Innovations in Hereditary Angioedema Pathophysiology

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Hereditary angioedema (HAE) is a rare, inherited disease mostly associated with mutations in the SERPING1 gene (serpin family G member 1), which encodes the C1 inhibitor (C1-INH) protein. Regulation can lead to plasma deficiency and ensuing repeated attacks of severe angioedema. This disease was first described clinically and genetically in 1888 by William Osler, who named it “hereditary angioneurotic edema (HANE).” It took 75 years until Donaldson and Evans identified the fundamental role of C1-INH in the pathophysiology of so-called HANE by Osler. Significant progress has been made in the research of this genetic disease when the role of neural factors was documented as being too small to lead to edema, the name was changed as HAE. Therefore, the name of more than 490 different mutations have been reported in the region of the C1-INH gene (SERPING1) until mid-2018. It is now known that C1-INH deficiency overstimulates the plasma contact (kallikrein-kinin) system, which eventually results in the overproduction of bradykinin. By binding to the bradykinin B2 receptor, bradykinin increases vascular permeability (vasodilation) and causes contraction of nonvascular smooth muscle, and acts as a main/major mediator in the pathophysiology of HAE. Reports since 2000 have described a new type of HAE with “normal” CI-INH levels, primarily in Caucasians. A number of abnormalities in the genes encoding for factor XII, angiopoietin-1, and plasminogen have been identified in this novel disease entity. The establishment of treatment modalities for HAE with normal CI-INH is also expected.

INTRODUCTION

This review provides a definition and a short history of angioedema, followed by previous knowledge of the epidemiology, pathogenesis, and types of angioedema, as well as new information reported in the literature.[1,2]

Brief history of hereditary angioedema

The term angioedema was first used by Donati[3] in 1586. Milton[4] contributed the first scientific description of angioedema as “giant urticaria” in 1876. In 1882, Quincke reported a series of patients with swelling (angioedema), the term “angioneurotic edema” was first proposed by Strübing[5] in 1885, and soon after, a hereditary form was described in 1888 as “hereditary angioneurotic edema (HAE)” by Osler.[6]

The biochemical basis of this form was clarified in 1963 by Donaldson and Evans.[7] Rosen et al.[8] described HAE type II in 1965. The SERPING1 gene was cloned in the mid-1980s. In 2000, Bork[9] described the most commonly studied type, today known as C1 inhibitor (C1-INH) normal HAE, which had previously been called HAE type III. De-wald[10] subsequently reported 2 missense mutations in the factor XII (FXII) gene in 2006. In 2018, other mutagenesis subtypes of the plasminogen (PLG) gene and angiopoietin-1 (ANGPT1) were reported: PLG-HAE and ANGPT1-HAE, which forms in the absence of an interaction of ANGPT1 with its vascular endothelial receptor.[11–13]

Definition of angioedema

Angioedema may be defined as regional, non-inflammatory, self-limiting edema. It is swelling that develops due to increased plasma leakage from capillary vessels in the deep layer of the skin (reticular dermis), the subcutaneous, or submucosal layers. Apart from the skin, it may also occur in submucosal layers of the upper respiratory or gastrointestinal tract.[14]

Although vasodilation due to the accumulation of endogenous inflammatory elements (histamine, prostaglandin, leukotriene, bradykinin, etc.) and leakage of plasma into interstitial tissue due to an increase in the permeability of endothelial cells occur, this condition does not follow a typical inflammatory process.[15] There is a local accumulation of non-inflammatory fluid. Other than eosinophilic...
infiltration seen in allergic angioedema, there is no cellular infiltration in angioedema tissue. Only the tumor, that is, the swelling (edema) component of the Celsus tetrad of inflammation (tumor, rubor, dolor, and calor) is present. This local limited inflammation may have a sudden onset and heal without adverse sequela in less than 5 days.[14]

