

Special Article

International Consensus on the Use of Genetics in the Management of Hereditary Angioedema

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Hereditary angioedema (HAE) is becoming much more genetically complex than was initially considered. Thus, the role of HAE genetics is expanding beyond research laboratories, and the genotyping of subjects suffering from HAE has become diagnostically indispensable in clinical practice. The synthesis and interpretation of the clinical and biochemical analyses to facilitate appropriate genetic test selection has thus also become significantly more complex. With this in mind, an international multidisciplinary group of 14 experts in HAE genetics and disease management was convened in October 2018. The objective was to develop clear, actionable, evidence- and consensus-based statements aiming to facilitate the communication between physicians treating patients with HAE and clinical geneticists, and thus promote the effective use of genetics in the management of the disease. Eleven consensus statements were generated, encompassing considerations regarding the clinical indications for genotyping patients with angioedema, the methods of detection of HAE-causative variants, the variant pathogenicity curation, the genotyping of patients with HAE in the clinic, and genetic counseling. These statements are intended both to guide clinicians and to serve as a framework for future educational and further genetic testing developments as the field continues to evolve rapidly. © 2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;■:■-■)

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INTRODUCTION

Hereditary angioedema (HAE) is a potentially fatal genetic disorder manifested clinically by episodes of nonpruritic and nonpitting swelling of the subcutaneous and/or submucosal tissues. The prototype HAE with C1-inhibitor (C1-INH) deficiency (C1-INH-HAE) caused by mutations of the *SERPING1* gene encoding C1-INH is diagnosed biochemically by the detection of low antigenic and/or functional levels of C1-INH with HAE genetics generally considered not to be required for the diagnosis of C1-INH-HAE and being reserved for selected cases where results are ambiguous.¹ Furthermore, the description of HAE with normal C1-INH (nl-C1-INH-HAE) in 2000² rekindled interest for HAE genetic testing. As a result, novel sequence variants are increasingly detected not only for *SERPING1* gene but also for a rapidly increasing number of genes that are associated with HAE.¹

At the same time, high-throughput sequencing technologies facilitate the detection of sequence variants whose effects on gene expression or protein function are unclear. Moreover, the various techniques in use for genotyping patients with HAE are not validated and the concordance between them is largely unknown. As a consequence, HAE genetic diagnosis has become much more complex and the establishment of the genetic defect underlying individual cases represents an increasingly challenging task.

As the reach of HAE genetics is expanding beyond research laboratories, the emerging key issue is which genetic testing is

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Abbreviations used

ACMG- American College of Medical Genetics and Genomics
AMP- Association for Molecular Pathology
ANGPT1-HAE- HAE due to angiopoietin-1 mutations
HAE- Hereditary angioedema
HAEdb- C1-INH gene mutation database
C1-INH- C1-inhibitor
nl-C1-INH-HAE- HAE with normal C1-INH
C1-INH-HAE- HAE with C1-inhibitor deficiency
FXII-HAE- Hereditary angioedema due to FXII mutations
HGMD- Human Genome Mutation Database
PLG-HAE- HAE due to plasminogen mutations
VUS- Variant of uncertain significance

appropriate for any given patient with HAE and how this evidence can be used to inform medical management decisions. Thus, this consensus paper is primarily aimed at physicians treating patients with HAE to facilitate the communication between them and clinical geneticists and therefore the effective use of genetics in the management of the disease (Table I).

OVERVIEW OF HAE GENETICS

C1-INH-HAE genetics

Initially identified for its regulatory activity in the complement system, C1-INH (UniProt ID: P05155) is a plasma serine protease inhibitor (Serpin), which controls the complement pathway through inhibition of C1r, C1s, MASP1, MASP2, the contact system by inhibiting factor XII and plasma kallikrein, the intrinsic coagulation cascade through factor XI and thrombin inhibition, and the fibrinolytic system through plasmin and tissue-plasminogen activator inhibition.³ In the 1960s, biochemical studies identified the deficiency of C1-INH in patients with HAE (C1-INH-HAE),^{4,5} but only in the 1980s were mutations in *SERPING1* (OMIM #606860; GeneBank NM_000062.2), the gene encoding C1-INH, described in families with HAE.⁶⁻¹⁰

