXII, studies of PKK-deficient mice indicate that there may be other FXII-independent contributions of PKK to arterial thrombosis.[41] Deletion of PKK was found to suppress bradykinin signaling, which resulted in compensatory upregulation of Mas receptor expression that under conditions of normal levels of angiotensin II lead to increased prostacyclin generation.[41] Prostacyclin exhibits direct antithrombotic effects acting as a potent inhibitor of platelet aggregation and indirect effects through Sirtuin 1 and Kruppel-like factor 4 dependent suppression of TF expression.[41,43,44]

It has proven challenging to compare results between intrinsic pathway knockouts due to the differing models and conditions used to initiate arterial thrombosis. Importantly, comprehensive assessments of intrinsic pathway knockouts have been conducted in the carotid artery ferric chloride model

Table 1. Intrinsic Pathway Knockouts and Thrombosis

Protein	Gene	Arterial Thrombosis	Phenotype	Reference	Venous Thrombosis	Phenotype	Reference
FVIII	*F8*	...			St Thomas' model (Mu)	↓	Singh et al[31]
FIX	*F9*	Carotid $FeCl_3$ (Mu) Carotid $FeCl_3$ (Rat)	↓ ↓	Wang et al,[32] Cheng et al[33]	...		
FXI	*F11*	Carotid $FeCl_3$ (Mu) Ear arteriole laser (Mu)	↓ NC	Wang et al,[32] Rosen et al,[34] Cheng et al[33]	IVC $FeCl_3$ (Mu) IVC Stenosis (Mu)	↓ ↓	Wang et al,[35] von Brühl et al[36]
FXII	*F12*	Carotid $FeCl_3$ (Mu) Mesenteric $FeCl_3$ (Mu) Carotid $FeCl_3$ (Rat)	↓ ↓ ↓	Renné et al,[37] Cai et al,[38] Cheng et al,[33] Kokoye et al[39]	IVC Stenosis (Mu)	↓	von Brühl et al[36]
PKK	*Klkb1*	Carotid $FeCl_3$ (Mu) Carotid rose bengal (Mu)	↓ ↓	Kokoye et al,[39] Bird et al,[40] Stavrou et al[41]	...		
HK	*Kng1*	Carotid $FeCl_3$ (Mu) Carotid rose bengal (Mu)	↓ ↓	Kokoye et al,[39] Merkulov et al[42]	...		

F indicates factor; HK, high molecular weight kininogen; IVC, inferior vena cava; Mu, murine; NC, no change; and PKK, prekallikrein.

in which vascular injury was initiated using a range of ferric chloride concentrations.[33,39] Interestingly, a moderately stronger level of thromboprotection was observed in FXII-deficient mice than either FXI- or FIX-deficient mice.[33] These findings suggest that there may be FIX and FXI-independent functions of FXII. A stronger level of thromboprotection was also observed in FXII-deficient mice when compared with PPK- and HK-deficient mice.[39] This data suggests that PPK independent FXII autoactivation occurs during arterial thrombosis and that PPK and HK likely support further FXIIa generation. These findings are also consistent with the differential prolongation of activated partial thromboplastin times observed in patients with congenital intrinsic pathway deficiencies. A deficiency of FXII is associated with longer prolongation of activated partial thromboplastin time (aPTT) than that for FXI or PKK deficiency.

The observed protection of intrinsic pathway knockouts from arterial thrombosis has led to the evaluation of numerous inhibitors of this pathway in models of thrombosis (Table 2). A variety of inhibitory strategies have been used, including function-blocking antibodies, antisense oligonucleotides (ASO), small interfering RNAs (siRNAs), and small molecules. The anti-FXI antibody 14E11 that selectively prevents activation of FXI by FXIIa, but not thrombin, inhibited thrombus formation in the carotid artery ferric chloride model providing and almost equivalent strength of phenotype as FXI-deficient mice.[33] This finding suggests that FXIIa is the primary driver of FXI activation. More recently, the anti-FXIa antibody C24 was found to dose-dependently inhibit thrombus formation in the carotid artery ferric chloride model.[47] The antithrombotic potential of a number of FXII-targeting antibodies has also been assessed. The anti-FXII antibodies 15H8 and 9A2 inhibited thrombus formation in the carotid ferric chloride model.[53] Additionally, an antibody that binds specifically to FXIIa, but not FXII, 3F7, inhibited thrombus formation in the carotid artery ferric chloride model.[54]

Nucleotide-based gene silencing therapies have also been developed against components of the intrinsic pathway. Administration of FIX-targeted siRNA at doses that reduced FIX activity by ≈90% inhibited arterial thrombosis in a rat carotid artery ferric chloride.[45] Surprisingly, siRNA-mediated FIX gene knockdown was not associated with increased bleeding suggesting a differential threshold in the amount of FIX required to promote hemostasis versus thrombosis.[45] A FXI-targeting ASO administered at a dose that reduced FXI activity by ≈80% reduced thrombosis in an aortic ferric chloride model.[51] Similarly, ASOs against FXII and PKK that reduced levels of their respective target proteins by ≈80% also inhibited thrombosis formation in a mesenteric arteriole ferric chloride model.[52] Interestingly, the antithrombotic effect of FXII-targeted ASO was maintained at lower doses than for PKK-targeted ASO.[52] This finding is consistent with the observation that FXII knockouts demonstrate more robust protection than PKK knockouts in the carotid artery ferric chloride model.[39] Complementary findings have been made with FXII-targeting siRNA administration resulting in a dose-dependent reduction in thrombus formation in a rat carotid artery ferric chloride model.[38] Although a mild prolongation in cuticle bleeding time was observed after FXII knockdown off-target effects of the FXII-targeting siRNA could not be excluded.[38]

