Factor XII Contact Activation

Clément Naudin, PhD[1,2] Elena Burillo, PhD[1,2] Stefan Blankenberg, MD[3] Lynn Butler, PhD[1,2] Thomas Renné, MD, PhD[1,2]

[1] Clinical Chemistry, Department of Molecular Medicine and Surgery, Karolinska Institutet and University Hospital, Stockholm, Sweden
[2] Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[3] Department of General and Interventional Cardiology, University Heart Center Hamburg-Eppendorf, Hamburg, Germany

Address for correspondence Thomas Renné, MD, PhD, Clinical Chemistry, Department of Molecular Medicine and Surgery, Karolinska Institutet and Karolinska University Hospital Solna (L2:05), SE-171 76 Stockholm, Sweden; Institute for Clinical Chemistry and Laboratory Medicine (O26), University Medical Center Hamburg-Eppendorf, D-20246 Hamburg, Germany, Sweden (e-mail: thomas@renne.net).

Semin Thromb Hemost 2017;43:814–826.

Abstract

Contact activation is the surface-induced conversion of factor XII (FXII) zymogen to the serine protease FXIIa. Blood-circulating FXII binds to negatively charged surfaces and this contact to surfaces triggers a conformational change in the zymogen inducing autoactivation. Several surfaces that have the capacity for initiating FXII contact activation have been identified, including misfolded protein aggregates, collagen, nucleic acids, and platelet and microbial polyphosphate. Activated FXII initiates the proinflammatory kallikrein-kinin system and the intrinsic coagulation pathway, leading to formation of bradykinin and thrombin, respectively. FXII contact activation is well characterized in vitro and provides the mechanistic basis for the diagnostic clotting assay, activated partial thromboplastin time. However, only in the past decade has the critical role of FXII contact activation in pathological thrombosis been appreciated. While defective FXII contact activation provides thromboprotection, excess activation underlies the swelling disorder hereditary angioedema type III. This review provides an overview of the molecular basis of FXII contact activation and FXII contact activation–associated disease states.

Keywords
- FXII
- contact activation
- aPTT
- polyphosphate
- thrombosis

Blood coagulation is essential to terminate bleeding at sites of vascular injury (hemostasis). However, uncontrolled coagulation also contributes to vascular occlusions (thrombosis). Thrombosis may occur in the venous or arterial circulation, causing pulmonary embolism or myocardial infarction and stroke. Cumulatively, these thromboembolic diseases have remained the primary cause of morbidity and mortality in the western world.[1] Coagulation and inflammation are considered as two distinct processes; however, they mutually interact and closely cooperate at multiple levels.[2] The crosstalk of proinflammatory and procoagulant reactions constitutes the unifying principle for a variety of disorders affecting the cardiovascular system, including atherothrombosis, acute coronary artery disease, and ischemia/reperfusion injury. Understanding the role of coagulation in inflammation and vice versa impact of inflammation on coagulation will introduce new perspectives to improve diagnostics and therapies for both disease entities.[3]

Activated platelets, leukocytes, and fibrin fibers form thrombi and in turn fibrin is produced by the plasma coagulation system. Fibrin formation can be initiated by exposure of the transmembrane protein tissue factor (TF, extrinsic pathway) or factor XII (FXII) (Hageman factor, intrinsic pathway) activation. Both pathways converge at the activation of factor X, which, in complex with the cofactor factor Va, converts prothrombin into thrombin that cleaves fibrinogen to insoluble fibrin that forms fibers. The intrinsic coagulation pathway is initiated by negatively charged surfaces that promote activation of zymogen FXII (►**Fig. 1**). FXII surface-binding induces a conformational change in the zymogen and generates proteolytic activity resulting in a low level of activated FXII (FXIIa).[4] FXIIa activates factor XI (FXI) and plasma prekallikrein (PPK) to FXIa and kallikrein (PK), respectively. In a positive feedback loop, PK hydrolyzes FXII, amplifying FXIIa formation.[5] Contact system-formed PK cleaves the

published online March 27, 2017

Issue Theme Recent Advances in Thrombosis and Hemostasis—Part II; Guest Editors: Sam Schulman, MD, PhD, and Md. Shahidul Islam, MD, PhD.

Copyright © 2017 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.

DOI https://doi.org/10.1055/s-0036-1598003.
ISSN 0094-6176.

Downloaded by: Harvard Library. Copyrighted material.

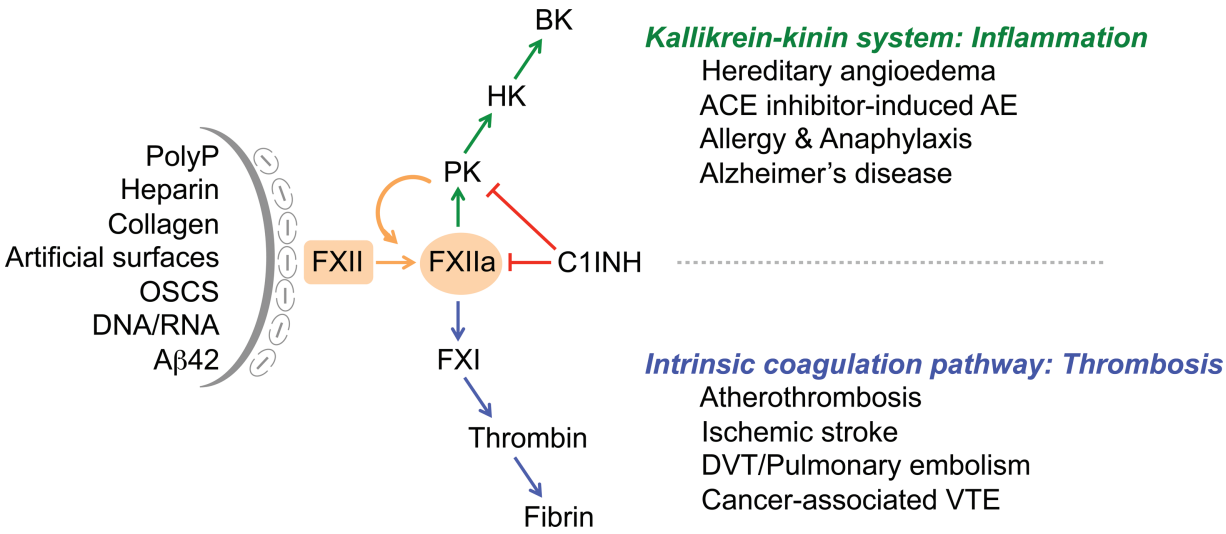

Fig. 1 Factor XII contact activation and contact system–mediated thrombotic and inflammatory disorders. FXII binding to negatively charged surfaces leads to the active protease FXIIa. Activators that initiate FXIIa formation in vivo include polyphosphate (polyP), heparin, collagen, nucleic acids (DNA and RNA), oversulfated chondroitin sulfate (OSCS), amyloid β peptide 1–42 (Aβ42), and artificial surfaces (e.g., glass or some polymers). FXIIa activates the intrinsic coagulation pathway through FXI, leading to thrombin and fibrin formation. Alternatively, FXIIa activates the kallikrein-kinin system via plasma kallikrein (PK), which releases the proinflammatory mediator bradykinin (BK) from high-molecular-weight kininogen (HK). PK reciprocally activates FXII in a positive feedback loop. The main inhibitor of contact system proteases FXIIa and PK is C1 esterase inhibitor (C1INH). The contact system contributes to an array of proinflammatory and procoagulant disorders. ACE, angiotensin-converting enzyme; AE, angioedema; DVT, deep vein thrombosis; VTE, venous thromboembolism.

nonenzymatic cofactor high-molecular-weight kininogen (HK) to liberate the proinflammatory mediator bradykinin (BK) from its precursor. FXIIa-produced FXIa initiates fibrin formation via its substrate factor IX. Both PPK and FXI circulate in plasma bound to HK. The plasma protease inhibitor, C1 esterase inhibitor (C1INH, a serpin), regulates activity of PK and FXIIa. Collectively, the five proteins FXII, PK, FXI, HK, and C1INH are referred to as plasma contact system (PCS) or contact activation system (CAS).[6,7]

Contact System Proteins

Plasma Kallikrein

Human PPK is a 619 amino acid single-chain glycoprotein coded by the *KLKB1* gene located on chromosome 4 (4q34–35). It circulates in plasma at a concentration of 35 to 50 µg/mL in two differentially glycosylated forms of 85 and 88 kDa apparent molecular weight, respectively. Following limited proteolysis, the active enzyme PK is composed of a disulfide-bond linked heavy (residues 1–371, 55 kDa) and light (378–619, 30 kDa) chains. The heavy chain is organized in four apple domains, with apple domains 1, 2, and 4 mediating PPK/PK binding to HK.[8,9] The light chain harbors the peptidase domain and established PK substrates are FXII, HK, plasminogen, and urokinase-type plasminogen activator (uPA). Thus, the protease amplifies contact-produced FXIIa, releases the proinflammatory mediator BK from HK, and promotes fibrinolysis directly and indirectly.[10]

Factor XI

FXI shares high homology with PPK, consisting of an N-terminal heavy chain with four apple domains and a C-terminal light chain that harbors a trypsin-like protease activity. However, while PK is a monomer, human FXI is a 160-kDa homodimer consisting of two identical 607 amino acid PK-like subunits that are linked by a disulfide bond between apple domains 4.[11] Similarly with PPK, FXI circulates in noncovalent complex with HK; however, active FXI has no capacity for cleaving HK and fails to liberate BK. HK functions as a docking protein and enhances FXI binding to platelets and zymogen activation. FXI is activated by FXIIa, thrombin, or by activated FXI molecules (autoactivation) and promotes coagulation via Ca^{2+}-dependent activation of its substrate factor IX of the intrinsic coagulation pathway.

