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Pathophysiology of Hereditary Angioedema (HAE) Beyond the *SERPING1* Gene

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Abstract

Hereditary Angioedema (HAE) is an autosomal dominant disorder characterized clinically by recurrent episodes of swelling involving subcutaneous tissues, gastrointestinal tract, and oro-pharyngeal area. Gene mutations are the most common genetic cause of HAE and observed in more than 90% of patients. More than 700 mutation variants have been described so far. Patients with angioedema who have no mutations in the gene for C1-INH and normal levels and activity of this inhibitor are labelled: normal C1 inhibitor HAE. These include genetic mutations in factor 12 gene, plasminogen gene, angiopoietin gene, kininogen 1, and myoferlin genes. The clinical manifestations of patients with these mutations are similar to with patients with C1-INH gene mutations. However, a later age of onset, oro-pharyngeal involvement, and higher female preponderance have been reported in these rare subtypes of hereditary angioedema. With the advent and increased accessibility of whole-exome sequencing, it is expected that new genetic defects and novel pathophysiological pathways will be identified in families with HAE of unknown cause or normal C1-INH angioedema. This review covers some of the recent advances in the field of HAE. The review focuses on pathophysiology of HAE beyond the well-known C1-INH deficiency phenotypes, including various biomarkers that can serve the diagnosis and management of these rare disorders.

Keywords Angiopoietin · Factor XII · Hereditary · Angioedema · Kininogen · Myoferlin · Plasminogen

Abbreviations

ACE-I	Angiotensin convertase enzyme inhibitors
ANGPT1	Angiopoietin gene
ARB	Angiotensin receptor blockers
AP2	Amino peptidase-2
BK	Bradykinin
C1-INH	C1-inhibitor
EREs	Estrogen responsive elements
FXIa	Activated factor XI
FXII	Factor XII
F12	Factor 12 gene,
GWAS	Genome-wide association studies
HAE	Hereditary angioedema
KKS	Kallikrein-kinin system
KNG1	Kininogen 1,
LK	Low molecular weight kininogens
MYOF	Myoferlin gene
nC1-INH-HAE	Normal C1 inhibitor HAE
PLG	Plasminogen gene
siRNA	Small interfering RNA
Tie-2	Tunica interna endothelial cell kinase 2
U-HAE	HAE with unknown mechanism
VEGF	Vascular endothelium growth factor
VEGFR2	Vascular endothelial growth factor receptor-2
WES	Whole-exome sequencing

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Introduction

The term "Angioedema" is a combination of angio (blood or lymph vessels) and oedema (swelling) and refers to swelling of vascular origin [1]. Angioedema may be hereditary or acquired. Hereditary angioedema (HAE) is an autosomal

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dominant disorder characterized clinically by recurrent episodes of swelling involving the subcutaneous tissue, oro-pharyngeal mucosa, and gastrointestinal tract [2]. There has been significant progress in our understanding of pathophysiology of HAE. This is based on two landmark discoveries: (i) identification of C1-inhibitor (C1-INH) protein deficiency in serum of patients with C1-INH-HAE (Type I and Type II) and (ii) identification of bradykinin (BK) as the main mediator of swelling. Mutation in the gene coding for C1-INH, SERPING1 gene, are the most common genetic cause of C1-INH-HAE and are seen in more than 90% of patients. Patients who have no mutations in *SERPING1* gene are referred as normal C1-inhibitor HAE (nC1-INH-HAE) [3, 4].

New Types of Hereditary Angioedema

Several other genetic mechanisms also have a role in pathogenesis of HAE [3, 5]. These include genetic defects in *factor 12* gene (*F12*), *plasminogen* gene (*PLG*), and *angiopoietin* gene (*ANGPT1*) [3, 6–9]. Recently, defects in *kininogen 1* (*KNG1*) and *myoferlin* (*MYOF*) genes have also been identified in some patients with nC1-INH-HAE [10, 11]. With the advent and increased accessibility of modern genetic technologies, such as whole-exome sequencing (WES), it is expected that new genetic defects will also be identified in families with nC1-INH-HAE and HAE with unknown mechanism (U-HAE).

In this review, we attempt to cover some of the recent advances in the field of the new types of HAE, beyond the more familiar SERPING1 mutations typical for C1-INH-HAE.

Pathophysiology and Genetics of Coagulation Factor XII Gene Mutation (FXII-HAE)

Factor XII (FXII) is a multi-domain serine protease first identified by Ratnoff et al. in 1955 [12]. It is present as inactive zymogen in human plasma and activated upon contact with negatively charged surfaces and by plasma kallikrein (PKa). PKa is generated from pre-kallikrein (PK) by the activated form of FXII (FXIIa). Furthermore, high molecular weight kininogen (HK) acts as a co-factor for reciprocal activation of PK and FXII [13]. FXII is a mosaic protein with 19 bp long leader peptide and 596 amino acid long mature plasma protein that can become activated by PKa, plasmin, and other proteases. FXII is also activated through an autoactivation loop that is normally controlled by C1-INH [14]. *F12* gene is composed of 14 exons, and its promoter region shows similarity with the gene for estrogen responsive elements (EREs). Moreover, estrogen has been shown to increase concentration of FXII in plasma and excess estrogen may produce clinical features mimicking HAE [15, 16].

Mutations in *F12* gene have been associated with familial angioedema [17–22] and account for up to 25% patients with nC1-INH-HAE [23]. This rare type of HAE was first described separately by Bork et al. and Binkley et al. in 2000, in families in which females only were affected and all affected had normal levels and function of C1-INH and normal C4 [24, 25]. It was initially thought to be inherited in an X-linked dominant pattern, but the actual genetic defect was identified in 2006 [9]. Missense mutations (c.1032C > A, p.Thr309Lys and c.1032C > G, p.Thr309Arg) are the most frequently detected at exon 9 of *F12* gene by several authors. Five different mutations located in a region coding for proline-rich linker peptide, between the kringle and trypsin-like serine protease domains, have been identified in this gene. These include 2 missense variants in exon 9 (c.1032C > A, p.Thr309Lys and c.1032C > G, p.Thr309Arg), a 72 bp deletion variant at exon 9-intron 9 junction (c.971_1018 + 24del72), 18 bp duplication (c.892_909dup) on exon 9, and another missense mutation on exon 10 (c.1027G > C, Ala324Pro) [17, 23, 26, 27]. These putatively *gain of function* mutations in *F12* gene show autosomal dominant pattern of inheritance and lead to over-production of FXIIa [16, 28, 29]. However, a few reports have refuted the concept of such gain-of-function activity. Bork et al. showed no differences in surface expression of FXII or kallikrein-like activity in these patients [30]. Normally, FXIIa further leads to overproduction of PKa and subsequently an increased production of BK (Fig. 1a and b). In nC1-INH-HAE It has been shown that the mutations: c.1032C > A, p.Thr309Lys, c.1032C > G, p.Thr309Arg, and c.971_1018 + 24del72, increase the sensitivity of the mutant FXII protein for cleavage of plasmin [16]. Plasmin cleaves the variant FXII protein at the mutated site (i.e. proline rich domain) and separates its surface binding domain from the protease domain.