A small molecule irreversible inhibitor of FXIa, BMS-262084, reduced thrombus formation in a rat carotid artery ferric chloride model and a rabbit carotid artery electrolytic injury model.[48,49] Likewise, a small molecule reversible and competitive inhibitor of FXIa, BMS-654457, also inhibited thrombus formation in the rabbit carotid artery electrolytic injury model in a dose-dependent manner.[50] Although other FXI small molecule inhibitors have been developed, the in vivo antithrombotic effects of these agents have not yet been reported.[61] A small peptide-based inhibitor of FXIIa, Phe-Pro-Arg-chloromethylketone, reduced thrombus formation in the carotid ferric chloride model.[12] A protein-based FXIIa inhibitor has also been developed from the fourth domain of the hematophagus insect *Triatoma infestans* protein infestin, named infestin 4.[62] An albumin conjugated derivative of infestin 4, rHA-infestin 4 (recombinant human albumin conjugated infestin 4), inhibited thrombus formation in the carotid

Table 2. Inhibitors Targeting the Intrinsic Pathway

Agent	Description	Arterial Thrombosis	Phenotype	Reference	Venous Thrombosis	Phenotype	Reference
LE2E9	anti-FVIII antibody	...			IVC St Thomas' (Mu)	↓	Singh et al[31]
FIX siRNA	Small interfering RNA	Carotid $FeCl_3$ (Rat)	↓	Metzger et al[45]	...		
14E11	Anti-FXI antibody	Carotid $FeCl_3$ (Mu)	↓	Cheng et al[33]	...		
FXI-175	Anti-FXI antibody	...			IVC $FeCl_3$ (Mu)	↓	van Montfoort et al[46]
FXI-203	Anti-FXI antibody	...			IVC $FeCl_3$ (Mu)	↓	van Montfoort et al[46]
C24	Anti-FXIa antibody	Carotid $FeCl_3$ (Mu)	↓	David et al[47]	Femoral thread (Rab)	↓	David et al[47]
BMS-262084	Small molecule FXIa inhibitor	Carotid $FeCl_3$ (Rat); Carotid EIM (Rab)	↓ ↓	Schumacher et al,[48] Wong et al[49]	...		
BMS-654457	Small molecule FXIa inhibitor	Carotid EIM (Rab)	↓	Wong et al[50]	...		
FXI ASO	Antisense oligonucleotide	Aortic $FeCl_3$ (Mu) Plaque Rupture (Mu)	↓ ↓	Zhang et al[51]	IVC St Thomas' (Mu) IVC $FeCl_3$ (Mu) Mesenteric $FeCl_3$ (Mu)	↓ ↓ ↓	Schumacher et al,[51] Revenko et al[52]
15H8	Anti-FXII antibody	Carotid $FeCl_3$ (Mu)	↓	Matafonov et al[53]	...		
9A2	anti-FXII antibody	Carotid $FeCl_3$ (Mu)	↓	Matafonov et al[53]	...		
3F7	Anti-FXIIa antibody	Carotid $FeCl_3$ (Mu)	↓	Larsson et al[54]	...		
rHA-Infestin 4	Protein-based FXIIa inhibitor	Mesenteric $FeCl_3$ (Mu) Carotid $FeCl_3$ (Mu) Femoral $FeCl_3$ (Rab)	↓ ↓ ↓	Hagedorn et al,[55] Barberry et al,[56] May et al,[57] Kuijpers et al[58]	Jugular ligation (Rab)	↓	May et al[57]
CTI	Small molecule FXIIa inhibitor	Plaque Rupture	↓	Kuijpers et al,[58] van Montfoort et al[59]			
PCK	Peptide FXIIa inhibitor	Carotid $FeCl_3$ (Mu)	↓	Kannemeier et al[12]	IVC Stenosis (Mu)	↓	von Brühl et al[36]
FXII ASO	Antisense oligonucleotide	Mesenteric $FeCl_3$ (Mu)	↓	Revenko et al[52]	IVC St Thomas' (Mu) IVC $FeCl_3$ (Mu)	↓ ↓	Revenko et al[52]
FXII siRNA	Small interfering RNA	Carotid $FeCl_3$ (Rat)		Cai et al[38]	...		
PKK ASO	Antisense oligonucleotide	Mesenteric $FeCl_3$ (Mu)	↓	Revenko et al[52]	IVC St Thomas' (Mu) IVC $FeCl_3$ (Mu)	↓ ↓	Revenko et al[52]
Ir-CPI	Protein-based FXIa/FXIIa/ PKK inhibitor	...			IVC Stasis (Rat) IVC $FeCl_3$ (Mu)	↓ ↓	Decrem et al[60]

ASO indicates antisense oligonucleotides; CTI, corn trypsin inhibitor; EIM, electrolytic injury model; F, factor; Ir-CPI, *Ixodes ricinus* contact phase inhibitor; rHA-infestin 4, recombinant human albumin conjugated infestin 4; IVC, inferior vena cava; Mu, murine; PKK, prekallikrein; PCK, Phe-Pro-Arg-chloromethylketone; and Rab, rabbit.

artery, femoral artery, and mesenteric arteriole ferric chloride models.[55–57] It is important to note that rHA-infestin 4 inhibits plasmin and FXa activity at higher concentrations.[63]