High-Molecular-Weight Kininogen

In humans, a single gene on chromosome 3q35 codes the nonenzymatic cofactor HK. Alternative splicing of the gene-transcript leads to a second kininogen-form, low-molecular-weight kininogen (LK). In mice, the situation is more complex and two kininogen genes exist. Both transcripts are subject to alternative splicing and thus four kininogens, two distinct HK and LK molecules, circulate in murine blood. Human HK is a 120-kDa glycoprotein that belongs to the α-globulin fraction. HK circulates in plasma at 80 µg/mL (half of LK concentration). HK is a single-chain polypeptide that is subdivided into a heavy and light chain with the BK moiety (domain 4) in between. HK binds to cell surface via the glycosaminoglycan chains of heparan- and chondroitin-type proteoglycans and Zn^{2+} ions promote this interaction.[12] Proteoglycan binding protects HK from proteolytic cleavage and impedes contact-triggered BK formation.[13]

C1 Esterase Inhibitor

Plasma protease inhibitors regulate amidolytic activity of FXIIa and PK with C1INH being the most important molecule. C1INH plasma concentration is 0.25 mg/mL and levels of the

Downloaded by: Harvard Library. Copyrighted material.

105-kDa serpin increase up to twofold in inflammatory states. C1INH was originally acknowledged for neutralizing complement proteases C1r and C1s, hence its name. C1INH is a suicide inhibitor and the C-terminal part represents the inhibitory protease-binding region, called "reactive center loop." The inhibition mechanism consists in the recognition of the reactive center loop, which mimics the substrate specificity, by the protease and cleavage of Arg444-Thr445 peptide bond. The cleavage leads to an irreversible conformational change with formation of a non-hydrolysable bond between the protease active site and the serpin. Deficiency in a functional C1INH results in excess BK formation that underlies the inherited swelling disorder, hereditary angioedema (HAE) types I and II (see Section "Medical Implication of FXII Contact Activation"), indicating that other PK inhibitors, such as antithrombin, α2-macroglobulin, plasminogen activator inhibitor-I, tissue factor pathway inhibitor (TFPI)-2, and protein C inhibitor, are not sufficient for controlling PK/FXIIa proteolysis.[14]

Factor XII

FXII Structure and Biochemistry

Factor XII (Hageman Factor, EC 3.4.21.38) is mostly, if not exclusively, produced by hepatocytes (http://www.proteinatlas.org/ENSG00000131187-F12/tissue) and is secreted into the circulation as an 80-kDa zymogen form (596 residues). FXII zymogen circulates in plasma at an estimated concentration of 30 µg/mL (375 nM) with a half-life of 50 to 70 hours. Upon activation by limited proteolysis, the single-chain polypeptide is converted into a two-chain molecule, composed of a heavy chain and a light chain of 353 and 243 amino acids, respectively. The chains remain connected by a disulfide bond. FXII protein is composed of six domains corresponding to the exon-intron organization of the *F12* gene: the N-terminal 19 amino acids signal peptide is followed by a fibronectin type-II domain (Fib-II), an epidermal growth factor like domain (EGF-I), a fibronectin type-I domain (Fib-I), a second EGF-like domain (EGF-II), a kringle domain, and a unique proline-rich domain.[5] These six domains constitute the heavy chain of the coagulation factor that mediates contact to other proteins and negatively charged surfaces. The protease domain forms the light chain and residues His393, Asp442, and Ser544 are the catalytic triad of the serine protease (►**Fig. 2**). FXII is the product of a single gene of 12 kb, located on human chromosome 5 (5q35.3) [13 in mouse, 13 B2] that is composed of 13 introns and 14 exons. The *F12* gene has evolved late during evolution and is absent in birds and fish, but is highly conserved among mammalians. Although FXII is not a typical acute phase protein, estrogen seems to modulate *F12* gene expression through an estrogen-response element (ERE) in the promotor.[15]

FXIIa Protease Forms

FXIIa is a trypsin-like serine protease that cleaves peptide bonds following a positively charged amino acid such as Lys or

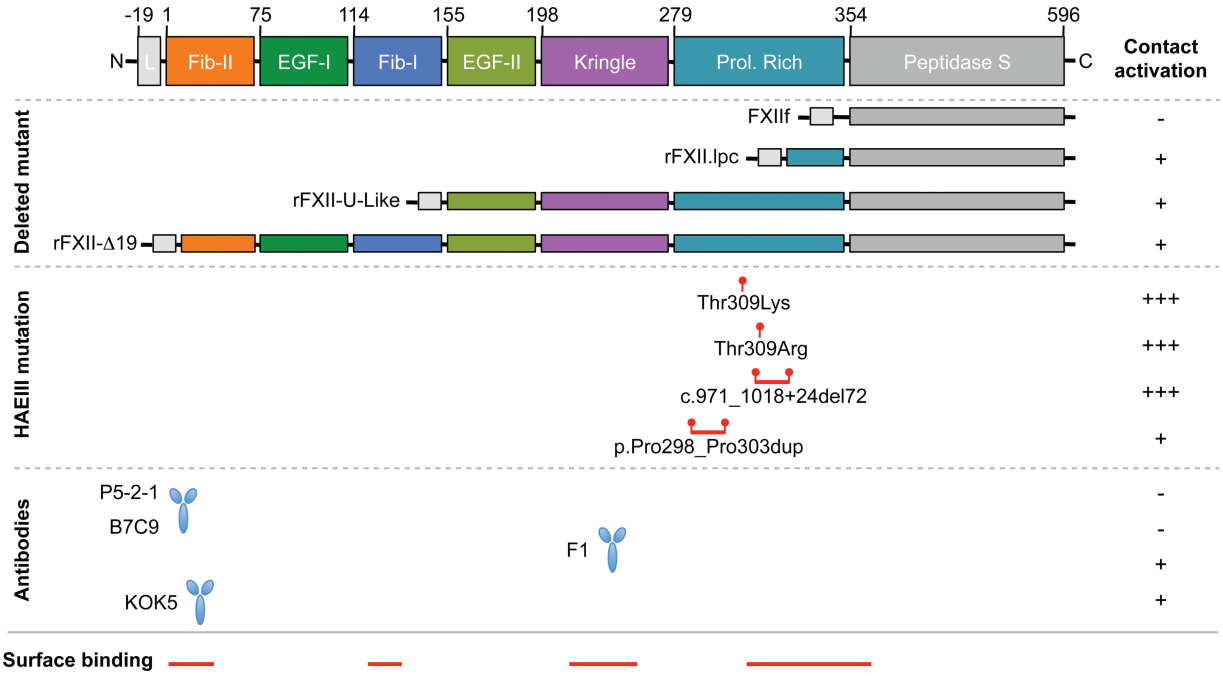

Fig. 2 Domain structure of factor XII with suggested surface-binding site involved in contact activation. Top: scheme of the FXII domain organization: L, leader signal peptide; Fib-II, fibronectin type II domain; EGF-I, EGF-like domain I; Fib-I, fibronectin type I domain; EGF-II, EGF-like domain II; Kringle domain; Pro., Rich, proline-rich domain; Peptidase S, catalytic light chain. Upper middle panel: natural and recombinant FXII-deleted mutant (FXIIf ref,[108] rFXII.lpc ref,[68] rFXII-U-Like ref,[19] rFXII-Δ19 ref[64]) relative to the native FXII structure. Middle panel: hereditary angioedema type III (HAE III) mutations (Thr309Lys ref,[78] Thr309Arg ref,[109] c.971_1018 + 24del72 ref,[110] p.Pro298_Pro303dup ref[111]). Lower middle panel: epitopes of FXII contact activation interfering antibodies (P5–2-1 ref,[61,63] B7C9 ref,[62,63] F1 ref,[66,67] KOK5[19,65]). Bottom panel: suggested surface-binding sites of FXII involved in contact activation. Right panel: effect of the FXII mutants and antibodies on contact system (−), defective activation; (+), activatable; (+++), excess activation.

Downloaded by: Harvard Library. Copyrighted material.

