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**Hereditary Angioedema:
looking for bradykinin production and triggers of vascular permeability**

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Abstract

Since the Osler's identification of the inherited nature of Hereditary Angioedema, a huge array of information was collected on pathogenetic mechanisms of the disease. Over the last years, information grew fast and mutations in different genes, in addition to *C1-inhibitor*, were found to be causative. All types are inherited as autosomal-dominant traits with incomplete penetrance and little or no genotype-phenotype correlation. As a result, the clinical expression is characterized by a large heterogeneity. The acknowledgement of mechanisms leading to heterogeneity of the clinical phenotype is likely to provide important information not only for a better understanding of the pathogenesis but also for therapy. Regardless of which gene is mutated, similar pathways seem to play a pivotal role, triggering the up-regulation of contact activation system/kallikrein kinin system and giving rise to an unbalanced increase of bradykinin. However, notwithstanding the increase of bradykinin in bloodstream, the phenomenon is localized and no general vascular leakage and oedema is recognized. Thus, is conceivable that there exist one or more localized factors that stimulate the production of bradykinin, which does not become a systemically event. Uncovering of these factors may shed lights on the missing part of the pathogenesis of Hereditary Angioedema. The present review, collecting information on pathogenesis from biochemical and genetics investigations, tries to provide a comprehensive view of the pathogenesis of Hereditary Angioedema. This can allow for a better understanding of the disease and lead to focused investigations that can further improve our knowledge.

History of a search for the (genetic) pathogenesis

Angioedema is now a well-known acute transient phenomenon due to increased vascular permeability allowing diffusion or extravasation of fluid from the vascular bed to the interstitial space. It results from overproduction or failure to inactivate vasoactive stimulants such as histamine, bradykinin, and leukotrienes.¹ In a seminal work, the hereditary component of the disease was firstly identified by Sir William Osler, in 1888, who provided a detailed description of an index case, a 24-year-old woman (Mrs. H) with recurrent episodes of non-pruritic swelling, and other 28 family members over five generations with recurrent attacks of oedema, thus recognizing “hereditary angio-neurotic oedema”.² The biochemical basis for hereditary angioedema (HAE) – the absence of C1-inhibitor (C1-INH) – was discovered several decades later and first published by Donaldson and Evans in 1963.³ Since then, a large series of studies ascertained the up-regulation of contact activation system/kallikrein kinin system, inhibited at several points from C1-INH, and in turn an increasing of bradykinin, the mediator responsible for vascular hyperpermeability and appearance of recurrent angioedema. Acknowledgment of the link between complement system, including C1-INH, and bradykinin-forming contact system led to the development of a series of therapies for HAE.

Starting from the late ‘80s, several hundreds of different mutations throughout the C1-INH gene (*SERPING1*) have been reported,^{4,5} which account for a large number of HAE cases and most families (OMIM #106100). In addition to mutations in *SERPING1*, mutations in coagulation Factor XII (FXII) gene (*F12*) were found to account for a variant of HAE characterized by normal activity of C1-INH in plasma (OMIM # 610618).⁶ The pathogenesis was defined and the same mediator appears responsible also for FXII-HAE.^{7,8} In patients without *SERPING1* or *F12* mutations and a positive family history of swelling attacks, a third form of HAE was described (U-HAE) that showed penetrance and symptoms very similar to other HAEs.⁹ Causes of disease were unknown and the underlying genetic defects remained not identified.¹⁰ Observation that the phenotype, clinical symptoms and swellings, is largely similar among the various HAE types and that pruritic rash is not a typical feature in U-HAE patients, indicated that histamine is not of critical importance for the pathology of the disease.¹¹ On the other hand, the efficacy of treatment with bradykinin-2 (B2) receptor antagonists in many attacks gave the clinical evidence that bradykinin is the mediator responsible for the disease.¹² Very recently, mutations in the plasminogen gene (*PLG*), in the Angiotensin 1 gene (*ANGPT-1*), and in the kininogen gene (*KNG1*) have been found in a sub-setting of U-HAE patients.^{13,14,15} Treatment of antihistamines,

combined or not with corticosteroid, was not effective, whereas the use of B2 receptor antagonists was effective in a high number of attacks and prophylaxis with oral tranexamic acid reduced the number and severity of acute episodes.