In approximately 85% of C1-INH-HAE cases, deleterious mutations affecting *SERPING1* lead to quantitative C1-INH deficiency (type I), with plasma levels below 50% of the reference range.¹¹ Although C1-INH-HAE is a dominant disease and most of the patients carry heterozygous mutations in *SERPING1*, C1-INH plasma levels usually range from 5% to 30% of normal in these patients, in contrast to the 50% expected to be produced by the wild-type allele.⁶ Recently, some mutated C1-INH were shown to form intracellular aggregates resulting in the normal C1-INH being trapped in the endoplasmic reticulum, in a dominant-negative mechanism that may in some cases explain the remarkably decreased secretion of C1-INH.¹² Variants giving rise to severely truncated C1-INH, like nonsense mutations and deletions or insertions causing frameshift and premature termination codons, usually lead to mRNA degradation by mRNA decay pathways.^{13,14} Mutations responsible for C1-INH-HAE type I are found across the *SERPING1* gene. Currently, more than 500 variants in *SERPING1* are described in the Human Genome Mutation Database (HGMD)¹⁵ and in the C1-INH gene mutation database (HAEdb).¹⁶ From 567 variants listed in HGMD, 544 were reported to be disease-causing mutations for HAE and 5 as likely disease-causing. The remaining 18 variants were associated with primary immunodeficiency and macular degeneration.^{17,18} Less frequent, mutations mainly

affecting exon 8 of *SERPING1*, the region encoding the reactive center loop of C1-INH, lead to the secretion of a dysfunctional C1-INH protein (C1-INH-HAE type II).^{9,10} Only 1 mutation outside exon 8 (p.Lys273del) is described to cause C1-INH-HAE type II.¹⁹ However, in about 5% of C1-INH-HAE cases, no mutation could be detected in the coding region of *SERPING1* gene.^{20,21}

Although family history is an important indication of C1-INH-HAE, it must be remembered that about 25% of cases are due to *de novo* mutations^{21,22} and that variable clinical expression of the disease may lead to misdiagnosis and significant diagnostic delay.

Animal models have been helpful in demonstrating that the increased vascular permeability in C1-INH deficiency is mediated by bradykinin²³ and that overexpression of the bradykinin B2 receptor leads to increased vascular permeability and spontaneous bowel edemas.²⁴ More recently, a new C1-INH-deficient mouse model was successfully treated by gene therapy mediated by a single intravenous infusion of an adeno-associated viral vector expressing the human *SERPING1*. This gene therapy treatment raised C1-INH levels for 24 weeks, abrogating the increased vascular permeability phenotype with reduced vascular leakage in skin and internal organs.²⁵ Haslund et al¹² demonstrated that viral gene transfer of *SERPING1* in fibroblasts of a patient carrying a mutation leading to C1-INH-HAE was able to increase secretion of normal C1-INH. This suggests that it may in future be possible to achieve successful transduction and gene delivery in patients with C1-INH deficiency and should encourage new studies using viral-vector gene delivery of *SERPING1* in C1-INH-HAE.

HAE due to FXII mutations genetics

In the year 2000, a new form of recurrent angioedema was described in patients without quantitative or qualitative C1-INH deficiency.^{2,26} This type of HAE presented with a similar clinical phenotype to that of types I and II, and has now been termed nl-C1-INH-HAE. An important precipitating factor in this new form, which occurs predominantly in women, is exposure to elevated levels of estrogen (eg, pregnancy or treatment with oral contraceptives or hormone replacement therapy). Analyzing the genetic background of this new condition has revealed the presence of 2 missense mutations (Thr328Lys and Thr328Arg or Thr309Lys/Arg as referred on the basis of nomenclature without the signal peptide) affecting the same residue of the *F12* gene (OMIM#610619) encoding for coagulation factor XII (FXII, Hageman factor)²⁷ in a proportion of the affected families. FXII, a serine protease the expression of which is increased by estrogen,²⁸ is involved in the release of bradykinin, as the activated enzyme has the capacity to cleave plasma prekallikrein—generating kallikrein, an enzyme responsible for the release of bradykinin from high-molecular-weight kininogen. The identified mutations are harbored by approximately 15% to 30% of nl-C1-INH-HAE cases in heterozygous form; however, a few homozygous carriers were also identified.²⁹ Several additional mutations affecting the same proline-rich region of the FXII protein as the point mutations were latterly reported including a deletion of 72 bp (c.971_1018+24del72)³⁰ and a duplication of 18 bp (c.892_909dup).³¹ The first article that determined the function of these FXII mutations demonstrated that plasma samples from patients carrying the Thr328Lys mutation showed increased FXII amidolytic activity compared with controls, suggesting a gain-of-function nature of the variants.³² Later studies revealed that this