Arg. Autoactivation or PK-induced limited proteolysis at Arg353-Val354 and cleavage leads to the αFXIIa form, where the two chains are linked by a disulfide-bond spanning Cys340-Cys467. This form represents the principal protease activating the intrinsic coagulation pathway and the kallikrein-kinin system. PK may further process αFXIIa at peptide bonds Arg334-Asn335 and Arg343-Leu344. The latter cleavage releases a 28-kDa FXII fragment containing the protease domain with few additional residues of the heavy chain termed βFXIIa or FXIIf. βFXIIa has limited activity for binding to anionic surfaces and activation of FXI, however, it may trigger PPK cleavage in plasma.[5]

FXII Structure–Function Relationship

FXII has a unique structure that is distinct from other coagulation proteases (►**Fig. 2**). The individual FXII domains have been functionally characterized in detail. Fib-I and -II domains mediate FXII binding to fibrin with implications for clot stability and fibrinolysis.[16] EGF-like I and II domains mediate mitogenic activity conferred by the coagulation factor. FXII/FXIIa stimulates proliferation of smooth muscle, HepG2[17] and endothelial cells in culture involving activation of the MAPK/ERK signaling cascade.[18] In culture, FXII induces phosphorylation of ERK1/2 and Akt through urokinase-type plasminogen activator receptor (uPAR) signaling, promoting proliferation of endothelial cells. Indeed, FXII stimulates uPAR-mediated aortic sprouting in wild-type mice and significantly fewer vessels are present in skin punch biopsies of FXII-deficient mice.[18] The precise mechanisms, involvement of EGF domains and potential in vivo relevance of FXII mitogenic activity, is not entirely clear and requires further analyses. Zinc ions facilitate FXII contact activation and four potential Zn^{2+} binding sites are localized in the Fib-II (residues 40–44 and 78–82) and EGF-like domains (residues 94–131 and 174–176). Zinc also promotes FXII binding to uPAR and cell surface proteoglycans facilitating Fib-II/EGF-like domain-driven FXII interaction with endothelial cells, neutrophils, or platelets.[5] Functions of the kringle domain and proline-rich domain have remained unclear, however, they might participate in FXII binding to artificial surfaces.[19]

Hepatocyte Growth Factor Activator

FXII domains differ from typical coagulation proteins, however, FXII shares homology with the serine protease hepatocyte growth factor activator (HGFA;) 39% homology on the amino acid sequence). Similar to FXII, the HGFA heavy chain has two fibronectin-type domains each followed by an EGF-like domain. While both FXII and HGFA share the kringle domain, the proline-rich domain is unique to FXII, suggesting that this sequence mediates specific function(s) of the coagulation factor.[20] HGFA is mainly synthetized in the liver and testis (http://www.proteinatlas.org/ENSG00000109758-HGFAC/tissue) and activates hepatocyte growth factor (HGF), which participates in tissue healing and cancer growth and invasion. Both FXII and the HFGA precursor (pro-HGFA) are activated by limited proteolysis, however, pro-HGFA does not undergo autoactivation. Binding to negatively charged surfaces is mediated by the pro-HGFA heavy chain and facilitates zymogen activation. When bound to anionic surfaces, such as dextran sulfate, chondroitin sulfate, and heparin, pro-HGFA is a substrate of thrombin, leading to a two-chain protease form that resembles αFXIIa. PK further processes active HGFA resulting in a variant that mostly comprises the catalytic domain homologous to βFXIIa. Similarities in structure and activation mechanisms of FXII and HGFA warrant further analyses and may pave the way to an in-depth understanding of plasma proteins binding to polyanions.

Mechanism of FXII Contact Activation

FXII activates upon adhesion to negatively charged surfaces (autoactivation, contact-phase activation; ►**Fig. 1**). Circular dichroism (CD) and sum frequency generation vibrational spectroscopy studies revealed that binding of FXII to surfaces induces a conformational change in the zymogen that is associated with protease activity.[21] Surface binding triggers a transition from the zymogen to the protease form and the intermediate form is stabilized by metal ions such as Zn^{2+}, Cu^{2+}, Co^{2+}, or Ni^{2+}. Platelets store Zn^{2+} in their intracellular granules and locally release the cation upon activation that may regulate FXII functions. In addition to stabilizing FXII/FXIIa intermediate forms, Zn^{2+} facilitates proteolytic FXII cleavage by proteases, particularly PK[22] and increases HK binding to dextran sulfate, liposome with high level of sulfatide, platelets, and endothelial surface. Thus, Zn^{2+} plays a significant role in the activation and regulation of the contact pathway. In addition to surface-driven autoactivation, surface-independent PK-mediated FXII activation (heteroactivation, fluid-phase activation) exists. In response to the potent contact activator polyphosphate (polyP; see later), hetero- and autoactivation accounts for about one-third and two-thirds, respectively, of FXIIa formed.

Natural Contact Activators

FXII-Mediated Platelet-Triggered Coagulation

For half a century, it has been known that procoagulant platelets activate FXII and promote coagulation in an FXII-dependent manner.[23] Indeed, multiple laboratories have shown a role for FXII in platelet-driven coagulation/clot formation. These studies include whole-blood assays[24] and experiments in platelet rich plasma, corroborating that activated platelets drive coagulation in a FXII-dependent manner independently of TF-driven extrinsic coagulation activity.[25,26] How do procoagulant platelets induce FXII contact activation? Platelets store an inorganic polymer, polyP, in their dense granules that is released upon platelet activation by collagen, ADP, thrombin, or Ca^{2+}-ionophore.[27] Using experiments in human plasma, thrombosis and inflammation models in mice, and a human disease model, our laboratory originally identified platelet polyP as FXII contact activator in vivo. PolyP-stimulated FXII activation drives platelet-mediated arterial and venous thrombosis in murine models. Additionally, polyP triggers BK-driven inflammatory reactions.[28] Supporting the in vivo results, synthetic polyP, of similar size to the platelet-derived soluble polymers, potently induces FXII contact activation in human plasma and drives FXIIa-mediated clotting.[29]

Downloaded by: Harvard Library. Copyrighted material.

Inorganic PolyP

Naturally occurring polyP is a linear macromolecule consisting of a few to several thousand residues of orthophosphate linked by phosphoanhydride bonds. In prokaryotic cell polyP synthesis, storage and functions are well understood.[30] However, polyP roles in mammalian systems are subject of ongoing research. Procoagulant platelets release polyP as soluble polymers with relatively short chain lengths of 60 to 100 phosphate units into the supernatant.[28] FXII activation induced by polyP molecules in solution increases with polymer chain length.[29] However, the hypothesis that merely chain length regulates polyP activities appears oversimplified. Long-chain polyP has very little solubility under physiological conditions, questioning a potential role of the molecule in plasma in the fluid phase. Disperse short-chain molecules are soluble; however, they form condensed aggregates in vivo. Aggregate formation largely increases activity of short-chain polyP for inducing FXII contact activation, however, it also decreases solubility. Supporting the hypothesis that aggregate formation is critical for polyP activity, short-chain polyP conjugated to colloidal gold nanoparticles is as potent as long-chain polyP for FXII contact activation.[31] Platelets store polyP in their dense granules and these organelles are Ca^{2+}-rich (molar range). Platelet-derived polyP binds to Ca^{2+} and Ca^{2+}-polyP readily forms stable nanoparticles, suggesting that polyP mostly operates as particles on platelet surfaces rather than in soluble form in the supernatant.[32]

Little is known about biosynthesis and degradation of polyP in mammalian systems. Inositol hexakisphosphate kinase 1 (IP6K1) is a key enzyme of platelet polyP biosynthesis. IP6K1-deficient mice have low platelet polyP and, similar to *F12*$^{-/-}$ mice, are protected from activated platelet-driven thrombosis in a fatal pulmonary embolism model.[33,34] The polyP/FXII pathway triggers thrombin formation independently of the extrinsic TF-stimulated pathway.[35] In addition to FXII contact activation, polyP has the capacity to promote other procoagulant mechanisms, including activation of factors V and XI, TFPI inhibition, and stabilization of fibrin structures.[36] While polyP drives these pathways in plasma ex vivo, possible in vivo roles of these FXII-independent mechanisms of the macromolecule remain to be elucidated.[7]

PolyP as a Drug Target

PolyP is a procoagulant and proinflammatory mediator in vivo and promising drug target for interference with thromboembolic and inflammatory disease states (►**Fig. 3**). Recombinant PdSP15a/b, a sand-fly odorant-binding protein, binds to polyP and inhibits polyP/FXII-mediated BK formation and vascular leakage in mouse edema models.[37] Furthermore, synthetic polycationic polymers, polyamidoamine dendrimers (PAMAM), inhibit polyP-driven clotting in human plasma and interfere with platelet-driven arterial and venous thrombosis in murine models. Despite the potent antithrombotic activities of PAMAM, treated mice have low hemostatic disturbances.[38,39] However, PAMAM are toxic in vivo, limiting potential preclinical use. Less toxic compounds such as universal heparin reversal agents (UHRAs) have high binding affinity for a variety of polyanions, including polyP. UHRAs provide thromboprotection in mouse models, but levels required for interference with thrombus