Distinguishing features of angioedema versus other types of edema
Unlike edema due to systemic disease, angioedema does not result in pitting edema. It is more asymmetric and typically occurs in areas not necessarily as dependent on the forces of gravity, including loose areas of tissue (face, genital area, etc.). The boundaries of the swelling are not sharp, and the overlying skin can often be colorless or slightly erythematous. The swelling is often painless, although a burning sensation without itching may be felt. Pain and tenderness can occur due to excessive distension of the cutaneous nerves. Sometimes a local increase in the temperature of the skin, pain, or occasionally, itching may be observed. Desquamation or urticarial spots are not seen, but bruising may develop as a result of scratching.[14-20] In particular, mast-cell mediated angioedema may be associated with urticaria.[14,16,20]

What is hereditary angioedema?
The most recent information indicates that hereditary angioedema may be due to the lack of C1-INH in the plasma (protein/functional), uncontrolled activation in the plasma contact system for various reasons, or disrupted interaction of the endothelial cell receptor with its ANGPT1 mediator in the vasculature.[21-25]

The most well-known and most often seen form is a type that develops in cases with C1-INH deficiency (protein/functional; C1-INH-HAE type I/II). Type I/II is a rare disorder with an autosomal dominant inheritance and an average frequency of 1/50,000. It occurs due to one or more mutations among the ≥490 mutations in the SERPING1 gene. The reactive center ring and the Arg444 residue in the crystalline structure of C1-INH are important. The hinge region is critical for movements in this region.[26]

The signal peptide C1-INH is an α2-globulin and a 110-kDa glycoprotein consisting of 478 amino acids. The C1-INH serine protease inhibitor (serpin) is similar to its prototype α1-antitrypsin (α1-AT). C1-INH is a suicide inhibitor that functions by forming a 1:1 stoichiometric complex with target proteases (prekallikrein, Mannan-binding lectin serine protease [MASP], etc.).[27]

C1-INH is known to inhibit C1r, C1s, MASP-1, MASP-2, FXII, and kallikrein, which are components of the classic complement pathway of the plasma contact system; factor XI and thrombin in the coagulation system; and tissue plasminogen activator and plasmin activities in the fibrinolytic system.[28]

Among its other functional roles, C1-INH plays a part in ischemia reperfusion damage in the bowels, liver, muscle, heart, and brain tissue; septic shock; bacterial infections (malaria, etc.); hyperacute transplant rejection; age-related macular degeneration; and other inflammatory disease models.[29]

Functional mechanism of C1-INH: Molecular trap (mousetrap)
The active site of the free target protease first interacts with the reactive central ring of active C1-INH. Upon separation of the reactive center ring, the protease is displaced downward on the serpin, which is conformationally flexible, and an irreversible complex is formed between the serpin and the target protease. This functional mechanism works like a molecular mousetrap.[30]

SERPING1 gene
The long arm of chromosome 11 is in the region of q12-q13.1. It consists of 8 exons and 7 introns. In type I HAE, the secretion of the protein is impaired due to synthesis defects, such as splicing or incorrect folding of the C1-INH protein due to mutations in this gene.[31]

In type II HAE, mutations of the C1-INH protein prohibit recognition as a substrate and inhibition of the target proteases.[32]
SERPING1 gene and C1-INH-HAE type I/II mutations

Nearly 500 (≥490) mutations result in C1-INH-HAE type I/II disease. Mutations occur frequently in exons 8, 5, and 6. They are less frequently seen at the amino-terminal end. More than 160 depend on missense (loss) mutations. Missense mutations have been reported in 34%, frameshift (frame) changes in 31%, wide gene regulations in 17%, splice-site defects in 10%, nonsense mutations in 7%, and regulatory region mutations in 1% of cases.[24]

Since the SERPING1 gene is unstable, de novo mutation in the hinge region of the 11th chromosome has been observed in 25% of patients, which may explain sporadic HAE cases occurring among non-relatives.