Similar effects may also be produced by thrombin and activated factor XI (FXIa) in patients with c.1032C > A, p.Thr309Lys and c.1032C > G, p.Thr309Arg mutations [30]. As a result, the activation loop of FXII is exposed and FXII becomes overtly sensitive to activation for plasmin or PKa. The same pathogenic mechanism has, however, not been reported in patients with c.892_909dup and c.1027G > C, p.Ala324Pro mutations. Therefore, the pathogenic mechanisms for increased BK production in patients with these 2 mutations remain unclear [27].

Based on these pathophysiological models, a small interfering RNA (Si-RNA) that would bind to and stop the transcription of FXII mRNA may become a potential prophylactic treatment for patients with HAE [31]. Two different mutations in *F12* gene (c1681-1G > A [intron 13] and c1027G > C [Exon 10] were also observed in

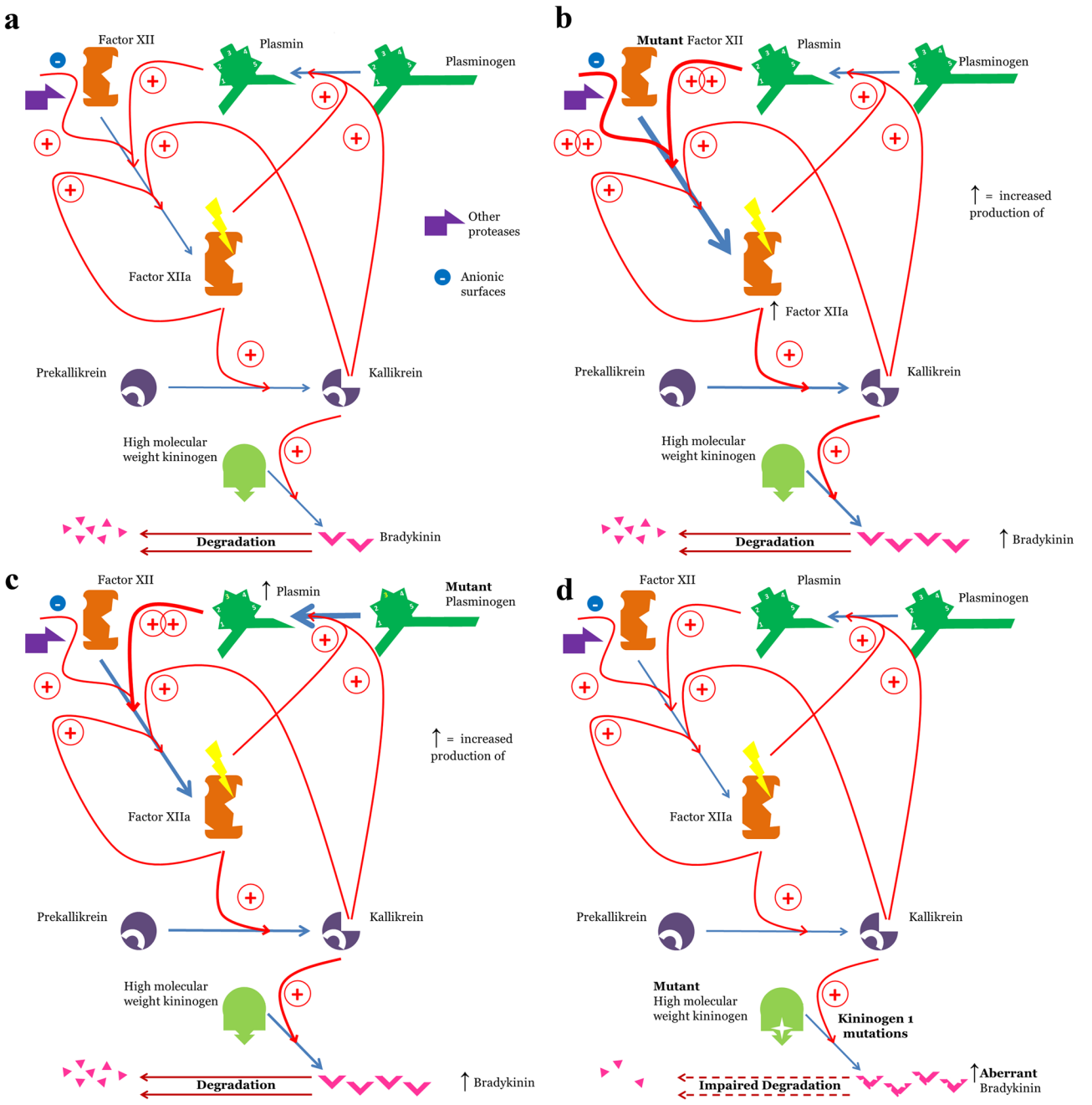


Fig. 1 The bradykinin generation pathway. **a** Normal individuals—FXII is activated through contact activation, facilitated by plasmin. XIIa facilitates conversion of PKa facilitates activation of FXII and PLN. **b** *F12* gene mutation—pathophysiology of FXII-HAE. As a result of mutation FXII becomes overtly sensitive to activation by plasmin, proteases, and anionic surfaces leading to enhanced production of activated XIIa. The end result is an increased production of BK that leads to angioedema. **c** *PLG* gene mutation- pathophysiology of PLG-HAE: Mutated Kringle 3 domain has been shown in a triangular shape (as opposed to rectangle with smooth corners) to denote increased affinity for corresponding ligands. There is increased affinity of mutant PLN towards tissue activators leading to overproduction of PL, XIIa, and BK. **d** *KNG-1* gene mutation—pathophysiology of KNG1-HAE. *KNG1* gene mutation affects both HK and LK isoforms as shown and produces an aberrant BK which is more resistant to degradation by various enzymes leading to its overactivity

patients with idiopathic nonhistaminergic angioedema. However, it is conjectural whether these mutations are primarily responsible for the disease per se. It is possible that some other gene polymorphisms associated with breakdown of BK may also have a role in nC1-INH-HAE [23].

Clinically, patients with FXII-HAE suffer similar manifestations to patients with C1-INH-HAE but with higher female preponderance (76–95%). Symptoms are noticeably exacerbated by estrogens during the menstrual cycle or use of contraceptives [18]. However, recently, a rare male majority was reported in a cohort of patients from Brazil [19]. Additionally, Tamoxifen (a selective estrogen receptor modulator) may trigger severe episodes of angioedema in patients with FXII-HAE [32]. Mean age of onset and frequency of disease-free intervals in patients with FXII-HAE is higher than in patients with C1-INH-HAE [23]. Unlike the clinical manifestations of C1-INH-HAE wherein extremity swelling is the predominant feature, patients with *F12* gene mutation often present with swelling of face, oropharynx, and abdominal attacks. Erythema marginatum, a characteristic prodromal clinical manifestation of patients with C1-INH-HAE, were rarely reported in patients with FXII-HAE [33]. Patients with FXII-HAE tend to develop symptoms during second decade of life or later, whereas in C1-INH-HAE, most patients develop symptoms during the first decade [34, 35].