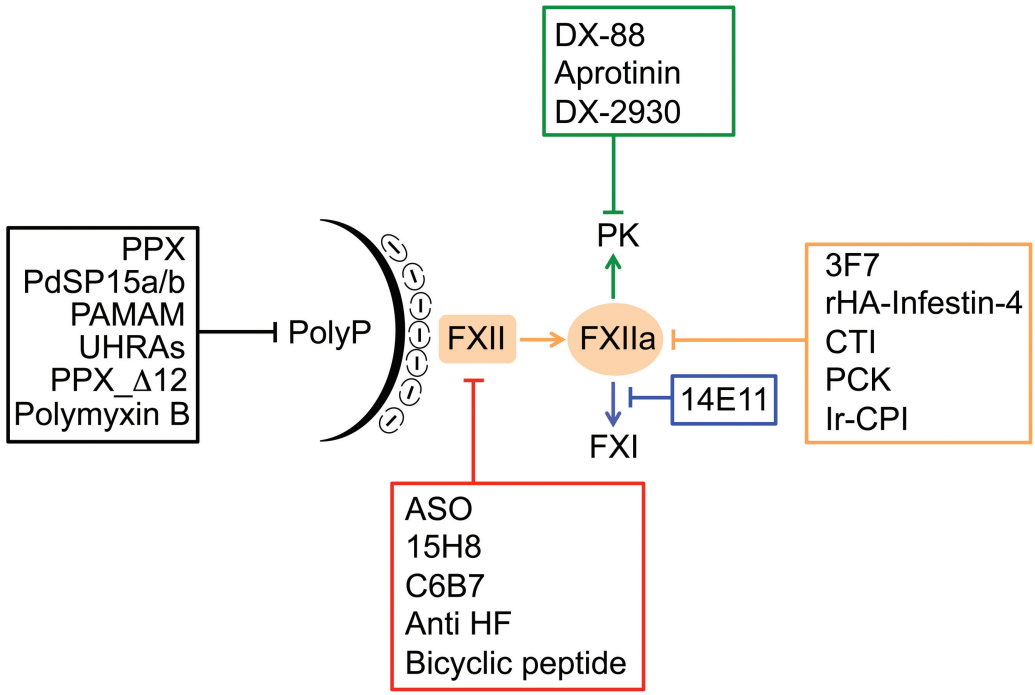

Fig. 3 *Targeting the contact system and its activator polyphosphate.* Anticoagulant and anti-inflammatory agents that target FXIIa, PK, zymogen FXII, and in vivo FXII activator platelets polyphosphate (polyP). rHA-Infestin-4, recombinant human albumin-tagged infestin-4; CTI, corn trypsin inhibitor; PCK, H-D-Pro-Phe-Arg-chloromethylketone; Ir-CPI, *Ixodes ricinus* contact phase inhibitor; DX-88, ecallantide; PPX, exopolyphosphatase; PdSP15a/b, sand fly salivary proteins; PPX_Δ12, PPX variant lacking domains 1 and 2; XII-binding antibodies 15H8, C6B7, anti HF (Hageman Factor); 3F7, anti FXIIa antibody; 14E11, anti FXI antibody that interferes with FXIIa-mediated activation; DX-2930, anti PK antibody; ASO, antisense oligonucleotide; PAMAM, polyamidoamine dendrimer; UHRAs, universal heparin reversal agents.

Downloaded by: Harvard Library. Copyrighted material.

formation also increase bleeding.[40] To test for specific interference with polyP, we have developed recombinant exopolyphosphatase (PPX) mutants that selectively target polyP.[41] Both binding and degradation of the polymer were sufficient to abrogate activated platelet-driven FXII activation. Neutralizing polyP abolished procoagulant activity of stimulated platelets. Targeting polyP with PPX variants also interfered with arterial and venous thrombosis models in normal, but not in FXII-deficient, mice, and impeded clotting in human plasma in a FXII-dependent manner. In sum, targeting of FXII or its activator polyP provides thromboprotection, which is not associated with increased bleeding.

Activated circulating cells release microparticles (MPs). Platelet- but not monocyte-derived MPs induce FXII activation and drive clotting by the intrinsic coagulation pathway.[42] Cancer is a strong and established risk factor for thrombosis. Prostate and pancreatic cancers and promyelocytic leukemia also release MPs. These MPs expose polyP on their surfaces that colocalizes with FXIIa and initiates coagulation by the FXII pathway, suggesting a role of polyP/FXII in malignancy associated thromboembolic disorders.[43]

FXII Activation by Heparin

In addition to polyP, a variety of other polyanions have the capacity for inducing FXII contact activation in vivo. Heparin is a highly sulfated/acetylated negatively charged polysaccharide that is exclusively produced by mast cells. Upon IgE/antigen activation, mast cells release the polysaccharide that in turn initiates FXII contact activation, resulting in BK formation via the kallikrein-kinin system.[5] BK produced by aberrant-activated mast cells participates in swelling and inflammatory reactions in animal models and in patients. In contrast, FXII or BK B2 receptor–deficient mice are protected from heparin and IgE/allergen-activated mast cell-induced edema and adverse cardiovascular reactions.[44] Anaphylaxis is a serious, possibly fatal, allergic reaction. Mast cells are maximally activated in anaphylaxis and patients' heparin levels are largely elevated during a reaction. Anaphylaxis is associated with transient decreased plasma FXII (as indicated by defective FXII- but normal TF- driven coagulation), suggesting that the factor is consumed.[45] Analysis of PPK and HK plasma levels in patients with anaphylaxis confirmed that the contact system is activated in anaphylaxis. Magnitude of FXII activation correlates with BK formation and severity of clinical symptoms (grade of anaphylaxis).[45] FXIIa that is formed in allergy and anaphylaxis selectively drives PK-mediated BK formation. Surprisingly, under these conditions, the intrinsic coagulation pathway is not activated, despite the fact that PPK and FXI share high homology. The mechanisms underlying specific activation of inflammatory reactions in the absence of a procoagulant state are currently unknown, but may reflect the fact that heparin potently amplifies anticoagulant pathways, such as antithrombin.

FXII Activation by Misfolded Proteins

Misfolded protein aggregates are another FXII contact activator that selectively drives the kallikrein-kinin system without associated FXI activation and coagulation.[46] Amyloid deposits of misfolded plasma proteins, such as transthyretin, immunoglobulin light chains, or glycated albumin, are found in systemic amyloidosis. FXIIa plasma levels are elevated in these patients and in vitro data show that misfolded protein aggregates, but not amyloid fibrillar structures, trigger FXII contact activation, leading to PK generation without FXI activation. Consistently, aggregated amyloid β peptide (Aβ) is found in the circulation and cerebrospinal fluid of Alzheimer's disease (AD). Aβ activates FXII in plasma, and recent studies showed elevated FXIIa, and consumption of PK and HK form in two AD patient cohorts and an AD mouse model, which overexpress human Aβ.[47] These data suggest a role of contact activation in increased thrombosis and vascular inflammation associated with AD.[48] Furthermore, the contact system seems to participate in neuronal function disruption and may provide a new drug target for interference with degenerative neuronal diseases.[49]

Matrix Components and Nucleic Acids

Some constituents of the subendothelial extracellular matrix, such as laminin[50] or collagen,[51] enhance FXII activation in vitro and plasma clotting in a FXII-dependent manner. However, the fact that FXII-driven coagulation has no function at the site of vascular injury suggests that matrix component-activated FXII triggers pathways distinct from fibrin formation in vivo. Nucleic acids are negatively charged polymers and there is emerging evidence of extracellular functions of the polyanions in inflammation and coagulation. Extracellular RNA is considered to promote blood coagulation by inducing FXII contact activation. The notion is based on the fact that infusion of RNase (an enzyme that degrades RNA) interferes with arterial thrombosis in a murine $FeCl_3$-driven vascular injury model.[52] However, RNase also readily hydrolyzes polyP,[41] offering an alternative explanation for the thromboprotective effects conferred by the enzyme. Both RNA and DNA are weak FXII contact activators in plasma,[52] indicating that nucleic acids operate in chronic contact system–mediated disease states. Single-stranded DNA activates FXII in vitro and DNA strains are a key component of neutrophil extracellular traps (NETs) that provide a surface for contact activation.[53] NETs locally assemble FXII zymogens and significantly increase thrombus propagation in the presence of platelets in a mouse deep vein thrombosis model.[54] FXII is activated in sepsis and nucleic acids released from bacteria or disintegrating cells may contribute to FXII contact activation that initiates plasma innate immunity mechanisms, including complement and coagulation.[55]

Nonphysiologic FXII Contact Activators

Silicates and Ellagic Acid

Glass provides a noncrystalline, negatively charged surface that binds and activates FXII. Similarly, the white clay material kaolin (a silicate) induces FXII contact activation. Kaolin is used to produce FXIIa in the diagnostic clotting assay, activated partial thromboplastin time (aPTT, see below). Locally applied kaolin potently triggers fibrin formation and has been used as a hemostatic agent to stop traumatic

Downloaded by: Harvard Library. Copyrighted material.

bleedings at the battlefield (combat gauze). In contrast, surface coating with sialine or plastic largely reduces activity of the negatively charged materials for producing FXIIa. Ionized polyphenolic ellagic acid (2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) is another FXII activator used to initiate aPTT assays. Ellagic acid precipitates in the presence of Ca^{2+} forming insoluble particles. These particles, but not monomeric Ca^{2+}-free ellagic acid, activate FXII.[56]

Sulfated Polysaccharides

Sulfated polysaccharides, such as dextran sulfate (DXS), have been useful to unravel the mechanism of FXII contact activation in experimental systems.[57] Similar to mast cell-derived heparin, DXS activates BK formation but not coagulation in vivo and infusion of high-molecular DXS induces hypotonic shock in large animal models.[58] The activity of DXS for FXII contact activation increases with polymer size and degree of sulfation; low-molecular DXS (5 kDa) fails to activate FXII.[59] The synthetic polysaccharide oversulfated chondroitin sulfate (OSCS) had been added to heparin to increase its activity. Charge density of OSCS exceeds that of heparin and the polymer potently activates FXII, leading to excessive BK release causing severe potentially lethal acute hypersensitivity reactions in patients.[60]