The effects of intrinsic pathway inhibition have also been explored in a model that more closely resembles human atherothrombotic disease. In this model, plaques in the carotid artery of western diet treated *Apoe*$^{-/-}$ mice are physically disrupted by ultrasound and the resultant thrombus formation monitored by intravital fluorescence video microscopy.[64] In this model, inhibition of FXIIa by administration of corn trypsin inhibitor, a FXIIa inhibitor purified from Indiana sweet corn, or rHA-infestin 4 reduced accumulation of platelets at the site of plaque disruption.[58] Similarly, gene-mediated silencing of FXI expression by targeted ASOs resulted in a modest but significant attenuation in the accumulation of platelets and fibrin at the site of plaque disruption.[59]

Several studies have also explored the contribution of intrinsic pathway activation to the thromboinflammatory response evoked by ischemia reperfusion injury. PKK, FXII, and FXI knockout mice demonstrated reduced cerebral infarction in a transient midcerebral artery occlusion stroke model.[65,66] In this same model, inhibition of FXII with Phe-Pro-Arg-chloromethylketone or rHA-infestin 4 and FXII mediated activation of FXI with the 14E11 antibody protecting against cerebral infarction.[65,67,68] In a murine microembolic injection stroke model inhibition of FXII with rHA-infestin 4

also reduced the severity of cerebral infarction.[69] Inhibition of the intrinsic pathway was consistently associated with reduced fibrin deposition in infarcted regions of the brain suggesting that the antithrombotic potential of these agents may contribute to the observed phenotype; however, anti-inflammatory effects cannot be discounted. Indeed, PKK knockouts demonstrated reduced expression of the inflammatory cytokine IL (interleukin) 1β in the cortex and basal ganglia after transient midcerebral artery occlusion.[66]

Findings made with intrinsic pathway inhibitors provide important complementary evidence to those made using gene knockout mice. It is possible that constitutive gene deletion may provide a strong selective drive for compensatory changes that could mask the phenotype of intrinsic pathway knockouts. However, the strong agreement between these 2 approaches in models of arterial thrombosis suggests that the effect of gene compensation is limited.

Venous Thrombosis in Animal Models

Studies of intrinsic pathway knockout mice indicate that this pathway also plays a major role in the formation of thrombi in the venous system (Table 1). FVIII-deficient mice were found to be completely protected from venous thrombosis in the St Thomas' model.[31] In the St Thomas' model, a combination of reduced flow and endothelial injury are used to initiate thrombus formation in the infrarenal inferior vena cava (IVC).[31] Deletion of FXI resulted in reduced thrombus formation in the IVC ferric chloride model.[35] Interestingly, at low doses of ferric chloride, a more robust inhibition of thrombus formation was observed in FXI-deficient mice than therapeutic doses of heparin, clopidogrel, and agatroban.[35] Mice deficient for FXII demonstrated reduced thrombus burden in the IVC stenosis model.[36] Thrombus formation in the IVC stenosis model is induced by reduced flow in the absence of additional thrombotic stimuli. Surprisingly, in the same study, no significant reduction in thrombus burden was observed in FXI-deficient mice, suggesting a potential FXI-independent function of FXII in venous thrombosis.[36] This finding is consistent with observations made in the carotid artery ferric chloride model.[33] Interestingly, the fibrin density of thrombi from FXII-deficient mice was significantly lower than thrombi from controls.[36] FXII binds directly to fibrinogen and increases fibrin network density independent of thrombin generation providing a potential FXI-independent procoagulant function of FXII.[70] In line with the role of PKK in the activation of FXII, *Klkb1* knockout mice also demonstrated reduced venous thrombosis in the IVC ferric chloride model.[40]

Inhibitors targeting the intrinsic pathway have been assessed for their ability to limit venous thrombosis in animal models (Table 2). An anti-FVIII antibody, LE2E9, potently inhibited thrombus formation in wild-type and FVIII humanized mice subject to the St Thomas' model.[31] Interestingly, in contrast to the severe bleeding phenotype of FVIII-deficient mice, bleeding in LE2E9-treated mice was comparable to that of controls.[31] In a rabbit femoral vein thread–induced model, a blocking antibody that binds to the enzymatic pocket of FXIa, referred to as DEF, reduced thrombus formation in a dose-dependent manner.[47] Importantly, DEF was not associated with increased bleeding in a rabbit cuticle model.[47] A pair of anti-FXI antibodies, αFXI-175 and αFXI-203, have also been found to significantly prolong time to occlusion in the IVC ferric chloride model.[46] An ASO targeting FXI robustly inhibited thrombus formation in the IVC ferric chloride, mesenteric vein ferric chloride, and St Thomas' models without an associated bleeding phenotype.[51] ASOs targeting FXII and PKK also demonstrated reduced thrombus formation in the IVC ferric chloride models and St Thomas' without affecting bleeding.[52] Interestingly, in this study, knockdown of PKK resulted in a gradual dose-dependent reduction in thrombus formation in the St Thomas' model. In contrast, FXII knockdown demonstrated a steep dose-response with significant reduction in thrombus formation only observed at FXII ASO doses that reduced the plasma FXII concentration by >80%.[52] This suggests that only relatively small amounts of FXII are required to promote venous thrombosis in this model. The FXIIa inhibitor, Phe-Pro-Arg-chloromethylketone, was also reported to reduce thrombus formation in the IVC stenosis model.[36] Similarly, rHA-infestin 4 markedly reduced the formation of occlusive thrombi in the IVC ferric chloride model.[57] A tick derived protein *Ixodes ricinus* contact phase inhibitor has been identified as a broad-acting antagonist against FXIa, FXIIa, and αKK.[60] *Ixodes ricinus* contact phase inhibitor administration reduced thrombus formation in both the rat IVC stasis and mouse IVC ferric chloride models at doses that did not affect bleeding after tail vein transection.[60]