Over about 150 years, information grew fast and acknowledged that mutations in different genes are responsible for HAE by means of similar pathways that trigger the up-regulation of contact activation system/kallikrein kinin system and give rise to an unbalanced increase of bradykinin (Fig. 1).

However, challenging open questions remain to be answered: 1) Is really HAE a monogenic/monofactorial disease? 2) Why swelling attacks are a localized event, which does not give rise to a generalized phenomenon? 3) Which is (are) the trigger(s) for the contact activation system/kallikrein kinin system and bradykinin upregulation?

Lightening the dark side of the pathogenesis

From genotype to phenotype: a complex relationship

Modelling genetic findings is of great importance in determining the clinical significance of a gene variant. Under the monogenic-like model, a trait is determined by the expression of a single (*target*) gene or allele and genetic variants have large effects on disease status, whereas a multifactorial model includes the influence of many genetic and environmental factors. Single-gene diseases run in families and are typically inherited in a classical Mendelian fashion (e.g. dominant or recessive). In a multifactorial disease several loci, but not an unlimited number, are involved in the expression of the trait; the loci act in concert in an additive fashion, each adding or detracting a small amount from the phenotype; and the environment interacts with the genotype to produce the final phenotype. None of them is either necessary or sufficient for the disease to occur but makes it more likely that one will become ill. In single-gene diseases, the occurrence of a mutation in the *target* gene is responsible for disease. In multifactorial ones, the liability to develop the disease is continuously distributed in the population because of the additive effects of genetic and environmental factors.

Often, the phenotypic heterogeneity of what is considered a monogenic disorder is further beyond disease-causing genes that have been recognized. In many monogenic diseases, although the causal *target* gene is well known, individuals carrying the same causal mutation either do not always develop the disorder, an effect called incomplete penetrance, or do not present the same

clinical symptoms, a phenomenon known as variable expressivity. All types of HAE are inherited as autosomal-dominant traits with incomplete penetrance and little or no genotype-phenotype correlation has been detected.¹⁶ Indeed, patients with HAE carrying the same mutation exhibit large variability of frequency and severity of symptoms, even among siblings. As a result, the clinical expression is characterized by a large inter- and intra-familial heterogeneity in regard to the age at disease onset, the number of attacks, triggers of acute episodes, the severity and localization of oedema, and prodromal signs and symptoms.¹⁷

Several factors other than different genes involved in the different sub-entities of the disease might help to explain disease variable penetrance or expressivity and significantly impact the ability to connect genotype to phenotype, as environmental factors and genetic background (*modifier* genes). Environmental factors may act as triggers for the occurrence of acute events. In HAE, known triggers, i.e. emotional distress, physical trauma and infection do not appear to directly result in swelling, but rather reduce the threshold for the initiation of an attack.¹⁸ Actually, the same trigger may not always provoke an attack in an individual patient; and triggers may change for an individual patient over time. Finally, many attacks occur without the presence of an identified trigger.^{19,20}

Modifier genes can explain disease expression variability, modulating the expression of the primary mutation in the *target* gene involved in the disease.^{21,22} *Modifier* genes have to be recognized as a genetic effect that is able to change the phenotypic outcome of a variant at the causal locus. *Modifier* genes have no obvious phenotype of their own and are effectively silent with respect to the phenotype under study, in the absence of the causative mutation. In this respect, *F12* represents a unique condition, because it embodies the role of both *target* and *modifier* gene. Indeed, besides known mutations causing the FXII-HAE, *F12* also harbours a gene variant that modulates the HAE phenotype. In HAE patients carrying a *SERPING1* mutation, the *F12* functional promoter polymorphism -46C/T modulates the age of onset of symptoms and the penetrance of the disease, the TT genotype being significantly associated with a 7-year delay and a 25-fold lower risk of developing symptoms.^{23,24} The T allele is acknowledged to affect the translation efficiency of the gene and results in low plasma levels of FXII activity and antigen.²⁵ In addition, other common variants within different genes involved in the kallikrein kinin system have been suggested to modulate the occurrence of the disease.²⁶

The traditional view of genetic diseases as monogenic or multifactorial is no longer supported and requires a different way of thinking towards factors that modify gene expression and clinical phenotype.