Therapeutic Solutions for FXII-HAE

Since FXII-HAE is thought to be BK-mediated, and despite having normal levels and function of C1-INH, these patients occasionally respond to C1-INH therapy. Additionally, Icatibant (bradykinin B2 receptor antagonist), plasma-derived C1-INH, tranexamic acid, and fresh frozen plasma have been tried successfully for the management of acute attacks in patients with FXII-HAE [22, 36–40]. This may suggests a concentration dependent control of C1-INH of the kallikrein-kinin system (KKS) [36]. Additionally, Tranexamic acid, danazol, and plasma-derived C1-INH have shown some benefits in the prevention of attacks (prophylaxis) in these patients [19, 20, 23, 36–39]. Mechanism of action of danazol in patients with FXII-HAE is likely related to increase in aminopeptidase P activity, leading to more effective inactivation of kinins [20]. Tranexamic acid (a lysine analog anti-fibrinolytic agent) is thought to prevent attacks of angioedema in these patients by suppressing plasmin activity. Progestin has also been observed to be beneficial in a case series [36, 41]. Future treatments based on SiRNA as a potential prophylactic treatment for patients with these patients are already in development [31].

Pathophysiology of *Plasminogen* Gene Mutation (*PLG-HAE*)

In 2017, Bork et al. identified a novel mutation in *PLG* gene in patients with nC1-INH-HAE [8]. This missense mutation (c.9886A > G, p.Lys330Glu) located in exon 9 of *PLG* gene was identified using whole exome sequencing in 4 different families and is transmitted in an autosomal dominant manner. Later, this mutation was also found in 9 other families with nC1-INH-HAE. Tongue swelling is more common in patients with this mutation, as compared with patients with FXII-HAE [8]. Again, facial swelling, oedema of the extremities and genitalia, abdominal symptoms, and laryngeal oedema are significantly less common in patients with PLG-HAE. The typical erythema marginatum prodromal skin rash in HAE was also not observed in these patients [8].

The *PLG* gene contains 19 exons and 18 introns, and the expressed PLG protein is 791 amino acids long. It contains N-terminal pre-activation short peptide region (11 amino acids), 5 kringle domains (K1-K5), an activation cleavage domain, and a catalytic domain. It circulates as an inactive zymogen and gets converted to the active enzyme plasmin by cleavage of peptide bond and removal of N-terminal region [8]. Unlike *F12* gene mutation wherein factor XII becomes more sensitive to activation by plasmin, factor XII in these patients has been found with normal sensitivity to plasmin [27]. *PLG* gene mutations in the kringle 3 domain may alter the protein structure and function (Fig. 2) [42]. Kringle 3 domain facilitates binding of PLG protein to multiple endothelial receptor proteins. The mutant PLG protein may thus exhibit higher affinity for tissue activators. This may lead to increased activation of the fibrinolytic system, subsequently leading to increased formation of plasmin, activation of the KKS, and eventually to increased BK production (Fig. 1c).

Subsequent to first descriptions by Bork et al., Dewald et al. reported a missense mutation in 3 patients from Germany-c.1100 A > G in exon 9 of *PLG* gene causing lysine to glutamic acid substitution at 311 amino acid position [43]. This mutation is similar to the original one reported by Bork et al. [8]. Dewald, however, studied the pattern of plasminogen protein using isoelectric focusing and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and found that patients with PLG-HAE had altered patterns referred to as "dysplasminogenemia" [43]. The PLG mutation was also reported by several European investigators, as well as from Japan and USA [43–48].

Belbezier et al. reported *PLG* gene mutation in 10 patients. Attacks were found to be precipitated by angiotensin converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARB) [45]. Tongue swelling was reported in most patients, while none of the patients developed oedema of the extremities. Bodian et al. carried out sequencing of PLG gene in 2820 apparently healthy adults and found the previously reported mutation (c.9886A > G, p.Lys330Glu) in only one (frequency of 3.54 in 10,000) [48]. The clinical features of this patient were consistent with phenotype of PLG-HAE.

Germenis et al. reported p.Lys330Glu mutation in *PLG* gene in patients with nC1-INH-HAEfrom Spain, Greece,

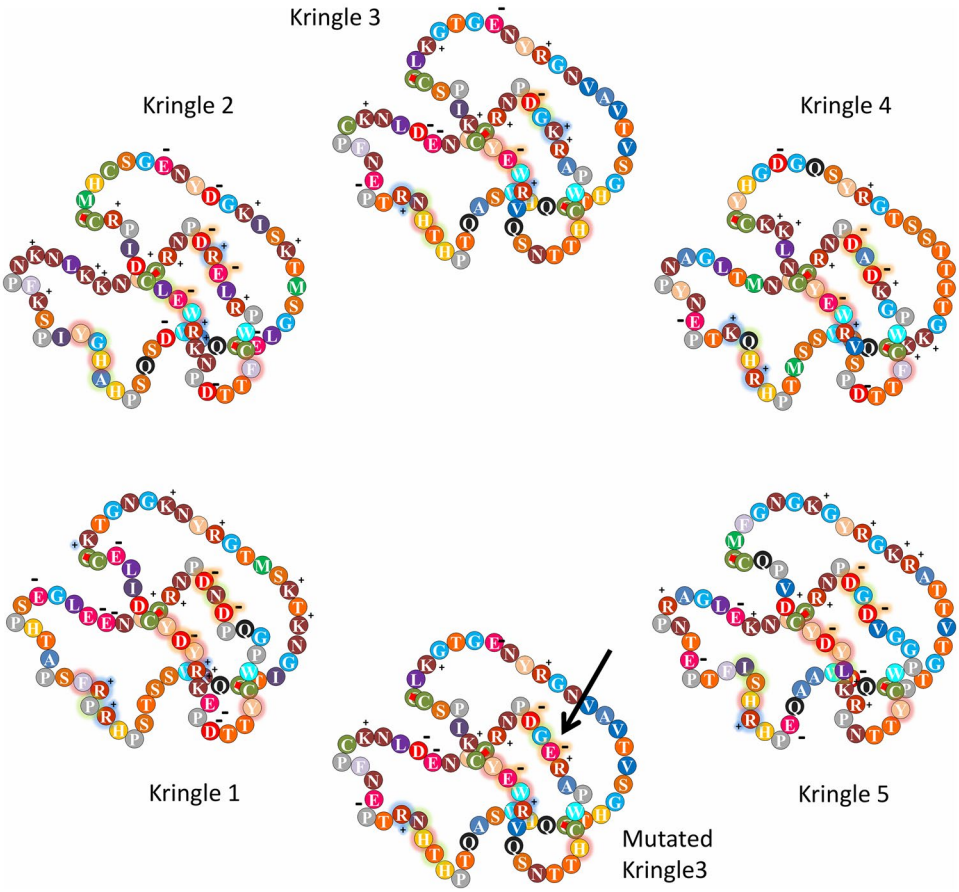


Fig. 2 Structure of human Kringle domains in plasminogen (PLN) protein. Pathogenic K330E mutation is indicated with a black arrow in the mutated Kringle domain. Individual circles with a single letter represent amino acids and the corresponding (+) or (−) sign denotes net charge on the side chain. Residues involved in ligand binding are highlighted with a glow around the circle and charge sign. K330E mutation leads to replacement of a positively charged amino acid (K) by a negatively charged one (E). This mutation, thereby, increases the affinity of mutated Kringle domain towards lysine-rich protein domains

and Bulgaria, using targeted next-generation sequencing (NGS) [47]. Detailed evaluation of these patients revealed that every symptomatic patient had a second variant. They included other variant(s) in *PLG* gene, in 1 family; a predicted deleterious variant in *carboxypeptidase N* gene; and a homozygous variant of the *PLG* gene in 1 patient each. Two relatives with heterozygous variant in *PLG* gene without other relevant variants were asymptomatic [47]. The authors concluded that heterozygous mutation in *PLG* gene alone may not be sufficient for causing HAE phenotype and would probably need a "second hit" from another variant in genes involved in coagulation or contact pathway. Therefore, the role of heterozygous *PLG* gene mutation in causing HAE remains conjectural.