Primate Models

In an attempt to bridge the gap between rodents and humans, numerous intrinsic pathway inhibitors have been assessed in a baboon model of graft thrombosis. In this model, a collagen-coated arteriovenous shunt serves as a thrombogenic surface with propagation facilitated by the presence of an uncoated region or expansion chamber.[53] Initiation of thrombus formation in response to collagen is intended to simulate exposure of the subendothelial matrix after arterial injury. In this model, inhibition of FXI by a goat anti-human FXI antibody and mouse anti-human FXI clone O1A6 (also referred to as aXIMab) reduced thrombus formation.[71–73] FXI ASOs also inhibited thrombus formation in this model.[73] Encouragingly, inhibition of FXIIa-mediated activation of FXI with the anti-FXI antibody 14E11 and FXII activation with the anti-FXII antibody 15H8 also reduced thrombus formation.[33,53] It is important to note, however, that the FXI-targeting antibody O1A6 demonstrated more potent inhibition of platelet accumulation in the collagen-coated segment of the graft than either 14E11 or 15H8. This suggests that targeting of FXIa-mediated activation of FIX may be more effective than targeting FXIIa-mediated activation of FXI in preventing thrombosis on collagen surfaces.

Epidemiology

Evidence from rodent models of arterial and venous thrombosis suggests that targeting of FXII may offer stronger protection against thrombosis than FXI.[33,36] However, there seems to be discordance between findings from rodent models and observations made in human studies. Patients with severe FXI have a reduced risk of stroke and VTE (venous

thromboembolism).[74–76] Conversely, elevated plasma FXI antigen levels is associated with an increased risk of stroke and VTE.[77–79] The involvement of FXII in the pathogenesis of thrombotic disorders, however, is not well established. Studies of patients with FXII deficiency are limited but have not demonstrated protection against thrombotic events.[80] Similarly, plasma FXII antigen levels have not been associated with changes in risk of stroke, myocardial infarction, and VTE.[78,79]

Clinical Studies

Therapies targeting components of the intrinsic pathway found to be effective in inhibiting thrombosis in preclinical studies have begun to be assessed in the clinical setting.[81] Studies to date have primarily focused on FXI inhibitors likely because of the available supporting human levels data for the role of this factor in thrombotic disorders. An FXI-targeting ASO developed by Ionis Pharmaceuticals, IONIS-416858, has successfully completed Phase I and II clinical trials.[82,83] In the phase I trial doses sufficient to lower FXI plasma levels by ≈80% were well tolerated with no evidence of excessive bleeding or agent associated toxicity.[82] In a phase 2 trial, prophylactic treatment with IONIS-416858 was compared with enoxaparin for the ability to prevent VTE in patients undergoing total knee arthroplasty.[83] Importantly, the higher 300 mg dose of FXI ASO was superior to enoxaparin in preventing VTE with less major or clinically relevant nonmajor bleeding.[83] FXIa-targeted agents, including the anti-FXIa antibody BAY1213790 (http://www.clinicaltrials.gov. Unique identifier: NCT03276143) and the small molecule FXIa inhibitor BMS-962212, are also currently undergoing clinical evaluation.[84]

Additional inhibitors of the contact pathway have been developed for the treatment of hereditary angioedema. The PKK inhibitor ecallantide has completed phase III trials and is approved for the treatment of acute attacks, with other agents currently undergoing clinical assessment.[85–87] It is interesting to consider whether PKK inhibitors developed for treatment of hereditary angioedema could find additional utility as antithrombotic agents.

Conclusions

Studies of intrinsic pathway knockouts highlight the critical contribution of this pathway to the pathogenesis of arterial and venous thrombosis. Numerous intrinsic pathway inhibitors have been developed that provide robust antithrombotic activity in animal models. Development of agents targeting FXI and FXII is of particular interest because of the limited contribution of these factors toward hemostasis. It remains to be determined if this approach can deliver a new class of clinically safe and effective anticoagulants.

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Disclosures

None.