TABLE 1. Summary of the 11 key statements made throughout the consensus

Statement 1	Ordinarily, genotyping is not necessary for the diagnosis of C1-INH-HAE with rare exceptions, such as inconclusive measurements of C4 and/or C1-INH antigenic or functional levels, and the diagnosis of the disease in newborns and children or in asymptomatic relatives.
Statement 2	Genotyping is required for the diagnosis of FXII-HAE, PLG-HAE, and ANGPT1-HAE.
Statement 3	<i>SERPING1</i> genotyping should cover all exonic, 5'- and 3'-untranslated regions and exon-intron boundaries for SNVs, MNVs, and large defects.
Statement 4	Segregation study of novel <i>SERPING1</i> variants with the disease in multiple family members can provide key evidence of their pathogenicity and penetrance, and should be performed where possible.
Statement 5	Exon 9 of <i>F12</i> is the only region of the gene that is recommended to be analyzed as a routine molecular diagnosis of FXII-HAE.
Statement 6	HGVS nomenclature should be used in the reports.
Statement 7	Clinical laboratories and researchers should submit all detected variants to ClinVar.
Statement 8	The constitution of an approved by ClinGen HAE Variant Curation Expert Panel is recommended to help evaluate the pathogenicity of individual genetic variants submitted to ClinVar, to design and validate gene-optimized rules for variant classification.
Statement 9	Laboratories performing and reporting results of HAE genetic testing are encouraged to incorporate the adapted ACMG-AMP guidelines.
Statement 10	There is an urgent need to establish and implement clinical practice guidelines, standardized procedures for gene testing and external quality assessment programs to provide a framework for best laboratory practice and reporting on the genetic diagnosis of HAEs.
Statement 11	It is recommended to implement genetic counseling—placed within the legal framework of each country—in collaboration with experts specialized in human genetics and in the management of HAE.

MNV, Multinucleotide variant; SNV, single-nucleotide variant.

increased FXII activity measured in patients' plasma is attributable to increased ability of the mutant FXII to become activated. Björkqvist et al³³ demonstrated an enhanced rate of contact-induced activation of FXII bearing either missense mutation by using low doses of dextran sulfate, which were sufficient to trigger FXIIa formation in mutation carriers but not in healthy individuals; however, raising the dextran sulfate concentration eliminated the difference. Albeit that they first suspected that the defective glycosylation pattern of the mutants underpinned these alterations, in a subsequent article they unraveled the role of plasmin as a natural trigger for bradykinin production in patients with HAE due to FXII mutations (FXII-HAE). As reported, the 2 FXII point mutations and the deletion (which results in the addition of 27 new amino acids by intron retention, PMID: 25113305) introduced new sites in the protein that were sensitive to enzymatic cleavage and therefore activation by plasmin, and this excessive activation temporarily rendered C1-INH inefficient at hindering bradykinin overproduction.³⁴ Moreover, Ivanov et al³⁵

recently showed that FXII with Lys/Arg substitutions for Thr309 can be cleaved by thrombin and factor XIa to generate the truncated species δ FXII, activation of which by kallikrein is markedly enhanced when compared with factor XII, increasing kininogen cleavage *in vivo*.