Type I mutations occur heterogeneously along the entire C1-INH gene, while type II mutations occur at exon 8, which encodes the active site of the C1-INH gene, or in the hinge region. The result is a secretory but dysfunctional (inactive) C1-INH protein. Most (≥70%) are localized at the reactive center/mobile ring: the so-called Arg444-Thr445 (P1-P1) link in the vicinity of missense point-mutations (a single amino acid change) or 2 critical hinge regions.[26]

C1-INH deficiency as a result of type I/II mutations leads to uncontrolled activation of the complement and plasma contact system as well as the fibrinolytic system, and results in increased production of vasoactive peptides such as bradykinin.[35,36]

New findings in the pathophysiology of hereditary angioedema SERPING1 mutations and genotype-phenotype relationships

More than 130 years have passed since HAE was defined by Osler, and thanks to recent genetic studies, the heterogeneity of the disease and genotype-phenotype relationships involved in the clinical expression have begun to be better illuminated. New mutations are being defined every day, original research is reported from around the world, and new techniques are applied, providing improved understanding of the disease.[34,37,38]

Next-generation sequencing has been used to study the genotype in C1-INH-HAE, and it has revealed that most SERPING1 mutations consist of single nucleotide variants (missense mutations [34%], splice-site area defects [10%], nonsense mutations [7%], regulatory mutations [1%]), and to a lesser extent, gene copy number variants (CNVs) (frameshifts, small insertions and deletions [small INDELS] [31%] and large gene changes [17%]).[34]

Relevant studies from our country[39] have been conducted and heterozygous mutations have been described in the 7th exon (p.Leu416X [c.1247T> A]) by Akoglu et al.[40] and by Büyüköztürk et al.[41] in the promoter region (CAAT box, c.−101A>G). Ozkars et al.[42] reported c. 601A>T nonsense variant mutation in the SERPING1 gene in C1-INH HAE patients.

Pathophysiology studies have identified biomarkers (endo-can, VE-cadherin) that can help to better clarify the etiology and/or the severity of the disease.[43,44]

Genotype-phenotype correlations in C1-INH-HAE disease are currently being extensively investigated.[38,45,46] Speletas et al.[45] conducted research in various European countries (Greece, Germany, Romania, and Hungary) and found that abdominal attacks were less common in Hungarians, and that the onset of the C1-INH-HAE type of the disease was delayed in Romanian patients.

Enzymes responsible for the degradation of bradykinin (aminopeptidase P [APP] and angiotensin-converting enzyme [ACE]) and a genetic polymorphism that leads to reduced kininase activity have been shown to affect the phenotype of FXII-HAE.[46] In another study, no correlation was found between the clinical phenotype of the disease and B1 and B2 bradykinin receptors (BDKR1/BDKR2) and mannos-binding lectin (MBL2) genes.[47] The F12-46C/T polymorphism was found to be correlated with a 7-year delay in onset of disease, but negatively associated with the need for long-term treatment, regardless of mutations in the SERPING1 gene.[48]

Estrogen-sensitive, estrogen-dependent, and estrogen-independent phenotypes have been described. It has also been shown that high estrogen levels increase the expression level of the overactive mutant FXII form but affect the phenotype of the disease by suppressing ACE and APP.[49,50]

Diagnostic criteria for normal C1-INH-level HAE (nC1-INH-HAE/type III)

The diagnostic criteria were first described in 2000: In addition to recurrent, urticarial, and non-drug-induced angioedema with normal C4 and C1-INH levels in the blood, an FXII gene mutation or family history, and angioedema unresponsive to antihistamines (40 mg/day cetirizine/ equivalent treatment that does not address intermittent episodes ≥3) were included in the definition.[31,52]

Distinctive features of normal C1-INH-level hereditary angioedema

This type is an autosomal dominant disease with low penetrance; the clinical symptoms are most often observed in adults, and particularly women. The interval between attacks may be lengthy, the symptoms appear less often, and in many patients, it manifests only with recurrent skin (lip) and tongue edema. Repetitive tongue swelling (F12-HAE: 40% of patients) and related choking-type episodes can occur. Multiple organ involvement and abdominal attacks are less common. Erythema marginatum is not seen, but bleeding may occur. Progesterone treatment has been reported to have a positive effect.[21,53]

Hereditary angioedema (FXII-HAE) due to factor XII gene mutation

This form is also referred to as HAE type B. Dewald et al.[19] reported on an FXII gene mutation in 20% to 30%
of nC1-INH-HAE/type III patients in 2006; however, the etiology is still unknown (HAE-unknown). Although there are fewer accounts from the US about this type, there are reports of this mutation from Turkey, Germany, Hungary, Spain, Brazil, Italy, the UK, Australia, and Morocco. The largest series, which included 57 cases, was reported by the French national angioedema reference center.[54]