References

1. Furie B, Furie BC. The molecular basis of blood coagulation. *Cell*. 1988;53:505–518.
2. Gale AJ. Continuing education course #2: current understanding of hemostasis. *Toxicol Pathol*. 2011;39:273–280. doi: 10.1177/0192623310389474
3. Furie B, Furie BC. Mechanisms of thrombus formation. *N Engl J Med*. 2008;359:938–949. doi: 10.1056/NEJMra0801082
4. Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol*. 2018;38(4):709–725.
5. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science*. 1964;145:1310–1312.
6. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature*. 1964;202:498–499.
7. Wiggins RC, Cochrane CC. The autoactivation of rabbit Hageman factor. *J Exp Med*. 1979;150:1122–1133.
8. van der Graaf F, Keus FJ, Vlooswijk RA, Bouma BN. The contact activation mechanism in human plasma: activation induced by dextran sulfate. *Blood*. 1982;59:1225–1233.
9. Tankersley DL, Alving BM, Finlayson JS. Activation of factor XII by dextran sulfate: the basis for an assay of factor XII. *Blood*. 1983;62:448–456.
10. Smith SA, Mutch NJ, Baskar D, Rohloff P, Docampo R, Morrissey JH. Polyphosphate modulates blood coagulation and fibrinolysis. *Proc Natl Acad Sci USA*. 2006;103:903–908. doi: 10.1073/pnas.0507195103
11. Müller F, Mutch NJ, Schenk WA, Smith SA, Esterl L, Spronk HM, Schmidbauer S, Gahl WA, Morrissey JH, Renné T. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. *Cell*. 2009;139:1143–1156. doi: 10.1016/j.cell.2009.11.001
12. Kannemeier C, Shibamiya A, Nakazawa F, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. *Proc Natl Acad Sci USA*. 2007;104:6388–6393. doi: 10.1073/pnas.0608647104
13. Noubouossie DF, Whelihan MF, Yu YB, Sparkenbaugh E, Pawlinski R, Monroe DM, Key NS. *In vitro* activation of coagulation by human neutrophil DNA and histone proteins but not neutrophil extracellular traps. *Blood*. 2017;129:1021–1029. doi: 10.1182/blood-2016-06-722298
14. Bendapudi P, Deceunynck K, Koseoglu S, Bekendam R, Mason S, Kenniston J, Flaumenhaft R. Stimulated platelets but not endothelium generate thrombin via a factor XIIa-dependent mechanism requiring phosphatidylserine exposure. *Blood*. 2016;128:258.
15. Meier HL, Pierce JV, Colman RW, Kaplan AP. Activation and function of human Hageman factor. The role of high molecular weight kininogen and prekallikrein. *J Clin Invest*. 1977;60:18–31. doi: 10.1172/JCI108754
16. Motta G, Rojkjaer R, Hasan AA, Cines DB, Schmaier AH. High molecular weight kininogen regulates prekallikrein assembly and activation on endothelial cells: a novel mechanism for contact activation. *Blood*. 1998;91:516–528.
17. Ivanov I, Matafonov A, Sun MF, Cheng Q, Dickeson SK, Verhamme IM, Emsley J, Gailani D. Proteolytic properties of single-chain factor XII: a mechanism for triggering contact activation. *Blood*. 2017;129:1527–1537. doi: 10.1182/blood-2016-10-744110
18. Engel R, Brain CM, Paget J, Lionikiene AS, Mutch NJ. Single-chain factor XII exhibits activity when complexed to polyphosphate. *J Thromb Haemost*. 2014;12:1513–1522. doi: 10.1111/jth.12663
19. Mast AE. Tissue factor pathway inhibitor: multiple anticoagulant activities for a single protein. *Arterioscler Thromb Vasc Biol*. 2016;36:9–14. doi: 10.1161/ATVBAHA.115.305996
20. Josso F, Prou-Wartelle O. Interaction of tissue factor and factor VII at the earliest phase of coagulation. *Thromb Diath Haemorrh Suppl*. 1965;17:35–44.
21. Lu G, Broze GJ Jr, Krishnaswamy S. Formation of factors IXa and Xa by the extrinsic pathway: differential regulation by tissue factor pathway inhibitor and antithrombin III. *J Biol Chem*. 2004;279:17241–17249. doi: 10.1074/jbc.M312827200
22. Taylor SA, Liddell MB, Peake IR, Bloom AL, Lillicrap DP. A mutation adjacent to the beta cleavage site of factor IX (valine 182 to leucine) results in mild haemophilia Bm. *Br J Haematol*. 1990;75:217–221.
23. Soeda T, Nogami K, Matsumoto T, Ogiwara K, Shima M. Mechanisms of factor VIIa-catalyzed activation of factor VIII. *J Thromb Haemost*. 2010;8:2494–2503. doi: 10.1111/j.1538-7836.2010.04042.x
24. Kamikubo Y, Mendolicchio GL, Zampolli A, Marchese P, Rothmeier AS, Orje JN, Gale AJ, Krishnaswamy S, Gruber A, Østergaard H, Petersen LC, Ruf W, Ruggeri ZM. Selective factor VIII activation by the tissue factor-factor VIIa-factor Xa complex. *Blood*. 2017;130:1661–1670. doi: 10.1182/blood-2017-02-767079

25. Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. *Science*. 1991;253:909–912.
26. von dem Borne PA, Meijers JC, Bouma BN. Feedback activation of factor XI by thrombin in plasma results in additional formation of thrombin that protects fibrin clots from fibrinolysis. *Blood*. 1995;86:3035–3042.
27. Kravtsov DV, Matafonov A, Tucker EI, Sun MF, Walsh PN, Gruber A, Gailani D. Factor XI contributes to thrombin generation in the absence of factor XII. *Blood*. 2009;114:452–458. doi: 10.1182/blood-2009-02-203604
28. Choi SH, Smith SA, Morrissey JH. Polyphosphate is a cofactor for the activation of factor XI by thrombin. *Blood*. 2011;118:6963–6970. doi: 10.1182/blood-2011-07-368811
29. Scott CF, Colman RW. Fibrinogen blocks the autoactivation and thrombin-mediated activation of factor XI on dextran sulfate. *Proc Natl Acad Sci USA*. 1992;89:11189–11193.
30. Pedicord DL, Seiffert D, Blat Y. Feedback activation of factor XI by thrombin does not occur in plasma. *Proc Natl Acad Sci USA*. 2007;104:12855–12860. doi: 10.1073/pnas.0705566104
31. Singh I, Smith A, Vanzieleghem B, Collen D, Burnand K, Saint-Remy JM, Jacquemin M. Antithrombotic effects of controlled inhibition of factor VIII with a partially inhibitory human monoclonal antibody in a murine vena cava thrombosis model. *Blood*. 2002;99:3235–3240.
32. Wang X, Cheng Q, Xu L, Feuerstein GZ, Hsu MY, Smith PL, Seiffert DA, Schumacher WA, Ogletree ML, Gailani D. Effects of factor IX or factor XI deficiency on ferric chloride-induced carotid artery occlusion in mice. *J Thromb Haemost*. 2005;3:695–702. doi: 10.1111/j.1538-7836.2005.01236.x
33. Cheng Q, Tucker EI, Pine MS, Sisler I, Matafonov A, Sun MF, White-Adams TC, Smith SA, Hanson SR, McCarty OJ, Renné T, Gruber A, Gailani D. A role for factor XIIa-mediated factor XI activation in thrombus formation in vivo. *Blood*. 2010;116:3981–3989. doi: 10.1182/blood-2010-02-270918
34. Rosen ED, Gailani D, Castellino FJ. FXI is essential for thrombus formation following FeCl3-induced injury of the carotid artery in the mouse. *Thromb Haemost*. 2002;87:774–776.
35. Wang X, Smith PL, Hsu MY, Gailani D, Schumacher WA, Ogletree ML, Seiffert DA. Effects of factor XI deficiency on ferric chloride-induced vena cava thrombosis in mice. *J Thromb Haemost*. 2006;4:1982–1988. doi: 10.1111/j.1538-7836.2006.02093.x
36. von Brühl ML, Stark K, Steinhart A, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med*. 2012;209:819–835. doi: 10.1084/jem.20112322
37. Renné T, Pozgajová M, Grüner S, Schuh K, Pauer HU, Burfeind P, Gailani D, Nieswandt B. Defective thrombus formation in mice lacking coagulation factor XII. *J Exp Med*. 2005;202:271–281. doi: 10.1084/jem.20050664
38. Cai TQ, Wu W, Shin MK, Xu Y, Jochnowitz N, Zhou Y, Hoos L, Bentley R, Strapps W, Thankappan A, Metzger JM, Ogletree ML, Tadin-Strapps M, Seiffert DA, Chen Z. Factor XII full and partial null in rat confers robust antithrombotic efficacy with no bleeding. *Blood Coagul Fibrinolysis*. 2015;26:893–902. doi: 10.1097/MBC.0000000000000337
39. Kokoye Y, Ivanov I, Cheng Q, Matafonov A, Dickeson SK, Mason S, Sexton DJ, Renné T, McCrae K, Feener EP, Gailani D. A comparison of the effects of factor XII deficiency and prekallikrein deficiency on thrombus formation. *Thromb Res*. 2016;140:118–124. doi: 10.1016/j.thromres.2016.02.020
40. Bird JE, Smith PL, Wang X, Schumacher WA, Barbera F, Revelli JP, Seiffert D. Effects of plasma kallikrein deficiency on haemostasis and thrombosis in mice: murine ortholog of the Fletcher trait. *Thromb Haemost*. 2012;107:1141–1150. doi: 10.1160/TH11-10-0682
41. Stavrou EX, Fang C, Merkulova A, Alhalabi O, Grobe N, Antoniak S, Mackman N, Schmaier AH. Reduced thrombosis in Klkb1-/- mice is mediated by increased Mas receptor, prostacyclin, Sirt1, and KLF4 and decreased tissue factor. *Blood*. 2015;125:710–719. doi: 10.1182/blood-2014-01-550285
42. Merkulov S, Zhang WM, Komar AA, Schmaier AH, Barnes E, Zhou Y, Lu X, Iwaki T, Castellino FJ, Luo G, McCrae KR. Deletion of murine kininogen gene 1 (mKng1) causes loss of plasma kininogen and delays thrombosis. *Blood*. 2008;111:1274–1281. doi: 10.1182/blood-2007-06-092338
43. Moncada S, Korbut R, Bunting S, Vane JR. Prostacyclin is a circulating hormone. *Nature*. 1978;273:767–768.
44. Barbieri SS, Amadio P, Gianellini S, Tarantino E, Zacchi E, Veglia F, Howe LR, Weksler BB, Mussoni L, Tremoli E. Cyclooxygenase-2-derived prostacyclin regulates arterial thrombus formation by suppressing tissue factor in a sirtuin-1-dependent-manner. *Circulation*. 2012;126:1373–1384. doi: 10.1161/CIRCULATIONAHA.112.097295