There is ample evidence that HAE has variable clinical pictures, even in patients carrying the same mutation. Looking only at which *target* gene is involved or the individual mutation occurring in each patient is an oversimplification of biological phenomena not sufficient to explain clinical phenotype. An encompassing model able to understand genetic modification phenomena, namely the effect of one or more *modifier* genes, will improve our ability to unravel HAE. Although much effort done in identifying genetic modifiers, a better knowledge of pathological mechanisms leading to phenotypic diversity needs. Knowledge of such mechanisms not only will allow to identify yet unknown genetic components of the disease, but also is likely to provide greater accuracy for phenotypic predictions and important information for therapy, as for other monogenic diseases.²⁷⁻²⁹

Swelling attacks: a localized event not a generalized phenomenon

An uncontrolled production of bradykinin by plasma contact system appears the common step for the development in most of all known types of HAE. Indeed, mice in which *C1-inh* has been knocked out present a constitutive increase in vascular permeability.³⁰ However, they do not spontaneously develop angioedema.

On the other hand, an excessive activation of contact system can cause hypotension, as observed in animal models and in patients who were administered injection of heparin contaminated with oversulfated chondroitin sulfate. The anaphylactoid response to contaminated heparin, which included also facial swelling, tachycardia, urticaria, and nausea, seemed to be modulated by circulating C1-INH levels.³¹⁻³³

In HAE, notwithstanding an increase of bradykinin in bloodstream, the phenomenon is localized and no general vascular leakage and oedema is recognized.³⁴ Thus, is conceivable that there exist one or more localised factors that stimulate the production of bradykinin, which does not become a generalised event. Uncovering of these factors may shed lights on the missing part of the pathogenesis of HAE.

FXII activation is the initial stage of contact system and modulates vascular permeability. A series of substances, such as calcium-preadsorbed Polyphosphate, have been reported to cause an increase of capillary leakage via activating FXII and initiating bradykinin formation in wild-type

mice, but not in mice lacking FXII or B2 receptor.³⁵ FXII is surface-activated and has limited proteolytic activity, when bound to contact-activating surfaces without undergoing proteolysis. The surface induces a conformational change of bound FXII and facilitates its activation³⁶ to α -FXIIa, which is responsible for FXII autoactivation.³⁷ During contact activation FXII, prekallikrein (PK) and coagulation Factor XII (FXI) bind to an activating surface.³⁸ Then, the formed α -FXIIa converts PK to kallikrein (KK) in presence of high molecular weight kininogen (HMWK). Additionally, α -FXIIa can be further cleaved by KK to generate β -FXIIa, which maintains the capability to activate PK but not FXI or FXII, and is able to dissociate from the surface.³⁹

Over the last years, it was ascertained that *F12* is a *target* gene for HAE. Information was gathered on biological processes that could help to explain how some variants of the factor that is considered to be the starting point of intrinsic coagulation pathway may affect HAE.^{7,8} Patients carrying *F12* variants did not show any abnormality of the coagulation pathway and, more importantly, of activated partial thromboplastin time test (aPTT). On the other hand, none of individuals presenting with *F12* variants that severely impair FXII production, and give a huge aPTT prolongation, has angioedema attacks. Thus, a different class of gene mutations is conceivable to occur in patients with HAE. After the demonstration that (some) *F12* variants associated with HAE give rise to a protein that shows a different pattern of glycosylation and is more prone to plasmin and KK activation,^{7,40} it seems clear that loss of function gene variants are causative of FXII reduction (and aPTT prolongation), whereas gain of function variants seemingly pave the way to HAE attacks.

An additional piece of the puzzle went from the detection of a unique *PLG* mutation in a series of HAE patients (and families).¹³ Although no experimental data exists that can help to understand the molecular mechanism, alignment with other protein containing *kringle* domains and the high conservation of the residue among different species strongly suggest that the substitution acts through a gain of function. Actually, the K330E variant may allow for a higher binding ability of the mutant protein to interact with exposed lysine residues of protein ligands.⁴¹ To further support that an increased action of plasmin is associated with HAE, patients carrying a severe loss of function *PLG* variant suffer from thickening of membranes, mainly *ligneous* conjunctivitis and gingivitis, but none has angioedema attacks.⁴² Finally, the reduction of attacks observed using antifibrinolytics, strengthens the biological plausibility of the mechanistic role of contact system activation in the pathogenesis.¹³

In patients with ischemic stroke, administration of recombinant tPA is associated with an increased risk of angioedema. When an attack occurs, patients usually manifest a hemi-oro-lingual oedema during or shortly after tPA administration that can be life threatening. A significant generation of bradykinin has been demonstrated after tPA infusion that is responsible for oedema and vascular leakage.⁴³ The effect was prevented in PK (*Klkbl*)-deficient and FXII-deficient mice and in wild-type mice treated with icatibant, a B2 receptor antagonist.^{43,44} Notably, most HAE patients carrying the *PLG* K330E variant suffered from tongue swellings.¹³ All this information derived from naturally occurring and drug-induced increase of plasmin formation conveys the hypothesis that plasmin generation plays a key role in the development of angioedema via FXII-driven activation of contact system.

