Introduction

While our understanding of hereditary angioedema (HAE) has evolved gradually since the condition was first described by Sir William Osler in 1888, it has been greatly enhanced in recent decades, particularly since the turn of the millennium, thanks to intensive research efforts, advances in laboratory techniques, increasing clinical observations, and improved understanding of the link between genetics and pathogenesis. HAE is characterized by recurrent non-pruritic, self-limiting subcutaneous or submucosal edema that can affect any part of the body (skin or mucous membranes or gastrointestinal and upper respiratory tract). Its severity and frequency varies among members of the same family and even within individual patients over time. Though initially believed to be an exclusively monogenic disorder, it has been postulated that the clinical expression of HAE is influenced by other conditions or cofactors and there is increasing evidence of multiple gene involvement.

There are two types of HAE: HAE due to C1-inhibitor (C1-INH) deficiency (C1-INH-HAE) and HAE with normal C1-INH (nC1-INH-HAE). C1-INH-HAE is further divided into type I HAE, which accounts for approximately 90% of cases and is characterized by low levels of C1-INH, and type II HAE, which accounts for the remaining 10% of cases and is characterized by normal or elevated levels of dysfunctional C1-INH protein. nC1-INH-HAE, in turn, is caused by an alteration in the F12 gene in up to 25-30% of cases (FXII-HAE). In the remaining 70-75% of cases, the genetic basis is unknown (unknown-HAE or U-HAE). The description of nC1-INH-HAE has driven a new field of study investigating the genetic basis of HAE in relation to bradykinin receptors and enzymes that act on fibrinolysis and the contact system. Much of the recent research has focused on gene polymorphisms.

The genetics of C1-INH-HAE

Mutations in the SERPING1 gene

C1-INH is a serine protease inhibitor that is a member of the serpin family, together with alfa-1-antitrypsin and antithrombin-III. Unlike other serpins, however, which have a single or small number of targets, C1-INH is a major inhibitor for several proteases. Its main function is to inhibit the complement system (C1; C1s, mannone-binding-lectin-associated-serine-proteases: MASP-1, and MASP-2) and other activation cascades involved in bradykinin...
production (factor XIIa, plasma kallikrein, and factor XIa from the contact/coagulation system; plasmin and tissue plasminogen activator from the fibrinolytic system)\textsuperscript{2}. It is encoded by the human C1-inhibitor gene (Gene Bank X54486; Swiss-Prot P05155), also known as the SERPING1 gene (OMIM no. 606860; GenBank NM_000062.2), located on chromosome 11q12-q13.\textsuperscript{1,3,4} The gene consists of eight exons (of which seven are protein-coding) and seven introns, distributed along a 17-Kb DNA segment. Exon 8 encodes the reactive center loop and the hinge region, which have an important role in protein function\textsuperscript{5}. The SERPING1 gene presents unusually dense clusters of Alu repeats in its introns, and is accordingly strongly predisposed to rearrangements (deletions and duplications) and genetic instability\textsuperscript{6-8}. De novo mutations are believed to exist in approximately 25% of all unrelated cases of C1-INH-HAE\textsuperscript{10}. This high frequency of de novo mutations seems to be related to Alu sequences\textsuperscript{7} and the existence of several mutation hotspots: the CpG dinucleotide at the end of exon 3 and exon 6, duplications of the ATG codon, and changes in the CpG dinucleotide of the reactive site (Arg444Cys and Arg444Leu) due to different mechanisms\textsuperscript{10}.

More than 450 SERPING1 mutations are currently listed in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac)\textsuperscript{11} and the HAE-specific HAEdb database (http://hae.enzim.hu/)\textsuperscript{12}. Type II HAE is the result of point mutations in or near the reactive center loop that result in inactive C1-INH\textsuperscript{11}. Up to 70% of patients with type II HAE have a mutation at Arg444 (the P1 residue) that produces dysfunctional C1-INH\textsuperscript{14}. Mutations in type I HAE, however, are highly heterogeneous and are distributed throughout the gene. Up to 20% of the cases are the result of large rearrangements (partial deletions, and less frequently, partial duplications)\textsuperscript{15}. The mutations most frequently observed are missense mutations (34%) and frameshift alterations and small indels (31%), followed by splice-site mutations (10%), nonsense mutations (7%), and regulatory mutations (1%)\textsuperscript{16}.

C1-INH-HAE has an autosomal dominant inheritance pattern with high penetrance, and most patients are heterozygous\textsuperscript{17}, although several cases of homozygosis\textsuperscript{18-22} and two cases of gonadal mosaicism have been described\textsuperscript{23,24}. Heterozygous patients have C1-INH plasma levels within 5-30% of normal values, rather than the 50% that might be expected. This seems to be the result not only of a synthesis defect but also of increased catabolism in patients with type I HAE\textsuperscript{25}, normal mRNA underexpression\textsuperscript{26}, or transinhibition of wild-type C1-INH translation by mutant mRNA and/or protein\textsuperscript{27}.