FXII is found on the long arm of the fifth chromosome (5q35.2-q35.3). Four mutations of the ninth exon of FXII were reported between 2006 and 2013. These mutation-related changes occur in the proline-rich region of FXII (Hageman’s Factor). FXII gene mutations are inherited through an autosomal dominant (low penetrance) pattern. Among asymptomatic carriers, 90% are men, while only 40% are women. In contrast to the SERPING1 gene, de novo mutations have not been detected. The known FXII-HAE-specific gene mutations include 2 missense/point mutations (p.Thr328Lys and p.Thr328Arg), a 72-bp wide deletion (c.971_1018 + 24del72), and a 18-bp duplication (c.892_909dup).

In addition to the 2 most common missense/point mutations observed, p.Thr328Lys (c.1032CA, Thr309Lys, T328K) mutations have also been reported more frequently in the literature. A large 72-bp deletion (c.971_1018 + 24del72) at the edge of exon 9/intron 9 in the proline-rich region of FXII was reported for the first time in 2 non-related Turkish families.[55–57]

**Pathophysiology of FXII-HAE**

The proline-rich region of FXII allows for the binding of coagulation FXII to negatively charged surfaces. Following mutations in this region, FXII is then replaced by threonine, arginine, or lysine localized at the 309th or 328th position of the protein. When the threonine is neutral, it becomes positively charged by the substitution and leads to the formation of excess bradykinin by easy activation of the mutant FXII.[54]

It has been thought that these mutations in the proline-rich region lead to a glycosylation defect and configurational change of FXII, and consequently to an increase in the sensitivity of FXII with contact (activation of the amidolytic enzyme activity of FXII). Soluble lysine analogues can be effective in treatment by alleviating this mechanism. It is also thought that mutant FXII, rather than prekallikrein, can be activated more quickly by plasmin and escape C1-INH inhibition, which helps explain the effectiveness of anti-fibrinolytic drugs in this disease.[57]

**Recent developments related to normal C1-INH level-HAE**

Aside from FXII, 2 new mutations have been reported in nC1-INH-HAE patients. Here also, the development of angioedema is mediated by bradykinin. The clinical phenotype and treatment options are similar. These are mutations of PLG and ANGPT1 genes. However, the gene mutation has not been identified in most of the patients with HAE of unknown etiology (uHAE). Since lower plasminogen activator inhibitor levels are also detected in these patients, as in other unknown HAE cases, increased plasmin formation is seen, leading to increased FXII activation and bradykinin.[51,58] The characteristics of the different types of HAE (C1-INH-HAE and nC1-INH-HAE) based on current literature data are provided in Table 1.

**Pathophysiology of hereditary angioedema due to plasminogen gene mutation**

The plasminogen gene consists of 19 exon+splice junctions+5 kringle domains. The missense mutation (c.9886A>G, p.Lys330Glu, K330E) in exon 9 of this gene leads to a negative charge of the resulting protein, resulting in the substitution of lysine in the amino acid at position 311 of the mature protein with glutamic acid (p.Lys311Glu). The heterozygote mutation has a dominant inheritance. This mutation, called K330E, is a very rare variant, with a prevalence in Europeans of 1/31.591. Although other dysplasminogenemias have been identified, angioedema formation has not been reported in these cases.[9–11]

The kringle domain facilitates the binding of PLG to large surfaces, such as fibrin, bacterial protein, the cell surface, and small molecular ligands. Mutant PLG exhibits greater affinity for such surfaces and/or is more accessible to plasminogen activators (such as tissue-type plasminogen activator/urokinase-type plasminogen activator). In these patients, PLG activity was within normal limits. Lower levels of PLG activator inhibitors 1 and 2 may be related to this phenomenon in these variations included in nC1-INH-HAE groups.[58]

**Hereditary angioedema due to plasminogen gene mutation (PLG-HAE: HAE type C)**

Bork et al.[11] first described this type of HAE in a report of 60 patients from 13 different German families. The male/female ratio was 13/47. The mean age of onset was 31 years, the patients had angioedema for a mean of 21 years, and it was much more pronounced in the tongue when compared with the type of HAE related to FXII gene mutation. In patients with FXII mutation, attacks affecting the extremities, genital area, abdominal region, and larynx are more frequently seen.