HAE due to plasminogen mutations genetics

The gene encoding plasminogen (*PLG*, OMIM#173350; GenBank NM_000301.3) is located on chromosome 6q26 and consists of 19 exons.³⁶ The protein encoded by the *PLG* gene is a proteolysis-activated zymogen that is converted to plasmin and angiostatin; the former dissolves fibrin in blood clots. Furthermore, plasmin was proposed to be a natural activator of FXII.³⁷ Up to now, more than 70 different mutations causing plasminogen deficiency have been reported in the HGMD, but only 1 specific missense *PLG* mutation, a substitution (c.9886A>G) causing a lysine to glutamic acid change (Lys330Glu), was observed in nl-C1-INH-HAE.^{38,39} The *PLG* Lys330Glu mutation was first described by Bork et al³⁸ who demonstrated the presence of this rare variant in 14 patients with nl-C1-INH-HAE of 4 families, and independently by Dewald³⁹ describing the identification of this mutation in 18 patients with nl-C1-INH-HAE from 3 families. Following this, the same mutation was described in further 3 French,⁴⁰ 1 Greek,⁴¹ 1 Spanish,⁴¹ 1 German,⁴² and also in an additional 2 Japanese families,⁴³ whose affected members suffer from nl-C1-INH-HAE, making this a global disorder. Patients with HAE due to plasminogen mutations (PLG-HAE) are characterized by a later disease onset (usually in adulthood) and by swellings affecting the face, tongue, and the larynx.^{38,42} Although the pathophysiology of PLG-HAE is not entirely known, Dewald³⁹ suggested that the mutant plasminogen may have an altered structure and function; thus, the mutation may modify the affinity of plasminogen to its binding partners.

HAE due to angiotensin-1 mutations genetics

HAE is attributable to bradykinin-mediated mechanisms. The detection of missense *ANGPT1* variants suggests that vascular leakage may also occur when the effect of inducers, such as bradykinin and vascular endothelial growth factor, is not counterbalanced. The variants p.Gln370His and p.Arg382Gln were found in single individuals, but have not been proven to segregate in the families where they were found. In fact, functional evidence of their role in HAE is still missing. Heterozygous gene variants can lead to a partially nonfunctional protein with an impairment of protein functions although the total amount of the protein expressed in the cell is normal. As a result, the *ANGPT1*'s reduced ability to counteract the increment of endothelial permeability produced by inducers, such as bradykinin and vascular endothelial growth factor, causes vascular leakage.⁴⁴ The p.Ala119Ser variant was shown to affect the ability of the functional protein to assemble in the correct fashion. The p.Ala119Ser substitution results in a pathogenic loss of function of the protein due to a mechanism of haploinsufficiency.⁴⁵ Haploinsufficiency is one of the primary mechanisms underlying many Mendelian diseases and may occur in several ways. Haploinsufficiency implies a heterozygous mutation resulting in a single functional copy of a gene that is inadequate to sustain the normal function of the protein. All mutations leading to loss-of-function alleles would be predicted to result in the expression of a functional *ANGPT1* protein below the threshold of expression required for proper function. However,

given the lack of detection of mutant protein, the clinical significance of different *ANGPT1* variants should be supported by robust evidence in keeping with the American College of Medical Genetics and Genomics (ACMG) guidelines (*in silico* analysis, etc).

METHODS

An international multidisciplinary group of 14 experts in HAE genetics and disease management was convened in 2018 and tasked with the development of an evidence- and consensus-based statement aiming to facilitate the communication between physicians treating patients with HAE and clinical geneticists and thus the effective use of genetics in the management of the disease. In October 2018, the group of experts held a preliminary meeting in Gazzada Schianno, Italy, where they identified 5 key topics for further discussion: the clinical indications for genotyping patients with angioedema, the methods of detection of HAE-causative variants, variant pathogenicity curation, the genotyping of patients with HAE in the clinic, and genetic counseling.

Thereafter, experts reviewed relevant literature via a detailed PubMed search and summarized the evidence on the above topics. Not unexpectedly, limited data were available to establish evidence-based clinical practice statements. Case reports and a limited number of case series were the primary evidence base available for the use of genetics in clinical decision making for angioedema. Thus, the group proceeded with the development of consensus recommendations.

All members of the group contributed on the basis of their expertise and a preliminary draft of statements was formulated. The experts reviewed this preliminary draft in 2 consecutive rounds to reach a minimum level of agreement. In mid-April 2019, the revised draft was distributed by e-mail for feedback to all Hereditary Angioedema International Working Group members as well as to 55 colleagues who were the coauthors of all publications of the last 5 years retrieved by searching PubMed for “angioedema” AND “genetics.” Feedback was returned by 15 of the above colleagues.