Therapeutic Solutions for PLG-HAE

Icatibant (specific bradykinin B2 receptor antagonist) has been used as an acute treatment, and tranexamic acid was used as LTP in patients with PLG-HAE [44–46]. The response to BK-icatibant) seen in PLG-HAE suggests that BK is the potential mediator leading to angioedema. High doses of the anti-fibrinolytic agent (tranexamic acid) have been reported to be effective in patients with *PLG* gene mutation, as increased activity of fibrinolytic system seems to be in the basic pathogenesis of this entity [8]. It should be noted that these patients do not respond to steroids and antihistamines.

Pathophysiology of *Angiopoietin-1* Gene Mutation (ANGPT1-HAE)

Baffuno et al. reported in 2018 another potential genetic defect in a cohort of patients with nC1-INH-HAE from Italy [7]. The clinical profile of these patients is similar to C1-INH-HAE. Transmission is autosomal dominant. However, it was observed that some of these patients also had nail-fold capillary abnormalities [7]. Using WES, a novel missense mutation (c.807G > T, p.A119S) in the *ANGPT1* gene was identified in a family who had no pathogenic mutation in *SERPING1* gene or *F12* gene [7]. This was an important observation inasmuch as a genetic defect had been identified in a protein not directly involved in coagulation, contact/KKS or fibrinolytic pathways. ANGPT1 protein stabilizes the blood vessels, promotes endothelial cell survival and inhibits bradykinin and vascular endothelium growth factor (VEGF)-mediated plasma leakage (Fig. 3a). Because of mutation in coiled-coil domain at position 119, the mutant ANGPT1 protein is unable to form multimers.

This crucial function is required for interaction with its receptor, tunica interna endothelial cell kinase 2 (Tie-2), on the vascular endothelium. This interaction is part of a signalling cascade involved in stabilizing and preventing vascular permeability [7] (Fig. 3b).

Interestingly, while the total plasma concentration of ANGPT1 protein was found normal, immunoblotting showed reduced multimers and increased concentrations of dimeric and trimeric forms of ANGPT1 protein. Further functional analysis showed that the mutant protein had reduced binding to a soluble form of Tie-2 protein [7]. It was later demonstrated by the same group that heterozygous substitution mutation at position 119 of the *ANGPT1* gene leads to loss-of-function through haploinsufficiency [49]. Firinu et al. later observed this mutation in two other families from Italy. While one family had a history suggestive of an autosomal dominant mode of inheritance, the second family had only one affected patient [50].

Therapeutic Solutions for ANGPT1-HAE

There are no known definitive therapeutic options for this subtype of HAE. However, it has been hypothesized that activation of the Tie-2 receptor (i.e. by a recombinant ANGPT1 protein or its analogues) may help in decreasing vascular leakage.

Meanwhile, treatment with tranexamic acid was found to reduce frequency and intensity of attacks in 2 out of 4 patients reported [7]. No improvement was noted with steroids or antihistamines.

Pathophysiology of *Myoferlin* Gene *Mutations* (*MYOF-HAE*)

Recently, Ariano et al. have recently reported *myoferlin* gene mutation (R217S) in 3 women (mother and her 2 daughters) with nC1-INH-HAE [11]. Myoferlin has been shown to prevent degradation of vascular endothelial growth factor receptor-2 (VEGFR2); therefore, a loss of myoferlin protein, either through gene silencing or knockout models, has been shown to decrease expression of VEGFR2 (and even Tie-2) on plasma membranes of endothelial cells [51, 52]. The *gain-of-function* mutation reported in *myoferlin* gene has been postulated to lead to an increased localization of VEGFR2 to the plasma membrane leading to increased VEGF signalling and increased vascular permeability (Fig. 3c) [11, 53].

Clinical manifestations included oedema of head and neck region with onset during adolescence. Menstruation and high temperature were found to be likely triggers. Affected patients had a relatively slower onset and delayed resolution of oedema. MYOF-HAE follows an autosomal dominant mode of inheritance with incomplete penetrance [11].

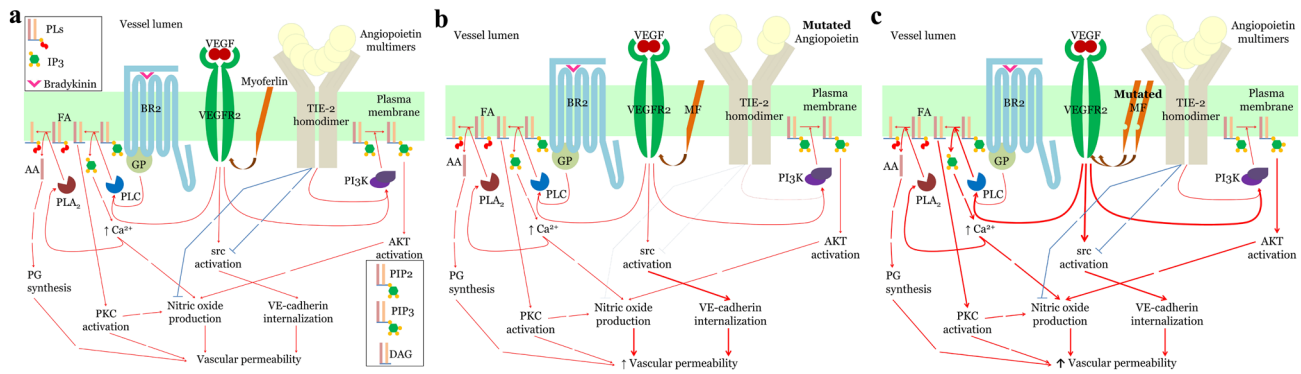


Fig. 3 The interplay between signalling cascades inside the endothelial cells. **a** Normal endothelial function. **b** Mutation in *angiopoietin-1* gene leads to defective multimerization of the protein required for normal intracellular signalling. Defective signalling is represented with thinner lines, while thicker arrows denote amplified molecular reactions. Because of defective signalling downstream of the ANGPT-1 and Tie-2 receptor, uncontrolled activation occurs downstream BK pathway of its receptor (BKRB2) leading to angioedema. **c** Schematic representation of pathophysiology of MYOF-HAE: Myoferlin R210S mutation has been postulated to lead to an increase in its function thereby leading to elevated VEGFR2 recycling (double arrows) that triggers an increase in vascular permeability. (Note: Arrows denote molecular reactions and stimulatory influences, while bar at the end denotes 638 inhibitory effect. Brown thick curved arrows denote role of myoferlin in recycling of VEGFR2) Abbreviations: AA arachidonic acid, AKT protein kinase B, BKRB2 bradykinin receptor type B2, Ca^{2+} ionic calcium, DAG diacyl glycerol, FA fatty acid, GP G protein, IP3 inositol 1,4,5-trisphosphate, PG prostaglandin, PI3K phosphoinositide 3-kinase, PIP2 phosphatidylinositol (4,5)-bisphosphate, PIP3 phosphatidylinositol (3,4,5)-trisphosphate, PKC protein kinase C, PLA_2 phospholipase A_2, PLC phospholipase C, PLs other phospholipids, MF myoferlin, Tie-2 tyrosine kinase with immunoglobulin and epidermal growth factor homology domains type 2, VEGF vascular endothelial growth factor, VEGFR2 vascular endothelial growth factor receptor type 2

Therapeutic Solutions for MYOF-HAE

The very few patients reported with MYOF-HAE do not respond to steroids or antihistamines. Nonetheless, Apatinib (a selective VEGFR2 inhibitor) has been found to be useful for the management of radiation induced vasogenic brain oedema [54], and based on this report and the pathogenetic mechanisms of MYOF-HAE, it can be speculated that VEGFR2 inhibitors may be a potential therapy for these patients.