Analysis of Contact Activation with Antibodies and Mutants

Monoclonal anti-FXII Antibodies

Anti-FXII antibodies that interfere with activation have been useful to analyze contact activation mechanisms. The monoclonal antibody P5–2-1 interferes with glass- and kaolin-triggered FXIIa formation in human plasma[61] but does not affect βFXIIa for activating PPK, indicating that P5–2-1 binds to the FXII heavy chain. Tryptic FXII fragments revealed that P5–2-1 epitope is localized in the amino-terminal portion of the molecule, and identified the Fib-II domain (1–28) as FXII surface-binding site (►**Fig. 2**). Anti-FXII antibody B7C9 interferes with kaolin-driven activation and FXII-fragments indicated that a 20 amino acid peptide in Fib-I domain (Thr134-Arg153) is crucial for zymogen surface activation.[62] Surprisingly, the same laboratory showed that P5–2-1 and B7C9 compete with each other for binding to FXII. Using peptides, the authors mapped P5–2-1/B7C9 to residues 1–28 of Fib-II domain in the extreme N-terminus of the mature FXII[63] (►**Fig. 2**). The region localized in Fib-II domain is positively charged, due to the presence of lysine residues, and this charged cluster has previously been shown to be involved in HK binding. Challenging a possible role of residues 1–28 in Fib-II for contact activation, a FXII deletion mutant lacking residues 3–19 was readily contact activatable. DXS readily triggered mutant FXII activation that even exceeded activation of normal FXII by fourfold, despite the fact that B7C9 failed to bind to mutant FXII.[64] Anti-FXII heavy chain antibody KOK5 inhibits kaolin-triggered FXII contact system activation.[65] Modeling experiments suggest that the antibody recognizes a discontinuous epitope in Fib-II domain involving residues 30–33, 40–47, and 57–60. A synthetic peptide spanning residues 39–47 was sufficient for interfering with FXII binding to both kaolin and DXS, and for inhibition of FXII activation. These data suggest that Fib-II domain, particularly residues 39–47, is involved in FXII binding to negatively charge surface and contact activation. F1 and F3 antibodies bind to the N- and C-terminal portion of the FXII heavy chain.[66] F1 binding to FXII mimicked contact activation and induced FXIIa formation in the presence of PPK and HK. Antibody-induced contact activation argues for conformational changes in FXII activation. Indeed, CD-spectroscopy revealed significant conformational changes induced by FXII binding to surfaces[59] and F1 binding enhances the FXII cleavage by PK. F1 epitope was mapped to the kringle domain using FXII deletion mutants[67] (►**Fig. 2**). In sum, the antibody-based studies suggest that Fib-I, Fib-II, and kringle domain mediate FXII contact activation.

FXII Domain-Deletion Mutants

Mutants have also been used to analyze mechanisms of FXII contact activation. The 319-amino acids–deleted mutant, rFXII. lpc, consisting of light chain and C-terminal part of the proline-rich domain binds to kaolin and is activated by negatively charged surface.[68] In contrast, a FXII fragment that completely lacked the heavy chain was inactive for contact activation.[69] Furthermore, a FXII mutant spanning EGF-II, kringle and proline-rich domain and the light chain (rFXII-U-like), binds to surfaces and undergoes contact activation.[19] Together, these studies underline the complexity in regulation contact activation and suggest a systematic approach to identify the portions of FXII involved in surface-triggered activation.

Contact-Independent FXII Activation

In addition to contact-driven FXII activation, several proteases including plasmin, FXIa, and trypsin proteolytically activate the zymogen, in a reaction called fluid-phase activation. Reciprocal FXII processing by PK is the basis for the positive feedback loop that amplifies FXIIa at surfaces. Recent studies indicate an unexpected role of plasmin for BK production in patients with HAE (see below) involving cleavage and activation of FXII.[70] Proteolytic activation of FXII by trypsin has no function in vivo, but represents a useful in vitro tool to produce FXIIa in experimental studies. FXII is a substrate for proteases derived from various pathogens such as clostripain, *Vibrio* metalloproteases, subtilisin, thermolysin, or aspartyl protease of *Candida albicans*.[71]

Medical Implication of FXII Contact Activation

In contrast to other proteins of the coagulation cascade, moderate and severe FXII deficiency is not associated with any hemostatic abnormalities and patients with low or no detectable FXII plasma levels do not bleed excessively, even during surgery. Starting with the index "patient" for FXII deficiency, Mr. John Hageman, an array of *F12* gene mutations has been described that result in deficiency in the protein (<10% of normal plasma levels; ►**Table 1**).

Downloaded by: Harvard Library. Copyrighted material.

Table 1 *F12* gene mutations associated with factor XII deficiency

Mutation	Remarks	FXII protein/activity	References
Exon 3, −224 bp: T > C (Intron B) (*Taq*I poly.)		FXII activity and antigen level <10%	80
Exon 14: TGT > AGT or TCT; p.Cys571Ser	CRM positive	No active; Factor XII Washington DC	81
Exon 10: CGC > CCC; p.Arg353Pro		No active; Factor XII Locarno	82
Exon 14: G11397A; p.Del from Ser544	CRM negative	No active; detectable transcript but unstable protein	83
Exon1, -8: G > C & *Taq*I poly.	CRM negative		84
Exon 12: 10586DelGfs	CRM negative		
Exon 10: G9998A; p.Arg398Gln	CRM negative		85
Exon 10: C9988A; p.Leu395Met	CRM negative		
Exon 11: G10372A; p.Asp442Asn	CRM positive		
Exon 14: G11482C; p.Gly570Arg	CRM positive		
Exon 12: 10590DelC & *Taq*I poly.	CRM negative		
Exon 1: c.C46T (5′-untranslated region)			86
Exon 3: A7832G; p.Tyr34Cys	CRM negative	Factor XII Tenri	87
Exon 11: C10310A; p.Gln421Lys	CRM negative	Reduced activity	88
Exon 6: G8694C; p.Arg123Pro	CRM negative	Severe FXII deficiency	
Exon 12: G10587C; p.Trp486Cys	CRM negative	FXII activity and antigen level <5%	89
Exon 10: G9979A; p.Ala392Thr	CRM negative	Activity <3% Antigen <10% Factor XII Shizuoka	90
Exon 13: C11185T; p.Gln501*Stop* and Exon1, -8: G > C		FXII activity and antigen level <1%	91
Exon 14: C11414T; p.Pro547Leu			
Promoter: C-13T)			
Exon 4: c.G218C; p.Cys73Ser			92
Exon 10: G9845C; p.Lys346Asn	CRM reduced	Factor XII Ofunato	93
Exon 13: c.C1583T; p.Ser528Phe		FXII activity <5%	94
Exon 14: c.G1744A; p.Gly582Ser		FXII activity <0.5%	
Exon 6: c.C405A; p.Cys135X		FXII activity <0.5%	
Exon 10: c.1093_1094insC; p.Lys365GlnfsX69		FXII activity <0.5%	
Exon 4: c.G251C; p.Arg84Pro		Activity <10% Antigen <17%	95
Exon 4: c.249delG; p.Gln83HisfsX12		FXII activity <0.5%	96
Exon 8: T9277G; p.Trp222Gly			97
Exon 12: C10468A; p.Arg447Ser	CRM negative	Activity <3% Antigen <10%	
Exon 5: 9160delC, 9161delA; p.His101Glnfs- > 137terminaison (exon6) and c.C46T		FXII activity and antigen level <1%	98
Exon 10: G9775C; p.Ala324Pro Exon 13: G11276A; p.Gly531Glu	Heterozygote CRM negative and positive	Factor XII Osaka	99

(*Continued*)

Downloaded by: Harvard Library. Copyrighted material.

Table 1 (*Continued*)

Mutation	Remarks	FXII protein/activity	References
Exon14: G11398A; p.Gly542Ser		FXII activity and antigen level <1%	100
Exon 7: c.583delC; p. His195fsX250		FXII activity <1%	101
Exon 9: 9499–9507del9bp		FXII activity and antigen level <1%	102
Exon 13: G11296A; p.Asp538Asn	CRM negative	Activity <5% Antigen <6.8%	103
Exon 13: G11188A; p.Glu502Lys		Activity <18% Antigen NS	104
Exon 8: G9357C; p.Arg267Gly		Activity <1% Antigen <5%	
Exon 13: G11188A; p.Glu502Lys		Activity <5% Antigen <5%	
Exon 8: G9334A; p.Gly259Glu		Activity <1% Antigen <1%	
Exon 8: C9357G; p.Arg267Gly		Activity <1% Antigen <5%	
Exon 10: G9826A; p.Gly341Arg		Activity <3% Antigen <6%	
Exon 5: 8440–8441delCA		Activity <3% Antigen <1%	105
Exon 9: 9452–9454delACA; p.Asn252del		Activity <3% Antigen <5%	

Abbreviations: CRM, cross-reactive material; del, deletion; fs, frameshift mutation; ins, insertion; NS, not significant.
Note: cDNA and genomic DNA sequences according to Cool and MacGillivray[106] and Tripodi et al.[107]

Activated Partial Thromboplastin Time

FXII contact activation provides the mechanistic basis for one of the most commonly used diagnostic clotting assays, the aPTT. Today, aPTT assays are performed more than 5 billion times annually and "patients" with FXII deficiency are mostly found by chance in preoperatively performed aPTT coagulation screening. The aPTT assay originates from the discovery that contact with glass reduces clotting time in platelet-poor plasma similarly with the recalcification clotting time performed in experimental settings today. In 1953, a one-stage antihemophilic factor assay was published that leads the way to the currently used aPTT. The term *partial thromboplastin* indicates addition of a tissue extract to the plasma sample that improved stability and reproducibility of the originally used recalcification clotting time in glass tubes. The tissue extract provides phospholipid surfaces and is named "partial" because it lacks TF. Addition of a defined amount of FXII activator kaolin instead of the poorly defined glass tube surface further improved the assay and produced a shortened, more sensitive, and standardized clotting time assay known today as aPTT.