A significant correlation has been observed between HAE severity scores and baseline C1-INH activity (but not other complement components)\textsuperscript{28}. This observation, however, contrasts with the findings of several studies that have failed to find such a correlation\textsuperscript{29-33}. Many studies have attempted to establish a correlation between clinical phenotype and SERPING1 gene mutations, but the results have been contradictory, and most authors have not found any clear evidence of a relationship\textsuperscript{31,32,34-35}. The largest study to date, conducted in 2014, investigated 256 patients from 117 unrelated families from 4 European countries, and found that missense mutations were associated with significantly lower probability of HAE attacks before the age of 10 years\textsuperscript{36}. Onset before this age has been linked to a severe disease course. The study, however, did not evaluate other aspects of disease severity, such as laryngeal or abdominal attacks or frequency of episodes. A smaller study detected a significant relationship between missense mutations and clinical severity score and laryngeal and facial angioedema, but found no association with disease onset\textsuperscript{37}. In our opinion, the lack of standardized criteria for classifying HAE severity makes it difficult to study the relationship between SERPING1 mutations and clinical phenotype. Nevertheless, the high variability in clinical expression between patients with the same mutation, and even in the same patient over time, led to the hypothesis that SERPING1 mutations alone might not be enough to explain the diversity of clinical expression.

Certain polymorphisms accompanying pathogenic mutations in the SERPING1 gene have been proposed as possible indicators of a severe phenotype in C1-INH-HAE, although no correlation has been found to date for the p.V480M polymorphism (c.1438G>A, rs4926)\textsuperscript{38,39} and contradictory results have been reported for the polymorphism c.-21T>C in exon 2\textsuperscript{20,33,40}. The distribution of SERPING1 alterations has been observed to vary among different countries with Caucasian populations\textsuperscript{46}, suggesting that additional factors such as epigenetic changes or environmental influences (e.g., hormones, radiation, dietary habits) might contribute to SERPING1 gene abnormalities and/or expression in at least some cases. More studies of this type, but with larger populations, are needed.

No evidence of SERPING1 mutations has been found in up to 10% of C1-INH-HAE families with typical clinical manifestations of HAE and diminished C4 and C1-INH levels and activity, giving rise to the hypothesis that the disease could, in some cases, be due to alterations to intronic or untranslated regions that may modify SERPING1 expression or other factors that result in increased post-translational consumption of C1-INH\textsuperscript{36}.

**Other alterations**

Much of the current evidence suggests that alterations to the SERPING1 gene are not the only factor that determines the clinical expression of C1-INH-HAE. Other genetic variations investigated to date include polymorphisms...
in the angiotensin-converting enzyme (ACE) gene. ACE is the main enzyme responsible for bradykinin degradation, and a defect in this degradation has been attributed a potential role in the clinical expression of HAE. The I/D polymorphism, which is the most relevant polymorphism in the ACE gene, is responsible for up to 47% of enzyme levels, but no evidence has been found for a link between clinical HAE manifestations and this polymorphism. No evidence has been found either for an association with polymorphisms investigated to date in certain cell receptors, such as the two known receptors for bradykinin (polymorphisms 58C/T and 181C/T in BDKRB1 gene and 669C/G and 1098G/C in BDKR2 gene), which is a key mediator of vascular permeability.

Alterations to the F12 gene, which encodes coagulation factor FXII, have also been studied because of their role in the kinin system. The functional promoter polymorphism 46C/T for instance, was significantly associated with delayed disease onset—independently of the accompanying SERPING1 gene mutation—and individuals with this polymorphism did not need long-term treatment. F12-46C/T carriage has, therefore, been postulated as an independent modifier of C1-INH-HAE severity.

The lectin pathway (LP) is a component of the innate immune defense formed by several pattern recognition molecules that form complexes with MASP-1 and MASP-2, which cleave C4 and C2. Mannose-binding lectin (MBL) is a component of this LP and because of its ability to activate the complement system, it might have an influence on HAE pathophysiology. Studies investigating possible links, however, have not found clinical HAE expression to be associated with either MBL plasma levels and their capacity for complement activation or polymorphisms that influence these levels.

Peripheral blood mononuclear cells (PBMCs) have also been analyzed, but no evidence has been found for a common altered PBMC expression pattern or for differential gene expression in PBMCs.

Emotional and physical stress both influence the hypothalamic-pituitary-adrenal axis, triggering the release of minerals and glucocorticoids. The N363S (rs6195) polymorphism in exon 2 of the gene encoding glucocorticoid receptor has been linked to an increase in glucocorticoid sensitivity. As emotional stress has been recognized as a common trigger of angioedema attacks, the possible involvement of this polymorphism in the clinical manifestations of C1-INH-HAE has also been investigated, but no association has been found between disease severity and the carriage or non-carriage of this polymorphism.

In a recent study, 15 genes associated with the contact system were investigated by next-generation sequencing in 23 members of a Brazilian family, 9 of whom had HAE symptoms. Although some genetic alterations (p.Ile197Met – HMWK-, p.Glu298Asp –NOS3-, and p.Gly354Glu – B2R) were found in almost all the symptomatic patients, suggesting a possible influence on symptom expression, they were also found in some of the asymptomatic individuals, highlighting the need for more studies.