Vascular Endothelium—a New Player in Pathophysiology of HAE

Vascular endothelium is a single layer of mesenchymal cells that demarcates tissues from circulating blood vessels. It plays an important role in regulating blood flow and movement of blood cells through the vessel wall [55]. Endothelial cells are heterogeneous in their ability to process signals generated by vasoactive agents, stress, or changes in chemical environment [56]. These cells control vasodilation and vasoconstriction by releasing mediators such as prostacyclin, thromboxane, nitric oxide, superoxide, and endothelin [57]. Bradykinin is a potent vasodilator and exerts its effects by releasing nitric oxide and prostacyclin from vascular endothelium. Bradykinin also mediates plasma leakage by interacting with vascular endothelium growth factor (VEGF) and bradykinin receptor. Vascular endothelium plays an active role in regulating plasma leakage in patients with HAE. Several other proteins (e.g. bradykinin B2 receptor and ANGPT1 receptor) that are not related to fibrinolysis and contact pathways but directly involved with vascular endothelium may potentially be involved in pathogenesis of HAE. Description of two mutations involving the vascular endothelium (ANGPT1 and MYOF) opens new frontiers in our understanding of the complex interplay between BKR2, Tie-2 and VEGFR2 signalling in the vascular endothelium in patients with HAE.

Pathophysiology of Kininogen-1 (KNG1) Gene Mutation (KNG1-HAE)

Bork et al. reported a hitherto unknown variant (c. 1136 T > A, p.Met379Lys) in all 6 affected members in a family with nC1-INH-HAE by using WES; a variant was not detected in other

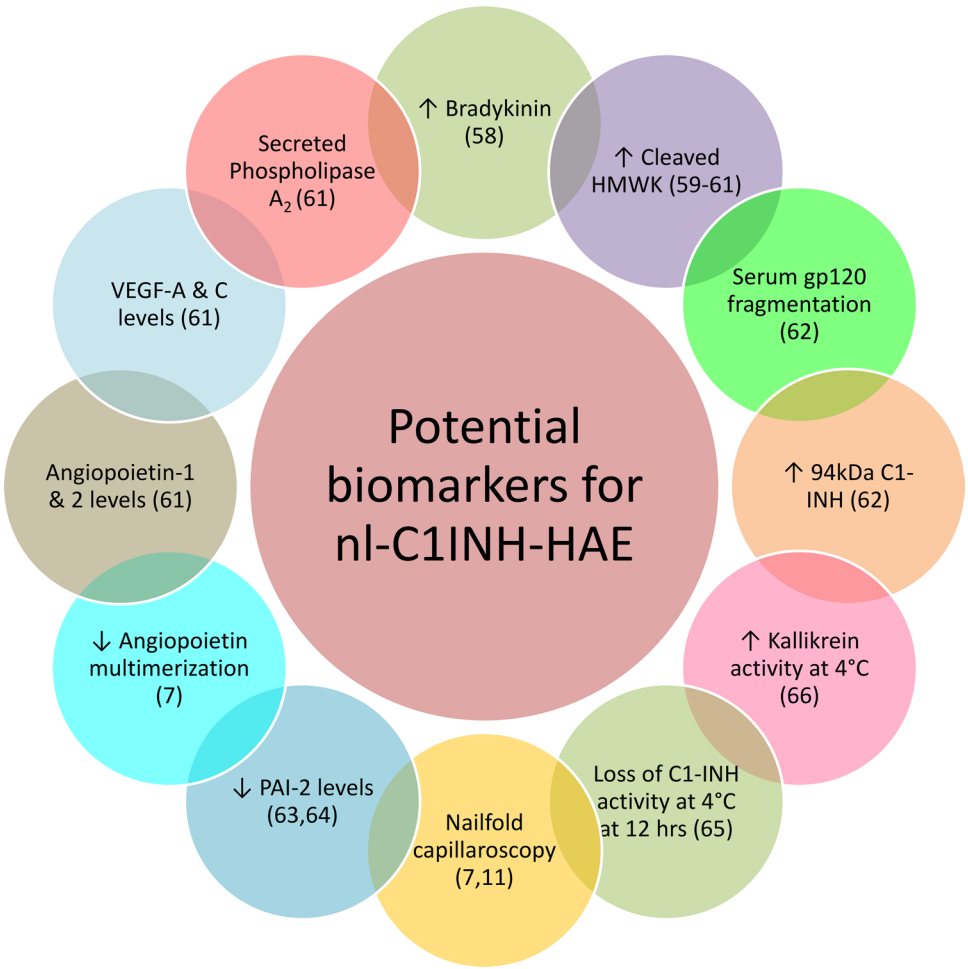


Fig. 4 Potential biomarkers for diagnosis and monitoring of disease activity of nC1-INH-HAE. (*See the article for common abbreviations*)

unaffected family members [10]. *Kininogen gene* (*KNG1*) gene contains 11 exons, and by alternate splicing, it transcribes into a unique mRNA transcript for both HK and low molecular weight kininogens (LK). This variant was identified in exon 10 of *KNG1* gene and was predicted to be pathogenic using in silico analysis. The variant leads to a change of the amino acid methionine to lysine and was found to be present in both isoproteins (i.e., HK and LK). The variant was found to be inherited in an autosomal dominant pattern. In all these patients, C1-INH antigen level, C1-INH function, C4, plasminogen activity, and FXII and FXI clotting activities are normal. Furthermore, PK and HK levels and activity were also found to be normal [10].

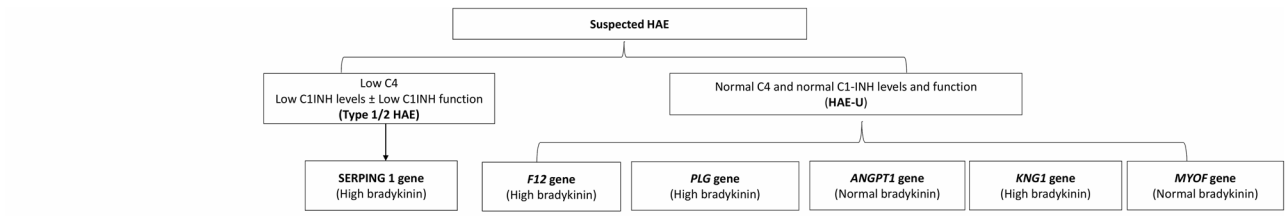
It has been hypothesized that in patients with mutation in *KNG1* gene, the variant potentially leads to change in the N-terminal cleavage site of HK and LK, thereby leading to production of an aberrant BK. This aberrant bradykinin is functionally active but may become resistant to cleavage by enzymes such as ACE or amino peptidase-2 (AP2), leading to its increased half-life and higher than usual activity (Fig. 1d).