Clinical Applications of the aPTT

Currently, the aPTT is extensively used for preoperative screening, the diagnosis of thrombosis-related autoimmune diseases, and monitoring of anticoagulant therapy (e.g., using heparin or new oral anticoagulant drugs targeting factor X). Ellagic acid, micronized silica, and kaolin are the mostly used contact activators in aPTT assays. The aPTT is prolonged in patients with acquired or congenital deficiencies in intrinsic pathway factors such as FXII, HK, PPK, FXI (but not C1INH), FXI, and factors VIII and IX. aPTT is also prolonged in patients with deficiency of fibrinogen (hypo-/dysfibrinogenemia), sepsis due to consumption of coagulation factors (disseminated intravascular coagulation, DIC), and defects in coagulation factor biosynthesis (vitamin K deficiency, liver cirrhosis). A prolonged aPTT is sensitive for the presence of circulating anticoagulant antibodies such as lupus anticoagulant and systemic lupus erythematosus. Inversely, a shortened aPTT is observed in early stage of DIC, acute hemorrhage, as well in advanced cancer.

Defective FXII Contact Activation Provides Thromboprotection

The role of FXII contact activation for clotting ex vivo has been acknowledged for decades; however, based on the fact that deficiency in FXII does not impair hemostasis, FXII was considered to have no role for coagulation in vivo. Challenging the dogma of a coagulation balance $F12^{-/-}$ mice are protected in various arterial and venous experimental thrombosis models without increased bleeding.[34] Infusion of prostasomes, which expose TF, in $F12^{-/-}$ mice is not sufficient to form vessel occlusive

Downloaded by: Harvard Library. Copyrighted material.

thrombi,[43] indicating a central role of the factor in thrombus growth. Indeed, studies from multiple laboratories have unraveled the role of FXII in thrombosis. PolyP exposed on activated platelets, MPs and cancer cells, activates FXII, and contact-activated FXIIa drives fibrin production within the developing thrombus via its substrate FXI. Genetic or pharmacological interference with FXII or FXI impairs coagulation within the thrombus leading to impaired thrombus stability and facilitated embolization[72,73] (►**Fig. 3**). Targeting polyP with synthetic[39,40] or recombinant[41] inhibitors ablates thrombosis, supporting a central role of the platelet polyP/FXII pathway for thrombus formation in mice. Impact of polyP/FXII is not restricted to murine systems. Large animal models have confirmed a critical function of polyP/FXII for arterial and venous thrombosis[69,74] and targeting FXII/FXIIa provides safe thromboprotection. Despite clear data originating from experimental animal models, there is a lack of clinical data that systematically compare the incidence or severity of thromboembolic events (i.e stroke, myocardial infarction, pulmonary embolism) in FXII-deficient patients. To provide a registry on FXII-deficient individuals, we encourage the reader to register their FXII-deficient "patients" at: www.factor12.net.

Due to the selective importance of FXII in pathological thrombosis, the protease is a promising pharmacologic target for anticoagulant drugs (►**Fig. 3**). Targeting FXIIa with inhibitors (CTI or rHA-infestin-4), neutralizing antibodies (3F7) or small molecules (bicyclic peptides) or interference with FXII expression (ASO) provide novel potential strategies for safe anticoagulation. For more detailed information on FXII and its various inhibitors in thrombosis, we refer to recent reviews.[7,75,76]

Excess Contact Activation Underlies Hereditary Angioedema Type III

Deficiency in FXII provides thromboprotection, however, excess activity of the protease is the underlying mechanisms of HAE, a life-threatening swelling disorder. Surprisingly, clinical and experimental data show that HAE is not associated with increased thrombotic risk. HAE types I and II are due to deficiency in a functional C1INH, leading to unregulated PK/FXIIa activity that culminates in excess BK production.[77] In contrast, a third HAE type exists that has normal levels of a functional C1INH. A genome-wide association study has associated HAE type III with single nucleotide polymorphisms in the *F12* gene that leads to amino acid exchanges at position 309 in FXII (Thr309Arg, Thr309Lys).[78] The FXII point mutation results in the loss of a single O-linked glycosylation and this loss of glycosylation increases capacity of mutant FXII to undergo contact activation.[79] Increased mutant FXII contact activatability initiates excessive BK formation in HAE type III patient plasma and edema in a genetically altered, humanized mouse model. Moreover, mutant FXII is more sensitive to plasmin cleavage.[70] Two more mutations of *F12* gene have been associated with HAE type III, which lead to deletion and/or duplications in the FXII sequence[77](►**Fig. 2**). Thus, a gain of FXII contact activation underlies HAE type III and the disease provides a proof-of-concept system to study mechanisms and therapy of excess FXII contact activation.

Competing Financial Interest
The authors declare that no competing financial interest exists.

Acknowledgments
This work was supported in part by grants from Vetenskapsrådet (K2013–65X-21462- 014–5), Hjärt Lungfonden (20140741), Stockholms läns landsting (ALF, 20140464), the German Research Society (SFB877, TP A11 and SFB841, TP B8), and a European Research Council grant (ERC-StG-2012–311575_F-12) to TR.

References

1 Mackman N. Triggers, targets and treatments for thrombosis. Nature 2008;451(7181):914–918
2 Dahlbäck B. Coagulation and inflammation–close allies in health and disease. Semin Immunopathol 2012;34(1):1–3
3 Long AT, Kenne E, Jung R, Fuchs TA, Renné T. Contact system revisited: an interface between inflammation, coagulation, and innate immunity. J Thromb Haemost 2016;14(3):427–437
4 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(9):1513–1522
5 Renné T, Schmaier AH, Nickel KF, Blombäck M, Maas C. In vivo roles of factor XII. Blood 2012;120(22):4296–4303
6 Maas C, Oschatz C, Renné T. The plasma contact system 2.0. Semin Thromb Hemost 2011;37(4):375–381
7 Kenne E, Nickel KF, Long AT, et al. Factor XII: a novel target for safe prevention of thrombosis and inflammation. J Intern Med 2015; 278(6):571–585
8 Renné T, Dedio J, Meijers JC, Chung D, Müller-Esterl W. Mapping of the discontinuous H-kininogen binding site of plasma prekallikrein. Evidence for a critical role of apple domain-2. J Biol Chem 1999;274(36):25777–25784
9 Herwald H, Renné T, Meijers JC, et al. Mapping of the discontinuous kininogen binding site of prekallikrein. A distal binding segment is located in the heavy chain domain A4. J Biol Chem 1996;271(22):13061–13067
10 Björkqvist J, Jämsä A, Renné T. Plasma kallikrein: the bradykinin-producing enzyme. Thromb Haemost 2013;110(3):399–407
11 Emsley J, McEwan PA, Gailani D. Structure and function of factor XI. Blood 2010;115(13):2569–2577
12 Renné T, Dedio J, David G, Müller-Esterl W. High molecular weight kininogen utilizes heparan sulfate proteoglycans for accumulation on endothelial cells. J Biol Chem 2000;275(43):33688–33696
13 Renné T, Schuh K, Müller-Esterl W. Local bradykinin formation is controlled by glycosaminoglycans. J Immunol 2005;175(5): 3377–3385
14 Björkqvist J, Nickel KF, Stavrou E, Renné T. In vivo activation and functions of the protease factor XII. Thromb Haemost 2014; 112(5):868–875
15 Inoue Y, Peters LL, Yim SH, Inoue J, Gonzalez FJ. Role of hepatocyte nuclear factor 4alpha in control of blood coagulation factor gene expression. J Mol Med (Berl) 2006;84(4):334–344
16 Konings J, Govers-Riemslag JW, Philippou H, et al. Factor XIIa regulates the structure of the fibrin clot independently of thrombin generation through direct interaction with fibrin. Blood 2011;118(14):3942–3951
17 Gordon EM, Venkatesan N, Salazar R, et al. Factor XII-induced mitogenesis is mediated via a distinct signal transduction pathway that activates a mitogen-activated protein kinase. Proc Natl Acad Sci U S A 1996;93(5):2174–2179

Downloaded by: Harvard Library. Copyrighted material.