45. Metzger JM, Tadin-Strapps M, Thankappan A, et al. Titrating haemophilia B phenotypes using siRNA strategy: evidence that antithrombotic activity is separated from bleeding liability. *Thromb Haemost*. 2015;113:1300–1311. doi: 10.1160/TH14-06-0505
46. van Montfoort ML, Knaup VL, Marquart JA, Bakhtiari K, Castellino FJ, Hack CE, Meijers JC. Two novel inhibitory anti-human factor XI antibodies prevent cessation of blood flow in a murine venous thrombosis model. *Thromb Haemost*. 2013;110:1065–1073. doi: 10.1160/TH13-05-0429
47. David T, Kim YC, Ely LK, Rondon I, Gao H, O'Brien P, Bolt MW, Coyle AJ, Garcia JL, Flounders EA, Mikita T, Coughlin SR. Factor XIa-specific IgG and a reversal agent to probe factor XI function in thrombosis and hemostasis. *Sci Transl Med*. 2016;8:353ra112. doi: 10.1126/scitranslmed.aaf4331
48. Schumacher WA, Seiler SE, Steinbacher TE, Stewart AB, Bostwick JS, Hartl KS, Liu EC, Ogletree ML. Antithrombotic and hemostatic effects of a small molecule factor XIa inhibitor in rats. *Eur J Pharmacol*. 2007;570:167–174. doi: 10.1016/j.ejphar.2007.05.043
49. Wong PC, Crain EJ, Watson CA, Schumacher WA. A small-molecule factor XIa inhibitor produces antithrombotic efficacy with minimal bleeding time prolongation in rabbits. *J Thromb Thrombolysis*. 2011;32:129–137. doi: 10.1007/s11239-011-0599-0
50. Wong PC, Quan ML, Watson CA, Crain EJ, Harpel MR, Rendina AR, Luettgen JM, Wexler RR, Schumacher WA, Seiffert DA. *In vitro*, antithrombotic and bleeding time studies of BMS-654457, a small-molecule, reversible and direct inhibitor of factor XIa. *J Thromb Thrombolysis*. 2015;40:416–423. doi: 10.1007/s11239-015-1258-7
51. Zhang H, Löwenberg EC, Crosby JR, MacLeod AR, Zhao C, Gao D, Black C, Revenko AS, Meijers JC, Stroes ES, Levi M, Monia BP. Inhibition of the intrinsic coagulation pathway factor XI by antisense oligonucleotides: a novel antithrombotic strategy with lowered bleeding risk. *Blood*. 2010;116:4684–4692. doi: 10.1182/blood-2010-04-277798
52. Revenko AS, Gao D, Crosby JR, Bhattacharjee G, Zhao C, May C, Gailani D, Monia BP, MacLeod AR. Selective depletion of plasma prekallikrein or coagulation factor XII inhibits thrombosis in mice without increased risk of bleeding. *Blood*. 2011;118:5302–5311. doi: 10.1182/blood-2011-05-355248
53. Matafonov A, Leung PY, Gailani AE, Grach SL, Puy C, Cheng Q, Sun MF, McCarty OJ, Tucker EI, Kataoka H, Renné T, Morrissey JH, Gruber A, Gailani D. Factor XII inhibition reduces thrombus formation in a primate thrombosis model. *Blood*. 2014;123:1739–1746. doi: 10.1182/blood-2013-04-499111
54. Larsson M, Rayzman V, Nolte MW, et al. A factor XIIa inhibitory antibody provides thromboprotection in extracorporeal circulation without increasing bleeding risk. *Sci Transl Med*. 2014;6:222ra17. doi: 10.1126/scitranslmed.3006804
55. Hagedorn I, Schmidbauer S, Pleines I, Kleinschnitz C, Kronthaler U, Stoll G, Dickneite G, Nieswandt B. Factor XIIa inhibitor recombinant human albumin Infestin-4 abolishes occlusive arterial thrombus formation without affecting bleeding. *Circulation*. 2010;121:1510–1517. doi: 10.1161/CIRCULATIONAHA.109.924761
56. Barbieri CM, Wang X, Wu W, Zhou X, Ogawa AM, O'Neill K, Chu D, Castriota G, Seiffert DA, Gutstein DE, Chen Z. Factor XIIa as a novel target for thrombosis: target engagement requirement and efficacy in a rabbit model of microembolic signals. *J Pharmacol Exp Ther*. 2017;360:466–475. doi: 10.1124/jpet.116.238493
57. May F, Krupka J, Fries M, Thielmann I, Pragst I, Weimer T, Panousis C, Nieswandt B, Stoll G, Dickneite G, Schulte S, Nolte MW. FXIIa inhibitor rHA-Infestin-4: Safe thromboprotection in experimental venous, arterial and foreign surface-induced thrombosis. *Br J Haematol*. 2016;173:769–778. doi: 10.1111/bjh.13990
58. Kuijpers MJ, van der Meijden PE, Feijge MA, Mattheij NJ, May F, Govers-Riemslag J, Meijers JC, Heemskerk JW, Renné T, Cosemans JM. Factor XII regulates the pathological process of thrombus formation on ruptured plaques. *Arterioscler Thromb Vasc Biol*. 2014;34:1674–1680. doi: 10.1161/ATVBAHA.114.303315
59. van Montfoort 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
60. Decrem Y, Rath G, Blasioli V, Cauchie P, Robert S, Beaufays J, Frère JM, Feron O, Dogné JM, Dessy C, Vanhamme L, Godfroid E. Ir-CPI, a coagulation contact phase inhibitor from the tick Ixodes ricinus, inhibits thrombus formation without impairing hemostasis. *J Exp Med*. 2009;206:2381–2395. doi: 10.1084/jem.20091007