Dewald et al.[59] later reported the same mutation in 3 of 8 women with angioedema of unknown etiology (uHAE) in 3 large, unrelated German families. In Japan, Yakushiji et al.[60] described similar mutations in 4 uHAE patients from 2 different families. Belbézier et al.[61] reported that the disease manifested at the mean age of 23 years in 8 patients (6 females, 2 males) from 3 French families, and that the attacks were triggered most frequently by the use of ACE-I and angiotensin II receptor blockers (ARBs).

**Common characteristics of the PLG-HAE type**

The presence of PLG-HAE has been reported in various ethnic groups. Women are 3 times more frequently af-
fected than men. Estrogen is less influential in the clinical picture of PLG-HAE compared with FXII-HAE. The use of ACE-Is and ARBs triggers attacks in these patients. Tongue (facial) swelling is a characteristic clinical finding and is observed in 89% of these cases. Tranexamic acid provides effective long-term prophylaxis. Icatibant can also be used in the treatment. The effect of tranexamic acid on the PLG/plasmin kringle domain is a result of competitive inhibition of lysine-binding sites. The formation of bradykinin via Factor XII activation of plasmin plays a role in the pathophysiology of angiogenesis.[11,59–61]

Pathophysiology of hereditary angioedema type (ANGPT1-HAE) due to angiopoietin-1 gene mutation

Unlike ANGPT2, ANGPT1 plays a role in the maintenance of the endothelial barrier by inhibiting the effects of inflammatory agents that increase permeability, such as vascular endothelial growth factor (VEGF) and bradykinin. As a result of ANGPT1 mutation, plasma levels of ANGPT1 decrease, and the multimerization function of ANGPT and its ability to recognize the receptor tunica interna endothelial cell kinase 2 (TIE2) are impaired.[12,13]

The discovery of this mutation has turned attention from the plasma contact system to the vascular structure in the pathophysiology of HAE. In animals, ANGPT1 regulates vascular integrity and prevents plasma leakage. In knockout mice, the lack of the ANGPT1 and TIE2 genes was embryonically fatal.[62] In humans, the p.Ala119Ser mutation in the ANGPT1 gene makes the endothelial barriers vulnerable to the effects of an inflammatory agent that increases permeability, such as VEGF or bradykinin, and induces formation of angioedema by increasing vascular permeability.

In March 2018, Bafunno et al.,[12] identified a missense mutation in the ANGPT1 gene in an Italian family. The structure of the ANGPT1 protein revealed a mutational change in serine (c.807G>T, p.A119S) rather than alanine (wild type) in the 119th amino acid position. A similar mutation has been reported in a Brazilian family, but the case has not been published.[12] An autosomal dominant inheritance has been suggested. Microvascular abnormalities (capillary ectasia, kinks, and hemorrhage) have been observed in the results of nailfold capillaroscopy performed on these patients.[12]

Angioedema type with unknown etiology (uHAE)

In this most frequently seen type of normal C1-INH-level HAE (nC1-INH-HAE/type III), as in similar clinical phenotypes, bradykinin mediates the development of angioedema, and the treatment options are similar. It is expected that other gene mutations of the plasma contact or related systems will be reported in the future in most of these patients with HAE of unknown etiology (uHAE).[21,58]

The common features of the different types of HAE (C1-INH-HAE and nC1-INH-HAE) based on current classifications are illustrated in Table 1.
Summary and expectations from future

Aside from FXII, 2 new mutations have been reported in nC1-INH-HAE patients. In patients who are thought to be type III (nC1-INH-HAE), it is imperative to look for the PLG and ANGPT1 genotypes before the diagnosis of HAE is made. Research of these mutations is ongoing, including in Turkey, and in the near future, we expect to report other gene mutations that lead to uHAE.

Peer-review
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Conflict of Interest
None declared.

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