Plasminogen takes also part into a well-known complex metabolic pathway; the fibrinolytic system.⁴⁵ Fibrinolysis is active at low rate in normal haemostasis. This is acknowledged by D-dimer formation, a fibrin degradation product. During a thrombotic episode, fibrinolysis is accelerated and D-dimers hugely increase.⁴⁶ Alpha(2)-antiplasmin (A2AP) is the primary inhibitor of plasmin. A2AP rapidly and specifically inactivates plasmin free circulating in bloodstream, resulting in the formation of a stable inactive complex, plasmin-A2AP.⁴⁷ In addition, A2AP is cross-linked to fibrin clot by coagulation factor XIII (FXIII) and binds to free plasmin, further modulating fibrinolysis. Speculation on how fibrinolytic pathway works may further add strength to the hypothesis that angioedema attacks follow a local activation of contact system and are not the consequence of a systemic process. In fibrinolysis, plasmin is active on a fibrin surface. Likewise, in the activation of contact system plasmin generation occurs on a surface, like endothelial cells. Indeed, endothelial cells present a heterotetramer, composed of 2 molecules of annexin A2 and 2 of the protein S100A10, which binds to plasminogen and colocalises with the complex formed by urokinase-type plasminogen activator and its receptor.⁴⁸ Colocalization of plasminogen and its activator results in a fast generation of plasmin. Plasmin generation is limited to cell surface and modulates extracellular matrix remodelling and angiogenesis.⁴⁹ In thrombosis, D-dimers can be detected in bloodstream. However, the formation occurs after plasminogen binds to lysine residues of fibrin clot and becomes activated to plasmin; that is a local phenomenon. Likewise, in HAE attacks plasmin-A2AP complex and biomarkers for contact activation can be measured in plasma. Nevertheless, free KK activity was detected in blister fluid and higher levels of bradykinin were measured in blood from oedematous arm.^{50,51} These data do not indicate a generalized plasmin activation.

Loss of function mutations occurring in the A2AP gene (*SERPINF2*) give rise to a hyperfibrinolytic state and cause a rare bleeding disorder, because of an increased plasmin activity. Bleeding tends to occur in response to local haemostatic challenges, such as trauma, surgery or dental procedures.⁵² Antifibrinolytics effectively treat bleeding episodes. Nonetheless, angioedema attacks were not reported in homozygous patients lacking A2AP.⁵³ Both in PLG-HAE and A2AP deficiency an increase of plasmin generation occurs but diseases have very different clinical phenotypes. In haemostasis, the key role of A2AP is not to inhibit plasmin-mediated fibrinolysis but to prevent premature clot dissolution. When A2AP lacks, formation of a physiologic fibrin clot is hampered and dissolution accelerated.⁵⁴ Plasmin generation plays a role in matrix remodelling and angiogenesis.⁵⁵ During activation of contact system, cellular receptor-mediated binding of plasminogen protects newly generated plasmin from inactivation by A2AP. When A2AP lacks, there is not an excess of plasmin activity,⁵⁶ because of the effect of a series of inhibitors. A2AP inhibitor fails to stop KK, FXIa, and FXIIa production; C1-INH does. It is attractive to hypothesize that inhibitors such as C1-INH do not inhibit matrix remodelling and angiogenesis but prevents an excessive plasma leakage. When the inhibitory effect of the C1-INH fails or is overcome, an angioedema attack realistically occurs. This view reconciles findings from different human genetic diseases and helps to better understand the pathogenesis of HAE.