Potential Biomarkers for Diagnosis and Disease Activity of nC1-INH-HAE

Diagnosis of nC1-INH-HAE is challenging at present time because of lack of valid biomarkers. Unlike patients with C1-INH-HAE, in which most patients would have either a low C1-INH antigen level or low C1-INH activity, and low serum complement C4 levels, these laboratory values are normal in patients with nC1-INH-HAE. Even though the clinical presentation of some of these patients may differ from patients with C1-INH-HAE, it may be extremely difficult to distinguish them clinically without laborious and expensive genetic analysis. Hence, it is very important to have a biomarker that can reliably distinguish the BK- mediated angioedema from other diseases. It is also important to identify a biomarker that may be useful for assessment of disease activity and may also be measured during prodromal phase to predict an attack [58, 59]. We have reviewed currently available biomarkers for diagnosis of nC1-INH-HAE (Fig. 4).

Over activation of the fibrinolytic and contact/KKS occurs in patients with C1-INH-HAE as well as nC1-INH-HAE [60, 61]. Thus, measurement of activated products of these pathways may prove to be useful biomarkers. Since overproduction of BK is central to the pathogenesis of most subtypes of HAE, serum levels might serve as a useful biomarker of disease activity. It should be noted that although plasma Lys-BK can be assayed by a competition enzyme linked immunosorbent assay (ELISA) or mass spectrometry (MS) [62, 63], BK has a very short half-life in plasma (a few seconds) and this limits its usefulness as a biomarker. To circumvent this problem, cleaved HK (cHK), the residua of HK after the nonapeptide BK was removed, has been proposed as a potential biomarker for BK mediated angioedema [64, 65]. The sensitivity and specificity of this noble technique are yet to be assessed.

Recently, Bova et al. studied plasma concentration of cHK, Angiopoietin, VEGF, and secreted phospholipase A_2 ($sPLA_2$) in a cohort of patients with FXII-HAE, ANGPT1-HAE, and U-HAE [66]. Indeed, cHK levels were significantly higher in patients with FXII-HAE and U-HAE as compared with normal serum controls. VEGF-A, VEGF-C, and ANGPT1 levels were



Age of onset	Usually <10 years	Later age of onset (2nd decade or beyond)	Later age of onset (usually after 2nd decade)	Later age of onset (2nd decade)	Later age of onset (usually after 2nd decade)	During adolescence
Gender	Male ~ female	Females >> Males	Female > Males	Females >> Males	Female > Males	Female >> Male
Edema over extremities	+++	+/-	-	NR	NR*	NR
Facial edema	+++	++	++	++	NR*	Edema of the head and neck
Abdominal attacks	++	++	++	++	NR*	NR
Laryngeal edema	++	++	+	NR	NR*	NR
Tongue edema	+/-	++	+++	NR	NR*	NR
Erythema marginatum	+++ (approximately 50% patients)	-	-	NR	NR	NR
Special features	Erythema marginatum	High estrogen dependency	Tongue swelling; responds to high dose tranexamic acid	Nail fold capillary abnormalities; slower onset and delayed resolution of edema	-	Menstruation and high temperature: potential triggers; slower onset and delayed resolution of edema

Fig. 5 Summary of clinical profile and pathophysiology of subtypes of HAE

significantly higher in patients with U-HAE, while only patients with FXII-HAE had elevated VEGF-C. Angiopoietin 2 levels and serum phospholipase A2 (sPLA2) activity was found to be normal. All the above biomarkers were not altered in patients with ANGPT1-HAE [66].

Other biomarkers studied in patients with nC1-INH-HAE include serum glycoprotein 120 (sgp120) fragmentation [67], plasminogen activator inhibitor 2 (PAI-2) [68, 69], assessment of C1-INH activity in plasma based on inhibition of activated factor XII and kallikrein [70], PKa activity [71], and multimers of ANGPT1 [7]. (Fig. 4).

In conclusion, despite studies carried out for validating a potential biomarker for nC1-INH-HAE, non-replicability of different biomarker signatures in different settings remains an issue for further research. Barriers include sample handling, processing, and different testing platforms. At the present time, it may be difficult to use these biomarkers for routine clinical practise for diagnosis, disease monitoring, or for prediction of an acute attack. Figure 5 summarizes important differences in the clinical profile and pathophysiology of various subtypes of HAE.

Conclusions and Future Directions

Over the last two decades, our understanding of pathophysiology of nC1-INH-HAE has significantly improved. Several genetic defects in pathways for BK production and the vascular endothelium are now better characterized. Next-generation genetic technologies, like Genome-wide Association Studies (GWAS) and WES offers opportunities, to diagnose other patients and families with yet unknown mechanisms of angioedema. The availability of advanced bioinformatic tools would also be applied to identification of several other target causative genes. This would, in turn, impact the development of novel treatment options for these patients.

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Compliance with Ethical Standards

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References

1. Kaplan AP, Greaves MW (2005) Angioedema. J Am Acad Dermatol 53(3):373–388
2. Busse PJ, Christiansen SC (2020) Hereditary angioedema. N Engl J Med 382(12):1136–1148
3. Banday AZ, Kaur A, Jindal AK, Rawat A, Singh S (2020) An update on the genetics and pathogenesis of hereditary angioedema. Genes & Diseases 7(1):75–83
4. Zuraw BL (2018) Hereditary angioedema with normal C1 inhibitor: four types and counting. J Allergy Clin Immunol 141(3):884–885
5. Germenis AE, Speletas M (2016) Genetics of hereditary angioedema revisited. Clin Rev Allergy Immunol 51(2):170–182
6. Marcelino I, Callero A, Mendoza-Alvarez A, Perez-Rodriguez E, Barrios-Recio J, García-Robaina JC, Flores C (2019) Bradykinin-mediated angioedema: an update of the genetic causes and the impact of genomics. Front Genet 10:900
7. Bafunno V, Firinu D, D'Apolito M, Cordisco G, Loffredo S, Leccese A, Bova M, Barca MP, Santacroce R, Cicardi M (2018) Mutation of the angiopoietin-1 gene (ANGPT1) associates with a new type of hereditary angioedema. J Allergy Clin Immunol 141(3):1009–1017
8. Bork K, Wulff K, Steinmüller-Magin L, Braenne I, Staubach-Renz P, Witzke G, Hardt J (2018) Hereditary angioedema with a mutation in the plasminogen gene. Allergy 73(2):442–450
9. Dewald G, Bork K (2006) Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem Biophys Res Commun 343(4):1286–1289
10. Bork K, Wulff K, Rossmann H, Steinmüller-Magin L, Brænne I, Witzke G, Hardt J (2019) Hereditary angioedema cosegregating with a novel kininogen 1 gene mutation changing the N-terminal cleavage site of bradykinin. Allergy 74(12):2479–2481
11. Ariano A, D'Apolito M, Bova M, Bellanti F, Loffredo S, D'Andrea G, Intrieri M, Petraroli A, Maffione AB, Spadaro G (2020) A myoferlin gain-of-function variant associates with a new type of hereditary angioedema. Allergy 75(11):2989–2992
12. Ratnoff OD, Colopy JE (1955) A familial hemorrhagic trait associated with a deficiency of a clot-promoting fraction of plasma. J Clin Investig 34(4):602–613
13. Cool D, Edgell C, Louie G, Zoller M, Brayer G, MacGillivray R (1985) Characterization of human blood coagulation factor XII Cdna. Prediction of the primary structure of factor XII and the tertiary structure of beta-factor XIIa. J Biol Chem 260(25):13666–13676
14. Kaplan AP, Joseph K (2014) Pathogenic mechanisms of bradykinin mediated diseases: dysregulation of an innate inflammatory pathway. In: Adv Immunol, vol 121. Elsevier, pp 41–89
15. Binkley KE (2010) Factor XII mutations, estrogen-dependent inherited angioedema, and related conditions. Allergy, Asthma Clin Immunol 6(1):16
16. de Maat S, Björkqvist J, Suffritti C, Wiesenekker CP, Nagtegaal W, Koekman A, van Dooremalen S, Pasterkamp G, de Groot PG, Cicardi M (2016) Plasmin is a natural trigger for bradykinin production in patients with hereditary angioedema with factor XII mutations. J Allergy Clin Immunol 138 (5):1414–1423. e1419
17. Bork K, Wulff K, Hardt J, Witzke G, Lohse P (2014) Characterization of a partial exon 9/intron 9 deletion in the coagulation factor XII gene (F12) detected in two Turkish families with hereditary angioedema and normal C1 inhibitor. Haemophilia 20(5):e372–e375
18. Bork K, Wulff K, Witzke G, Hardt J (2015) Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations. Allergy 70(8):1004–1012