Finally, newly circulating extracellular microRNAs have been postulated as potential predictors of HAE attack frequency in a small population studied, potentially opening up a new path of research in this field.

**Genetics of nC1-INH-HAE**

Patients with nC1-INH-HAE have no deficiencies in C1-INH levels or activity and no alterations in the SERPING1 gene. The underlying pathophysiology of nC1-INH-HAE remains unknown, although the role of contact pathway dysregulation is gaining increasing recognition.

**Mutations in the F12 gene**

Approximately 25% of patients with nC1-INH-HAE have a mutation in the F12 gene. This leaves 75% of patients with familial nC1-INH-HAE with no known genetic basis. The mutations described to date are located in exon 9 and intron 9, on chromosome 5q33-qter (OMIM no. 610619). The most frequent mutation, which is found in the majority of patients, results in a threonine-to-lysine substitution (c.983C>A, p.Thr309Lys) in the secreted zymogene protein (also referred to as p.Thr328Lys with the addition of the leader protein). Another mutation predicting a threonine-to-arginine substitution (c.983C>G, p.Thr309Arg; also referred to as p.Thr328Arg with the addition of the leader protein) in the same codon has been described in two families. There has also been a report of an 18-bp duplication and a 72-bp deletion in the same proline-rich region. The 18-bp duplication (c.892_909dup) is caused by the repeated presence of 6 amino acids (p.298–303) while the 72-bp deletion (c.971_1018 + 24del72) causes a loss of 48 bp in exon 9 (coding amino acids 324-340) and a loss of 24 bp in intron 9, including the splice site of exon 9. These deletions do not cause a reading frameshift or a premature stop codon, and the FXII catalytic domain remains preserved.

We do not yet fully understand how these genetic alterations contribute to the pathophysiology of nC1-INH-HAE. One initial theory proposed was that the p.Thr309Lys mutation might be associated with increased FXII enzymatic activity, but this was not confirmed in a subsequent study and is no longer believed to be the case. p.Thr309Lys (T309K) and p.Thr309Arg (T309R) have both been studied in vivo and in vitro mouse models and been observed to result in a loss of O-liked glycosylation of the amino acid residue. This loss increased the susceptibility...
of FXII zymogen autoactivation, leading to excessive activation of bradykinin formation through the kallikrein-kinin pathway. These mutations have also been found to result in an accelerated activation of FXII by plasmin, a natural activator of the contact system. Furthermore, this activation is not completely regulated by C1-INH regulation, possibly explaining why HAE occurs in patients with normal C1-INH levels and activity. It has also been hypothesized that variations in the plasminogen activation system could relate to disease expression and/or activity, but more studies are needed.

nC1-INH-HAE presents an autosomal dominant inheritance pattern but has a very low penetrance, particularly in males (over 90% of male carriers are asymptomatic compared with 40% of females). Mutations in the F12 gene are mainly heterozygous. There has just been one report of 2 Brazilian patients from unrelated families. Other genetic alterations have been described in a family with U-HAE62 and the 46C/T polymorphism in the F12 gene 43, suggesting a possible influence on phenotype. This theory, however, was not confirmed in a recent study, which found no correlation with disease expression or severity.

Recently, a missense mutation in the angiopoietin-1 gene (ANGPT1, c.807G>T, p.A119S), associated with a reduced ability to bind the natural receptor "tunica interna endothelial cell kinase-2" (TIE2) of the ANGPT1 p.A119S variant, has been described in a family with U-HAE, adding a further research field.

Other genetic alterations

Polymorphisms in bradykinin-degrading enzymes, which result in altered enzyme levels or activity, have also been studied in nC1-INH-HAE. In an initial study, the I allele of the I/D polymorphism of the ACE gene and the A allele of the XPNPEP2 gene were found in three symptomatic patients from a family with FXII-HAE, suggesting a possible influence on phenotype. This theory, however, was not confirmed in a recent study, which found no correlation with disease expression or severity.

Conclusions

The original hypothesis that HAE was an exclusively monogenic disease is losing weight. Growing understanding of the pathophysiology of HAE has triggered the search for genetic alterations at other points of the contact system and related systems, with evidence increasingly pointing to the involvement of multiple genes. The lack of a standardized system for measuring disease severity constitutes a clear obstacle. Recently missense mutations in the SERPING1 gene and the 46C/T polymorphism in the F12 gene, have been both associated with later disease onset (and hence less severe disease) in Caucasian patients with C1-INH-HAE. These results are promising but should be regarded with caution as they cannot explain the high variability of clinical presentation in carriers of the same mutation or even in the same patient over time. Studies are increasingly investigating the role of epigenetic and environmental factors in this variability. In the case of nC1-INH-HAE, detection of genetic alterations is essential for an accurate diagnosis, as there are no biological markers or clear clinical or laboratory diagnostic criteria.

References