Looking for the local trigger(s)

Predisposing circumstances, such as infection and trauma, may trigger acute episodes of angioedema. It could be speculated that localized pathogen invasion may precede swelling. Indeed, contact system becomes activated in response to viral and bacterial infections and stimulates bradykinin release, turning on an increase of endothelial cell permeability, which is hampered by the addition of a FXII inhibitor or B2 receptor antagonist.⁵⁷⁻⁵⁹

Non-fibrinolytic activity of plasmin occurs on the surface of cells, such as endothelial cells, and is essential for matrix remodelling, a key event that allows cell migration.⁶⁰ In physiologic conditions, the activation of contact system is counterbalanced by a series of inhibitors, as plasminogen activator-inhibitors PAI-1 and PAI-2, in addition to C1-INH. During HAE acute attacks, up-regulation of the Urokinase Plasminogen Activator Receptor gene (*uPAR*) has been shown to occur. The following increase of bradykinin suggests that a local plasmin generation plays an important role in the development of endothelial leakage.⁶¹ It is attractive to speculate that the occurrence of some different, and possibly external, stimuli provokes a local generation of

plasmin, which overwhelms plasmin inhibitors. In this scenario, a localised downregulation of inhibitors should result in the deliverance of bradykinin at the site where vascular leakage and oedema will follow.

Vascular endothelial growth factor (VEGF) is produced by vascular smooth muscle cells and induces blood vessel permeability and also promotes the formation and maintenance of capillary fenestrations.⁶²⁻⁶⁴ VEGF produces, if not offset, important effects on cell-cell interaction and disposition of cytoskeleton. In animal models, overexpression of VEGF induced leakage from blood vessels and VEGF antagonism reduced lung vascular leak.⁶⁵ In addition, increased concentrations of VEGF have been found in different human disorders characterized by increased vascular permeability, such as systemic capillary leak syndrome⁶⁶ and sepsis.⁶⁷ Finally, partial impairment of mechanism modulating VEGF stimulation on endothelial cells, such as an *ANGPT1* loss of function mutation, stimulates vascular leakage.⁶⁸

As compared with healthy controls, patients with C1-INH-HAE in remission displayed higher plasma levels of VEGF, particularly of VEGF-A the form that specifically exerts an effect on endothelial cells. Interestingly, plasma levels were even higher in patients with C1-INH-HAE experiencing more than 12 attacks per year in the last year and in those showing C1-INH plasma levels <30%.⁶⁹ Thus, VEGF appears to further reduce the threshold for vascular leakage already low for the occurrence of a series of gene variants.

In this scenario, an intriguing hypothesis is that VEGF, and possibly other acquired, circumstantial factors, represent a conditioning environment and act as a trigger to locally stimulate endothelial cells and/or contact system to prime the bradykinin generation on surfaces (Fig. 2). While this production is finely tuned in normal subjects, mainly by C1-INH, in HAE, where C1-INH plasma levels are genetically reduced or overwhelmed by hyperfibrinolysis or other conditions (e.g. reduced effect of Angiopoietin-1), a higher vascular permeability occurs and angioedema develops. This landscape might represent a working hypothesis that reconciles biological and clinical findings in which genetic predisposition (major gene variants), susceptibility factors (modifier genes) and local events (triggers) melt to yield HAE attacks.

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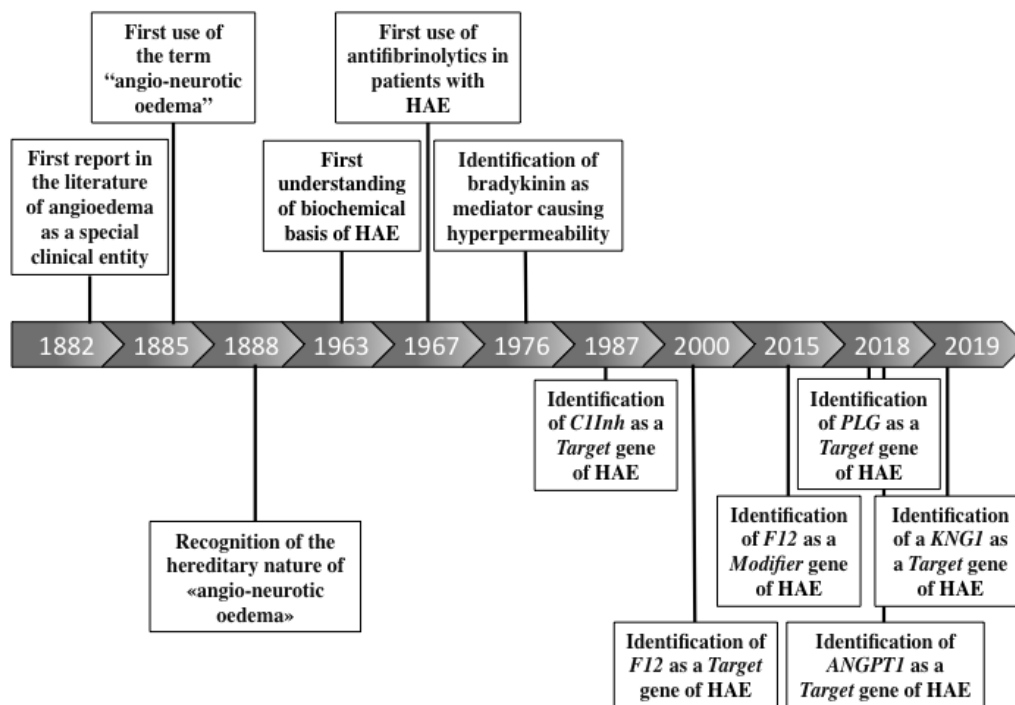
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Legend to the figures