19. Veronez CL, Moreno AS, Constantino-Silva RN, Maia LS, Ferriani MP, Castro FF, Valle SR, Nakamura VK, Cagini N, Gonçalves RF (2018) Hereditary angioedema with normal C1 inhibitor and F12 mutations in 42 Brazilian families. J Allergy Clin Immunol: In Practice 6 (4):1209–1216. e1208
20. Moreno AS, Valle SO, Levy S, França AT, Serpa FS, Arcuri HA, Palma MS, Campos WN, Dias MM, Ponard D (2015) Coagulation factor XII gene mutation in Brazilian families with hereditary angioedema with normal C1 inhibitor. Int Arch Allergy Immunol 166(2):114–120
21. Bork K, Gül D, Hardt J, Dewald G (2007) Hereditary angioedema with normal C1 inhibitor: clinical symptoms and course. Am J Med 120(11):987–992
22. Piñero-Saavedra M, González-Quevedo T, de San Pedro BS, Alcaraz C, Bobadilla-González P, Fernández-Vieira L, Hinojosa B, García-Lozano R (2016) Hereditary angioedema with F12 mutation: clinical features and enzyme polymorphisms in 9 Southwestern Spanish families. Ann Allergy Asthma Immunol 117(5):520–526
23. Magerl M, Germenis AE, Maas C, Maurer M (2017) Hereditary angioedema with normal C1 inhibitor: update on evaluation and treatment. Immunology and Allergy Clinics 37(3):571–584
24. Bork K, Barnstedt S-E, Koch P, Traupe H (2000) Hereditary angioedema with normal C1-inhibitor activity in women. The Lancet 356(9225):213–217
25. Binkley KE, Davis A III (2000) Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema. J Allergy Clin Immunol 106(3):546–550
26. Aulak K, Davis A III, Donaldson V, Harrison R (1993) Chymotrypsin inhibitory activity of normal C1-inhibitor and a P1 Arg to His mutant: Evidence for the presence of overlapping reactive centers. Protein Sci 2(5):727–732
27. Maas C (2019) Plasminflammation-an emerging pathway to bradykinin production. Front Immunol 10:2046
28. Cichon S, Martin L, Hennies HC, Müller F, Van Driessche K, Karpushova A, Stevens W, Colombo R, Renné T, Drouet C (2006) Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. Am J Hum Genet 79(6):1098–1104
29. Bork K, Wulff K, Meinke P, Wagner N, Hardt J, Witzke G (2011) A novel mutation in the coagulation factor 12 gene in subjects with hereditary angioedema and normal C1-inhibitor. Clin Immunol 141(1):31–35
30. Bork K, Kleist R, Hardt J, Witzke G (2009) Kallikrein–kinin system and fibrinolysis in hereditary angioedema due to factor XII gene mutation Thr309Lys. Blood Coag Fibrinol 20(5):325–332
31. Liu J, Qin J, Borodovsky A, Racie T, Castoreno A, Schlegel M, Maier MA, Zimmerman T, Fitzgerald K, Butler J (2019) An investigational RNAi therapeutic targeting Factor XII (ALN-F12) for the treatment of hereditary angioedema. RNA 25(2):255–263
32. Bork K, Wulff K, Witzke G, Rietz S, Hardt J (2017) Tamoxifen may cause life-threatening angioedema attacks in patients with hereditary angioedema. J Eur Acad Dermatol Venereol 31(5):e237–e239
33. Bork K (2010) Diagnosis and treatment of hereditary angioedema with normal C1 inhibitor. Allergy, Asthma Clin Immunol 6(1):15
34. Bork K, Meng G, Staubach P, Hardt J (2006) Hereditary angioedema: new findings concerning symptoms, affected organs, and course. Am Journal Med 119(3):267–274
35. Agostoni A, Cicardi M (1992) Hereditary and acquired C1-inhibitor deficiency: biological and clinical characteristics in 235 patients. Medicine 71(4):206–215
36. Deroux A, Boccon-Gibod I, Fain O, Pralong P, Ollivier Y, Pagnier A, Djenouhat K, Du-Thanh A, Gompel A, Faisant C (2016) Hereditary angioedema with normal C1 inhibitor and factor XII mutation: a series of 57 patients from the French National Center of Reference for Angioedema. Clin Exp Immunol 185(3):332–337
37. Vitrat-Hincky V, Gompel A, Dumestre-Perard C, Boccon-Gibod I, Drouet C, Cesbron J-Y, Lunardi J, Massot C, Bouillet L (2010) Type III hereditary angio-oedema: clinical and biological features in a French cohort. Allergy 65(10):1331–1336
38. Bork K, Wulff K, Witzke G, Hardt J (2017) Treatment for hereditary angioedema with normal C1-INH and specific mutations in the F12 gene (HAE-FXII). Allergy 72(2):320–324
39. Firinu D, Bafunno V, Vecchione G, Barca MP, Manconi PE, Santacroce R, Margaglione M, Del Giacco SR (2015) Characterization of patients with angioedema without wheals: the importance of F12 gene screening. Clin Immunol 157(2):239–248
40. Boccon-Gibod I, Bouillet L (2012) Safety and efficacy of icatibant self-administration for acute hereditary angioedema. Clin Exp Immunol 168(3):303–307
41. Saule C, Boccon-Gibod I, Fain O, Kanny G, Plu-Bureau G, Martin L, Launay D, Bouillet L, Gompel A (2013) Benefits of progestin contraception in non-allergic angioedema. Clin Exp Allergy 43(4):475–482
42. Christen MT, Frank P, Schaller J, Llinas M (2010) Human plasminogen kringle 3: solution structure, functional insights, phylogenetic landscape. Biochemistry 49(33):7131–7150
43. Dewald G (2018) A missense mutation in the plasminogen gene, within the plasminogen kringle 3 domain, in hereditary angioedema with normal C1 inhibitor. Biochem Biophys Res Commun 498(1):193–198
44. Recke A, Massalme EG, Jappe U, Steinmüller-Magin L, Schmidt J, Hellenbroich Y, Hüning I, Gillessen-Kaesbach G, Zillikens D, Hartmann K (2019) Identification of the recently described plasminogen gene mutation p. Lys330Glu in a family from Northern Germany with hereditary angioedema. Clinical and translational allergy 9(1):9
45. Belbézier A, Hardy G, Marlu R, Defendi F, Dumestre PC, Boccon-Gibod I, Launay D, Bouillet L (2018) Plasminogen gene mutation with normal C1 inhibitor hereditary angioedema: three additional French families. Allergy 73(11):2237
46. Yakushiji H, Hashimura C, Fukuoka K, Kaji A, Miyahara H, Kaname S, Horiuchi T (2018) A missense mutation of the plasminogen gene in hereditary angioedema with normal C1 inhibitor in Japan. Allergy 73(11):2244–2247
47. Germenis A, Loules G, Zamanakou M, Psarros F, González-Quevedo T, Speletas M, Bork K, Wulff K, Steinmüller-Magin L, Brænne I (2018) On the pathogenicity of the plasminogen K330E mutation for hereditary angioedema. Allergy 73(8):1751
48. Bodian DL, Vilboux T, Hauser NS (2019) Genotype-first analysis of a generally healthy population cohort supports genetic testing for diagnosis of hereditary angioedema of unknown cause. Allergy, Asthma Clin Immunol 15(1):1–4
49. d'Apolito M, Santacroce R, Colia AL, Cordisco G, Maffione AB, Margaglione M (2019) Angiopoietin-1 haploinsufficiency affects the endothelial barrier and causes hereditary angioedema. Clin Exp Allergy 49(5):626–635
50. Firinu D, Loffredo S, Bova M, Cicardi M, Margaglione M, Del Giacco S (2019) The role of genetics in the current diagnostic workup of idiopathic non-histaminergic angioedema. Allergy 74(4):810–812
51. Bernatchez PN, Acevedo L, Fernandez-Hernando C, Murata T, Chalouni C, Kim J, Erdjument-Bromage H, Shah V, Gratton J-P, McNally EM (2007) Myoferlin regulates vascular endothelial growth factor receptor-2 stability and function. J Biol Chem 282(42):30745–30753
52. Yu C, Sharma A, Trane A, Utokaparch S, Leung C, Bernatchez P (2011) Myoferlin gene silencing decreases Tie-2 expression in vitro and angiogenesis in vivo. Vascul Pharmacol 55(1–3):26–33