18 LaRusch GA, Mahdi F, Shariat-Madar Z, et al. Factor XII stimulates ERK1/2 and Akt through uPAR, integrins, and the EGFR to initiate angiogenesis. Blood 2010;115(24):5111–5120

19 Citarella F, Ravon DM, Pascucci B, Felici A, Fantoni A, Hack CE. Structure/function analysis of human factor XII using recombinant deletion mutants. Evidence for an additional region involved in the binding to negatively charged surfaces. Eur J Biochem 1996; 238(1):240–249

20 Miyazawa K. Hepatocyte growth factor activator (HGFA): a serine protease that links tissue injury to activation of hepatocyte growth factor. FEBS J 2010;277(10):2208–2214

21 Chen X, Wang J, Paszti Z, et al. Ordered adsorption of coagulation factor XII on negatively charged polymer surfaces probed by sum frequency generation vibrational spectroscopy. Anal Bioanal Chem 2007;388(1):65–72

22 Mutch NJ, Waters EK, Morrissey JH. Immobilized transition metal ions stimulate contact activation and drive factor XII-mediated coagulation. J Thromb Haemost 2012;10(10):2108–2115

23 Castaldi PA, Larrieu MJ, Caen J. Availability of platelet Factor 3 and activation of factor XII in thrombasthenia. Nature 1965; 207(995):422–424

24 Nielsen VG, Cohen BM, Cohen E. Effects of coagulation factor deficiency on plasma coagulation kinetics determined via thrombelastography: critical roles of fibrinogen and factors II, VII, X and XII. Acta Anaesthesiol Scand 2005;49(2):222–231

25 Bäck J, Sanchez J, Elgue G, Ekdahl KN, Nilsson B. Activated human platelets induce factor XIIa-mediated contact activation. Biochem Biophys Res Commun 2010;391(1):11–17

26 Walsh PN, Griffin JH. Contributions of human platelets to the proteolytic activation of blood coagulation factors XII and XI. Blood 1981;57(1):106–118

27 Ruiz FA, Lea CR, Oldfield E, Docampo R. Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. J Biol Chem 2004; 279(43):44250–44257

28 Müller F, Mutch NJ, Schenk WA, et al. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. Cell 2009; 139(6):1143–1156

29 Smith SA, Choi SH, Davis-Harrison R, et al. Polyphosphate exerts differential effects on blood clotting, depending on polymer size. Blood 2010;116(20):4353–4359

30 Rao NN, Gómez-García MR, Kornberg A. Inorganic polyphosphate: essential for growth and survival. Annu Rev Biochem 2009;78:605–647

31 Szymusiak M, Donovan AJ, Smith SA, et al. Colloidal confinement of polyphosphate on gold nanoparticles robustly activates the contact pathway of blood coagulation. Bioconjug Chem 2016; 27(1):102–109

32 Donovan AJ, Kalkowski J, Szymusiak M, et al. Artificial dense granules: a procoagulant liposomal formulation modeled after platelet polyphosphate storage pools. Biomacromolecules 2016; 17(8):2572–2581

33 Ghosh S, Shukla D, Suman K, et al. Inositol hexakisphosphate kinase 1 maintains hemostasis in mice by regulating platelet polyphosphate levels. Blood 2013;122(8):1478–1486

34 Renné T, Pozgajová M, Grüner S, et al. Defective thrombus formation in mice lacking coagulation factor XII. J Exp Med 2005;202(2):271–281

35 Nickel KF, Spronk HM, Mutch NJ, Renné T. Time-dependent degradation and tissue factor addition mask the ability of platelet polyphosphates in activating factor XII-mediated coagulation. Blood 2013;122(23):3847–3849

36 Morrissey JH. Polyphosphate: a link between platelets, coagulation and inflammation. Int J Hematol 2012;95(4):346–352

37 Alvarenga PH, Xu X, Oliveira F, et al. Novel family of insect salivary inhibitors blocks contact pathway activation by binding to polyphosphate, heparin, and dextran sulfate. Arterioscler Thromb Vasc Biol 2013;33(12):2759–2770

38 Jain S, Pitoc GA, Holl EK, et al. Nucleic acid scavengers inhibit thrombosis without increasing bleeding. Proc Natl Acad Sci U S A 2012;109(32):12938–12943

39 Smith SA, Choi SH, Collins JN, Travers RJ, Cooley BC, Morrissey JH. Inhibition of polyphosphate as a novel strategy for preventing thrombosis and inflammation. Blood 2012;120(26):5103–5110

40 Travers RJ, Shenoi RA, Kalathottukaren MT, Kizhakkedathu JN, Morrissey JH. Nontoxic polyphosphate inhibitors reduce thrombosis while sparing hemostasis. Blood 2014;124(22):3183–3190

41 Labberton L, Kenne E, Long AT, et al. Neutralizing blood-borne polyphosphate in vivo provides safe thromboprotection. Nat Commun 2016;7:12616

42 Van Der Meijden PE, Van Schilfgaarde M, Van Oerle R, Renné T, ten Cate H, Spronk HM. Platelet- and erythrocyte-derived microparticles trigger thrombin generation via factor XIIa. J Thromb Haemost 2012;10(7):1355–1362

43 Nickel KF, Ronquist G, Langer F, et al. The polyphosphate-factor XII pathway drives coagulation in prostate cancer-associated thrombosis. Blood 2015;126(11):1379–1389

44 Oschatz C, Maas C, Lecher B, et al. Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo. Immunity 2011;34(2):258–268

45 Sala-Cunill A, Björkqvist J, Senter R, et al. Plasma contact system activation drives anaphylaxis in severe mast cell-mediated allergic reactions. J Allergy Clin Immunol 2015;135(4):1031–43.e6

46 Maas C, Govers-Riemslag JW, Bouma B, et al. Misfolded proteins activate factor XII in humans, leading to kallikrein formation without initiating coagulation. J Clin Invest 2008;118(9): 3208–3218

47 Zamolodchikov D, Chen ZL, Conti BA, Renné T, Strickland S. Activation of the factor XII-driven contact system in Alzheimer's disease patient and mouse model plasma. Proc Natl Acad Sci U S A 2015;112(13):4068–4073

48 Zamolodchikov D, Renné T, Strickland S. The Alzheimer's disease peptide β-amyloid promotes thrombin generation through activation of coagulation factor XII. J Thromb Haemost 2016;14(5): 995–1007

49 Göbel K, Pankratz S, Asaridou CM, et al. Blood coagulation factor XII drives adaptive immunity during neuroinflammation via CD87-mediated modulation of dendritic cells. Nat Commun 2016;7:11626

50 White-Adams TC, Berny MA, Patel IA, et al. Laminin promotes coagulation and thrombus formation in a factor XII-dependent manner. J Thromb Haemost 2010;8(6):1295–1301

51 van der Meijden PE, Munnix IC, Auger JM, et al. Dual role of collagen in factor XII-dependent thrombus formation. Blood 2009;114(4):881–890

52 Kannemeier C, Shibamiya A, Nakazawa F, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. Proc Natl Acad Sci U S A 2007;104(15):6388–6393

53 Oehmcke S, Mörgelin M, Herwald H. Activation of the human contact system on neutrophil extracellular traps. J Innate Immun 2009;1(3):225–230

54 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(4):819–835

55 Nickel KF, Renné T. Crosstalk of the plasma contact system with bacteria. Thromb Res 2012;130(Suppl 1):S78–S83

56 Bock PE, Srinivasan KR, Shore JD. Activation of intrinsic blood coagulation by ellagic acid: insoluble ellagic acid-metal ion complexes are the activating species. Biochemistry 1981; 20(25):7258–7266

57 Björkqvist J, Lecher B, Maas C, Renné T. Zinc-dependent contact system activation induces vascular leakage and hypotension in rodents. Biol Chem 2013;394(9):1195–1204

58 Siebeck M, Cheronis JC, Fink E, et al. Dextran sulfate activates contact system and mediates arterial hypotension via B2 kinin receptors. J Appl Physiol (1985) 1994;77(6):2675–2680

Downloaded by: Harvard Library. Copyrighted material.

59 Samuel M, Pixley RA, Villanueva MA, Colman RW, Villanueva GB. Human factor XII (Hageman factor) autoactivation by dextran sulfate. Circular dichroism, fluorescence, and ultraviolet difference spectroscopic studies. J Biol Chem 1992;267(27):19691–19697

60 Blossom DB, Kallen AJ, Patel PR, et al. Outbreak of adverse reactions associated with contaminated heparin. N Engl J Med 2008;359(25):2674–2684

61 Saito H, Ishihara T, Suzuki H, Watanabe T. Production and characterization of a murine monoclonal antibody against a heavy chain of Hageman factor (factor XII). Blood 1985;65(5):1263–1268

62 Pixley RA, Stumpo LG, Birkmeyer K, Silver L, Colman RW. A monoclonal antibody recognizing an icosapeptide sequence in the heavy chain of human factor XII inhibits surface-catalyzed activation. J Biol Chem 1987;262(21):10140–10145

63 Clarke BJ, Côté HC, Cool DE, et al. Mapping of a putative surface-binding site of human coagulation factor XII. J Biol Chem 1989; 264(19):11497–11502

64 Citarella F, Fedele G, Roem D, Fantoni A, Hack CE. The second exon-encoded factor XII region is involved in the interaction of factor XII with factor XI and does not contribute to the binding site for negatively charged surfaces. Blood 1998;92(11):4198–4206

65 Citarella F, te Velthuis H, Helmer-Citterich M, Hack CE. Identification of a putative binding site for negatively charged surfaces in the fibronectin type II domain of human factor XII–an immunochemical and homology modeling approach. Thromb Haemost 2000;84(6):1057–1065

66 Nuijens JH, Huijbregts CC, Eerenberg-Belmer AJ, Meijers JC, Bouma BN, Hack CE. Activation of the contact system of coagulation by a monoclonal antibody directed against a neodeterminant in the heavy chain region of human coagulation factor XII (Hageman factor). J Biol Chem 1989;264(22):12941–12949