Fig.1. Graphic sketch of the history of acknowledgements on biochemistry and genetics of HAE pathogenesis. HAE: hereditary angioedema. ANGPT1: angiopoietin-1; C1-INH: C1-inhibitor; F12: gene coding for the coagulation factor XII (FXII); KNG1: gene coding for high molecular weight kininogen; PLG: plasminogen.

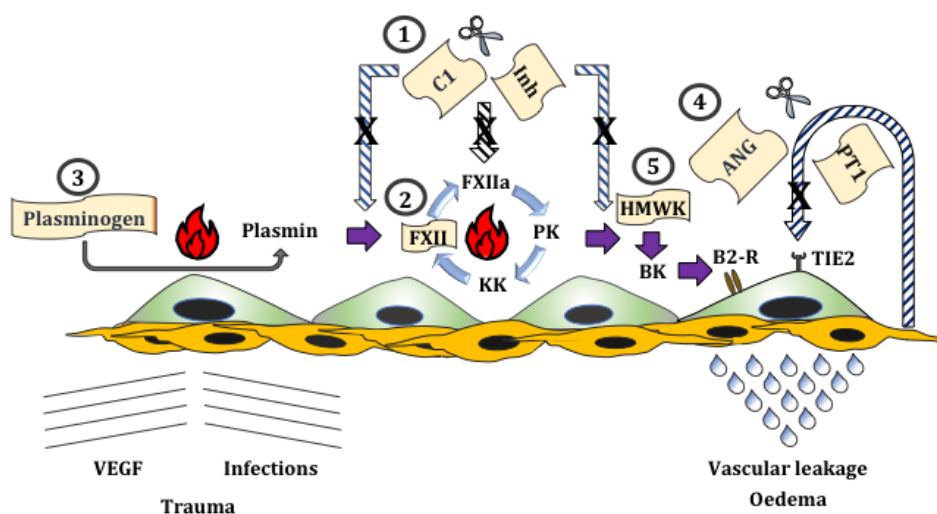
Fig. 2. Schematic view of the proposed pathway of regulation of the contact phase activation and bradykinin production. A series of stimuli, as infection, trauma, and substances liberation (VEGF, PolyP) gives rise to plasmin production and, in turn, to FXII and KK activation, which stimulate each other. Then, a burst of bradykinin is produced that stimulates endothelial cells. The C1Inh acts as the key regulator of the pathway, whereas effects produced by bradykinin are regulated by the action of ANGPT1. In HAE, *C1-INH* loss of function (1) mutations impair the regulation of the contact phase activation, whereas *F12* (2), coding for FXII, and *PLG* (3) gain of function mutations much more stimulate the contact phase. An *ANGPT1* (4) loss of function mutation impairs the regulation of endothelial cells permeability. Finally, a *KNG1* variant (5), coding for HMWK, was found to cosegregate with HAE. ANGPT1: angiopoietin-1; B2R: bradykinin type-2 receptor; BK: bradykinin; C1-INH: C1-inhibitor; F12: gene coding for the coagulation factor XII (FXII); HMWK: high molecular weight kininogen; KK: kallikrein; KNG1: gene coding for HMWK; PK: prekallikrein; PLG: plasminogen; TIE-2: endothelial cell receptor for ANGPT1.  : burst of production;  : low functionality.

Fig. 1



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Fig. 2



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