61. Quan ML, Pinto DJP, Smallheer JM, Ewing WR, Rossi KA, Luettgen JM, Seiffert DA, Wexler RR. Factor XIa inhibitors as new anticoagulants. *J Med Chem*. 2018;61:7425–7447. doi: 10.1021/acs.jmedchem.8b00173
62. Campos IT, Tanaka-Azevedo AM, Tanaka AS. Identification and characterization of a novel factor XIIa inhibitor in the hematophagous insect, Triatoma infestans (Hemiptera: Reduviidae). *FEBS Lett*. 2004;577:512–516. doi: 10.1016/j.febslet.2004.10.052
63. Maas C, Renné T. Coagulation factor XII in thrombosis and inflammation. *Blood*. 2018;131:1903–1909. doi: 10.1182/blood-2017-04-569111
64. Kuijpers MJ, Gilio K, Reitsma S, Nergiz-Unal R, Prinzen L, Heeneman S, Lutgens E, van Zandvoort MA, Nieswandt B, Egbrink MG, Heemskerk JW. Complementary roles of platelets and coagulation in thrombus formation on plaques acutely ruptured by targeted ultrasound treatment: a novel intravital model. *J Thromb Haemost*. 2009;7:152–161. doi: 10.1111/j.1538-7836.2008.03186.x
65. Kleinschnitz C, Stoll G, Bendszus M, Schuh K, Pauer HU, Burfeind P, Renné C, Gailani D, Nieswandt B, Renné T. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med*. 2006;203:513–518. doi: 10.1084/jem.20052458
66. Langhauser F, Göb E, Kraft P, et al. Kininogen deficiency protects from ischemic neurodegeneration in mice by reducing thrombosis, blood-brain barrier damage, and inflammation. *Blood*. 2012;120:4082–4092. doi: 10.1182/blood-2012-06-440057
67. Leung PY, Hurst S, Berny-Lang MA, Verbout NG, Gailani D, Tucker EI, Wang RK, McCarty OJ, Gruber A. Inhibition of factor XII-mediated activation of factor XI provides protection against experimental acute ischemic stroke in mice. *Transl Stroke Res*. 2012;3:381–389. doi: 10.1007/s12975-012-0186-5
68. Krupka J, May F, Weimer T, Pragst I, Kleinschnitz C, Stoll G, Panousis C, Dickneite G, Nolte MW. The Coagulation factor XIIa inhibitor rHA-infestin-4 improves outcome after cerebral ischemia/reperfusion injury in rats. *PLoS One*. 2016;11:e0146783. doi: 10.1371/journal.pone.0146783
69. Chen JW, Figueiredo JL, Wojtkiewicz GR, Siegel C, Iwamoto Y, Kim DE, Nolte MW, Dickneite G, Weissleder R, Nahrendorf M. Selective factor XIIa inhibition attenuates silent brain ischemia: application of molecular imaging targeting coagulation pathway. *JACC Cardiovasc Imaging*. 2012;5:1127–1138. doi: 10.1016/j.jcmg.2012.01.025
70. Konings J, Govers-Riemslag JW, Philippou H, Mutch NJ, Borissoff JI, Allan P, Mohan S, Tans G, Ten Cate H, Ariëns RA. Factor XIIa regulates the structure of the fibrin clot independently of thrombin generation through direct interaction with fibrin. *Blood*. 2011;118:3942–3951. doi: 10.1182/blood-2011-03-339572
71. Gruber A, Hanson SR. Factor XI-dependence of surface- and tissue factor-initiated thrombus propagation in primates. *Blood*. 2003;102:953–955. doi: 10.1182/blood-2003-01-0324
72. 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
73. 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
74. Salomon O, Steinberg DM, Koren-Morag N, Tanne D, Seligsohn U. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood*. 2008;111:4113–4117. doi: 10.1182/blood-2007-10-120139
75. Salomon O, Steinberg DM, Zucker M, Varon D, Zivelin A, Seligsohn U. Patients with severe factor XI deficiency have a reduced incidence of deep-vein thrombosis. *Thromb Haemost*. 2011;105:269–273. doi: 10.1160/TH10-05-0307
76. Preis M, Hirsch J, Kotler A, Zoabi A, Stein N, Rennert G, Saliba W. Factor XI deficiency is associated with lower risk for cardiovascular and venous thromboembolism events. *Blood*. 2017;129:1210–1215. doi: 10.1182/blood-2016-09-742262
77. Yang DT, Flanders MM, Kim H, Rodgers GM. Elevated factor XI activity levels are associated with an increased odds ratio for cerebrovascular events. *Am J Clin Pathol*. 2006;126:411–415. doi: 10.1309/QC259F09UNMKVP0R
78. Cushman M, O'Meara ES, Folsom AR, Heckbert SR. Coagulation factors IX through XIII and the risk of future venous thrombosis: the Longitudinal Investigation of Thromboembolism Etiology. *Blood*. 2009;114:2878–2883. doi: 10.1182/blood-2009-05-219915
79. Siegerink B, Rosendaal FR, Algra A. Antigen levels of coagulation factor XII, coagulation factor XI and prekallikrein, and the risk of myocardial infarction and ischemic stroke in young women. *J Thromb Haemost*. 2014;12:606–613.
80. Key NS. Epidemiologic and clinical data linking factors XI and XII to thrombosis. *Hematology Am Soc Hematol Educ Program*. 2014;2014:66–70. doi: 10.1182/asheducation-2014.1.66
81. Weitz JI, Chan NC. Advances in antithrombotic therapy. *Arterioscler Thromb Vasc Biol*. 2019;39:7–12. doi: 10.1161/ATVBAHA.118.310960
82. Younis H, Crosby J, Huh J, Lee H, Rime S, Monia B, Henry S. ISIS-FXIRx, a novel and specific antisense inhibitor of factor XI, caused significant reduction in FXI antigen and activity and increased aPTT without causing bleeding in healthy volunteers. *Blood*. 2011;118:209.
83. 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
84. Perera V, Luettgen JM, Wang Z, et al. First-in-human study to assess the safety, pharmacokinetics and pharmacodynamics of BMS-962212, a direct, reversible, small molecule factor XIa inhibitor in non-Japanese and Japanese healthy subjects. *Br J Clin Pharmacol*. 2018;84:876–887. doi: 10.1111/bcp.13520
85. Cicardi M, Levy RJ, McNeil DL, Li HH, Sheffer AL, Campion M, Horn PT, Pullman WE. Ecallantide for the treatment of acute attacks in hereditary angioedema. *N Engl J Med*. 2010;363:523–531. doi: 10.1056/NEJMoa0905079
86. Banerji A, Busse P, Shennak M, et al. Inhibiting plasma kallikrein for hereditary angioedema prophylaxis. *N Engl J Med*. 2017;376:717–728. doi: 10.1056/NEJMoa1605767
87. Aygören-Pürsün E, Bygum A, Grivcheva-Panovska V, et al. Oral plasma kallikrein inhibitor for prophylaxis in hereditary angioedema. *N Engl J Med*. 2018;379:352–362. doi: 10.1056/NEJMoa1716995