67 Ravon DM, Citarella F, Lubbers YT, Pascucci B, Hack CE. Monoclonal antibody F1 binds to the kringle domain of factor XII and induces enhanced susceptibility for cleavage by kallikrein. Blood 1995;86(11):4134–4143

68 Citarella F, Aiuti A, La Porta C, et al. Control of human coagulation by recombinant serine proteases. Blood clotting is activated by recombinant factor XII deleted of five regulatory domains. Eur J Biochem 1992;208(1):23–30

69 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(222):222ra17

70 de Maat S, Björkqvist J, Suffritti C, et al. Plasmin is a natural trigger for bradykinin production in patients with hereditary angioedema with factor XII mutations. J Allergy Clin Immunol 2016; 138(5):1414–1423.e9

71 Potempa J, Herwald H. Kinins in bacterial infections. In: Bader M, ed. Kinins. Berlin, Germany: De Gruyter; 2012:307–320

72 Kuijpers MJ, van der Meijden PE, Feijge MA, et al. Factor XII regulates the pathological process of thrombus formation on ruptured plaques. Arterioscler Thromb Vasc Biol 2014;34(8):1674–1680

73 van Montfoort ML, Kuijpers MJ, Knaup VL, et al. Factor XI regulates pathological thrombus formation on acutely ruptured atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2014; 34(8):1668–1673

74 Cheng Q, Tucker EI, Pine MS, et al. A role for factor XIIa-mediated factor XI activation in thrombus formation in vivo. Blood 2010; 116(19):3981–3989

75 de Maat S, Maas C. Factor XII: form determines function. J Thromb Haemost 2016;14(8):1498–1506

76 Labberton L, Kenne E, Renné T. New agents for thromboprotection. A role for factor XII and XIIa inhibition. Hamostaseologie 2015;35(4):338–350

77 Björkqvist J, Sala-Cunill A, Renné T. Hereditary angioedema: a bradykinin-mediated swelling disorder. Thromb Haemost 2013; 109(3):368–374

78 Cichon S, Martin L, Hennies HC, et al. Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. Am J Hum Genet 2006;79(6):1098–1104

79 Björkqvist J, de Maat S, Lewandrowski U, et al. Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III. J Clin Invest 2015;125(8):3132–3146

80 Bernardi F, Marchetti G, Volinia S, et al. A frequent factor XII gene mutation in Hageman trait. Hum Genet 1988;80(2):149–151

81 Miyata T, Kawabata S, Iwanaga S, Takahashi I, Alving B, Saito H. Coagulation factor XII (Hageman factor) Washington D.C.: inactive factor XIIa results from Cys-571——Ser substitution. Proc Natl Acad Sci U S A 1989;86(21):8319–8322

82 Hovinga JK, Schaller J, Stricker H, Wuillemin WA, Furlan M, Lämmle B. Coagulation factor XII Locarno: the functional defect is caused by the amino acid substitution Arg 353–>Pro leading to loss of a kallikrein cleavage site. Blood 1994;84(4):1173–1181

83 Schloesser M, Hofferbert S, Bartz U, Lutze G, Lämmle B, Engel W. The novel acceptor splice site mutation 11396(G–>A) in the factor XII gene causes a truncated transcript in cross-reacting material negative patients. Hum Mol Genet 1995;4(7):1235–1237

84 Hofferbert S, Müller J, Köstering H, von Ohlen WD, Schloesser M. A novel 5′-upstream mutation in the factor XII gene is associated with a TaqI restriction site in an Alu repeat in factor XII-deficient patients. Hum Genet 1996;97(6):838–841

85 Schloesser M, Zeerleder S, Lutze G, et al. Mutations in the human factor XII gene. Blood 1997;90(10):3967–3977

86 Kanaji T, Okamura T, Osaki K, et al. A common genetic polymorphism (46. C to T substitution) in the 5′-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. Blood 1998; 91(6):2010–2014

87 Kondo S, Tokunaga F, Kawano S, Oono Y, Kumagai S, Koide T. Factor XII Tenri, a novel cross-reacting material negative factor XII deficiency, occurs through a proteasome-mediated degradation. Blood 1999;93(12):4300–4308

88 Kanaji T, Kanaji S, Osaki K, et al. Identification and characterization of two novel mutations (Q421. K and R123P) in congenital factor XII deficiency. Thromb Haemost 2001;86(6):1409–1415

89 Wada H, Nishioka J, Kasai Y, et al. Molecular characterization of coagulation factor XII deficiency in a Japanese family. Thromb Haemost 2003;90(1):59–63

90 Oguchi S, Ishii K, Moriki T, et al. Factor XII Shizuoka, a novel mutation (Ala392Thr) identified and characterized in a patient with congenital coagulation factor XII deficiency. Thromb Res 2005;115(3):191–197

91 Lombardi AM, Bortoletto E, Scarparo P, Scapin M, Santarossa L, Girolami A. Genetic study in patients with factor XII deficiency: a report of three new mutations exon 13 (Q501STOP), exon 14 (P547L) and -13C>T promoter region in three compound heterozygotes. Blood Coagul Fibrinolysis 2008;19(7):639–643

92 Feng Y, Ye X, Pang Y, Dai J, Wang XF, Zhou XH. A novel mutation in a patient with congenital coagulation factor XII deficiency. Chin Med J (Engl) 2008;121(13):1241–1244

93 Suzuki K, Murai K, Suwabe A, Ishida Y. Factor XII Ofunato: Lys346Asn mutation associated with blood coagulation factor XII deficiency causes impaired secretion through a proteasome-mediated degradation. Thromb Res 2010;125(5):438–443

94 Kwon MJ, Kim HJ, Lee KO, Jung CW, Kim SH. Molecular genetic analysis of Korean patients with coagulation factor XII deficiency. Blood Coagul Fibrinolysis 2010;21(4):308–312

95 Matsuki E, Miyakawa Y, Okamoto S. A novel factor XII mutation, FXII R84P, causing factor XII deficiency in a patient with hereditary spastic paraplegia. Blood Coagul Fibrinolysis 2011;22(3):227–230

96 Kim HJ, Kim HJ, Kwon EH, Lee KO, Park IA, Kim SH. Novel deleterious mutation in the F12 gene in a Korean family with severe coagulation factor XII deficiency. Blood Coagul Fibrinolysis 2010;21(7):683–686

Downloaded by: Harvard Library. Copyrighted material.

97 Matsukuma E, Gotoh Y, Kuroyanagi Y, et al. A case of atypical hemolytic uremic syndrome due to anti-factor H antibody in a patient presenting with a factor XII deficiency identified two novel mutations. Clin Exp Nephrol 2011;15(2):269–274
98 Ye X, Feng Y, Ding Q, Dai J, Wang X. Genetic analysis of a pedigree with combined factor XII and factor XI deficiency. Blood Coagul Fibrinolysis 2011;22(2):118–122
99 Iijima K, Arakawa Y, Sugahara Y, et al. Factor XII Osaka: abnormal factor XII with partially defective prekallikrein cleavage activity. Thromb Haemost 2011;105(3):473–478
100 Zhang Y, Xie HX, Wang MS, Jin YH, Xie YS, Zheng FX. Analysis of an hereditary coagulation factor XII deficiency in a consanguineous pedigree [in Chinese]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2011;28(6):666–669
101 Singhamatr P, Kanjanapongkul S, Rojnuckarin P. Molecular analysis of factor XII gene in Thai patients with factor XII deficiency. Blood Coagul Fibrinolysis 2013;24(6):599–604
102 Xie H, Lv M, Yang X, et al. Identification of a novel mutation of factor XII gene in a family with coagulation FXII deficiency [in Chinese]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2013;30(3): 313–317
103 Li M, Xie H, Wang M, Ding H. Molecular characterization of a novel missense mutation (Asp538Asn) in a Chinese patient with Factor XII deficiency. Clin Lab 2015;61(12):1967–1971
104 Jin P, Jiang W, Yan H, et al. Novel mutations in congenital factor XII deficiency. Front Biosci (Landmark Ed) 2016;21:419–429
105 Yang L, Wang Y, Zhou J, et al. Identification of genetic defects underlying FXII deficiency in four unrelated Chinese patients. Acta Haematol 2016;135(4):238–240
106 Cool DE, MacGillivray RT. Characterization of the human blood coagulation factor XII gene. Intron/exon gene organization and analysis of the 5′-flanking region. J Biol Chem 1987;262(28): 13662–13673
107 Tripodi M, Citarella F, Guida S, Galeffi P, Fantoni A, Cortese R. cDNA sequence coding for human coagulation factor XII (Hageman). Nucleic Acids Res 1986;14(7):3146
108 Kaplan AP, Silverberg M. The coagulation-kinin pathway of human plasma. Blood 1987;70(1):1–15
109 Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem Biophys Res Commun 2006;343(4):1286–1289
110 Bork K, Wulff K, Meinke P, Wagner N, Hardt J, Witzke G. A novel mutation in the coagulation factor 12 gene in subjects with hereditary angioedema and normal C1-inhibitor. Clin Immunol 2011;141(1):31–35
111 Kiss N, Barabás E, Várnai K, et al. Novel duplication in the F12 gene in a patient with recurrent angioedema. Clin Immunol 2013; 149(1):142–145

Downloaded by: Harvard Library. Copyrighted material.