53. Oubaha M, Gratton J-P (2009) Phosphorylation of endothelial nitric oxide synthase by atypical PKCζ contributes to angiopoietin-1–dependent inhibition of VEGF-induced endothelial permeability in vitro. Blood, The Journal of the American Society of Hematology 114(15):3343–3351
54. Hu WG, Weng YM, Dong Y, Li XP, Song Q-B (2017) Apatinib in refractory radiation-induced brain edema: A case report. Medicine 96 (46)
55. Galley HF, Webster NR (2004) Physiology of the endothelium. Br J Anaesth 93(1):105–113
56. McCarron JG, Wilson C, Heathcote HR, Zhang X, Buckley C, Lee MD (2019) Heterogeneity and emergent behaviour in the vascular endothelium. Curr Opin Pharmacol 45:23–32
57. Sandoo A, van Zanten JJV, Metsios GS, Carroll D, Kitas GD (2010) The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J 4:302
58. Kaplan AP, Maas C (2017) The search for biomarkers in hereditary angioedema. Front Med 4:206
59. Hofman ZL, de Maat S, Suffritti C, Zanichelli A, van Doorn C, Sebastian SA, Veszeli N, Csuka D, Renné T, Pasterkamp G (2017) Cleaved kininogen as a biomarker for bradykinin release in hereditary angioedema. J Allergy Clin Immunol 140 (6):1700–1703. e1708
60. Veronez CL, Grumach AS (2020) Angioedema without urticaria: novel findings which must be measured in clinical setting. Curr Opin Allergy Clin Immunol 20(3):253–260
61. Reshef A, Zanichelli A, Longhurst H, Relan A, Hack C (2015) Elevated D-dimers in attacks of hereditary angioedema are not associated with increased thrombotic risk. Allergy 70(5):506–513
62. Marceau F, Rivard GE, Gauthier JM, Binkley KE, Bonnefoy A, Boccon-Gibod I, Bouillet L, Picard M, Levesque G, Elfassy HL (2020) Measurement of bradykinin formation and degradation in blood plasma: Relevance for acquired angioedema associated with angiotensin converting enzyme inhibition and for hereditary angioedema due to factor XII or plasminogen gene variants. Front Med 7:358
63. Baralla E, Nieddu M, Boatto G, Varoni MV, Palomba D, Demontis MP, Pasciu V, Anania V (2011) Quantitative assay for bradykinin in rat plasma by liquid chromatography coupled to tandem mass spectrometry. J Pharm Biomed Anal 54(3):557–561
64. Suffritti C, Zanichelli A, Maggioni L, Bonanni E, Cugno M, Cicardi M (2014) High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C 1-inhibitor deficiency. Clin Exp Allergy 44(12):1503–1514
65. Baroso R, Sellier P, Defendi F, Charignon D, Ghannam A, Habib M, Drouet C, Favier B (2016) Kininogen cleavage assay: diagnostic assistance for kinin-mediated angioedema conditions. PLoS One 11(9):e0163958
66. Bova M, Suffritti C, Bafunno V, Loffredo S, Cordisco G, Del Giacco S, De Pasquale TMA, Firinu D, Margaglione M, Montinaro V (2019) Impaired control of the contact system in hereditary angioedema with normal C1-inhibitor. Allergy
67. Larrauri B, Hester CG, Jiang H, Miletic VD, Malbran A, Bork K, Kaplan A, Frank M (2020) sgp120 and the contact system in hereditary angioedema: a diagnostic tool in HAE with normal C1 inhibitor. Mol Immunol 119:27–34
68. Joseph K, Tholanikunnel BG, Wolf B, Bork K, Kaplan AP (2016) Deficiency of plasminogen activator inhibitor 2 in plasma of patients with hereditary angioedema with normal C1 inhibitor levels. J Allergy Clin Immunol 137 (6):1822–1829. e1821
69. Marlu R, Deroux A, Du-Thanh A, Boccon-Gibod I, Launay D, Bouillet L (2017) Normal PAI-2 level in French FXII-HAE patients. J Allergy Clin Immunol 139(5):1719–1720
70. Joseph K, Bains S, Tholanikunnel BG, Bygum A, Aabom A, Koch C, Farkas H, Varga L, Ghebrehiwet B, Kaplan AP (2015) A novel assay to diagnose hereditary angioedema utilizing inhibition of bradykinin-forming enzymes. Allergy 70(1):115–119
71. Lara-Marquez ML, Christiansen SC, Riedl MA, Herschbach J, Zuraw BL (2018) Threshold-stimulated kallikrein activity distinguishes bradykinin-from histamine-mediated angioedema. Clin Exp Allergy 48(11):1429–1438