During the 11th C1 Inhibitor Deficiency and Angioedema Workshop in Budapest, in May 2019,⁴⁶ an open consensus meeting took place where the revised statements and the returned comments were presented and discussed with the participants of the workshop, and a consensus was developed using a modified Delphi survey via voting. The participants were asked to rate their agreement with each of 11 recommendations on a 3-point scale (1 = disagree, 2 = neutral, 3 = agree). An *a priori* threshold of greater than or equal to 80% affirmative votes was required for acceptance of each statement.

Clinical indications for genotyping patients with angioedema

Statement 1. *Ordinarily, genotyping is not necessary for the diagnosis of C1-INH-HAE, with rare exceptions, such as inconclusive measurements of C4 and/or C1-INH antigenic or functional levels, and the diagnosis of the disease in newborns and children or in asymptomatic relatives.*

Despite the genetic nature of C1-INH-HAE, the disease is generally diagnosed exclusively on the basis of clinical and biochemical findings. Genetic screening of the *SERPING1* gene, though economically and technically affordable to many laboratories, can be obviated in most cases by the measurement of C4 concentration and antigenic and functional C1-INH levels, especially when a family history of angioedema is reported.¹

However, certain special situations may benefit from genetic studies. One of such is the diagnosis of suspected *de novo* cases.

When dealing with patients with HAE lacking family history, *SERPING1* screening can be useful both as a confirmation for a C1-INH-HAE diagnosis in the *propositus* and to rule out the disease in asymptomatic relatives.⁴⁷ Furthermore, measurement of C1-INH and C4 antigenic levels can yield inconclusive results in the form of borderline values while C1-INH functional assays (Berichrom or Microvue assays) are sensitive to the preanalytical handling of plasma samples and can cause artefactually low C1-INH functional levels.

In addition, the levels and reference ranges of biochemical parameters used in HAE diagnosis change throughout life, which can hamper the identification of the disease in newborns and very young children for whom concentration reference ranges are not well defined,^{48,49} although there is scarce evidence indicating that testing for antigenic and functional C1-INH levels is a good tool to diagnose or discard C1-INH-HAE despite the age of the patient.⁵⁰ Genetic screening is not affected by such artifacts and biases and can be performed in newborns and children for diagnostic purposes, especially when dealing with relatives of already genetically diagnosed patients. Nevertheless, asymptomatic newborns and children carrying the same *SERPING1* mutation as their suffering family member(s) should be considered as having inherited C1-INH deficiency accepting that the clinical expression of the condition may occur much later. Finally, prenatal and preimplantation genetic diagnosis of C1-INH-HAE has also been successfully performed.^{51,52}

Statement 2. *Genotyping is required for the diagnosis of FXII-HAE, PLG-HAE, and HAE due to angiotensin-1 mutations (ANGPT1-HAE).*

For patients with nl-C1-INH-HAE, the diagnosis of FXII-HAE, PLG-HAE, or ANGPT1-HAE can be established only after genotyping of the corresponding genes. In addition, the diagnosis of unknown HAE in patients with HAE with normal C1-INH levels is dependent on the absence of pathogenic variants in these genes. Moreover, approximately 40 cases of patients initially diagnosed or classifiable as idiopathic nonhistaminergic acquired angioedema have been reported, which, when appropriately genotyped, were found suffering from FXII-HAE, PLG-HAE, or ANGPT1-HAE.⁵³ Therefore, genotyping of *F12*, *PLG*, and *ANGPT1* is recommended for the diagnosis of idiopathic nonhistaminergic acquired angioedema to be confirmed. Finally, there are anecdotal cases of C1-INH-HAE, which were clinically expressed after receiving angiotensin-converting enzyme inhibitors and diagnosed as such by the measurement of C4 and C1-INH levels. This could also be true with FXII-HAE, PLG-HAE, or ANGPT1-HAE especially given the older age at their onset. Thus, genotyping of *F12*, *PLG*, and *ANGPT1* should be considered for the diagnosis of acquired angioedema related to angiotensin-converting enzyme inhibitors to be established, particularly in cases with normal C1-INH levels that relapse after the discontinuation of the drug. In some cases of acquired angioedema with C1-INH deficiency, in particular if C1q is normal and anti-C1-INH antibodies cannot be detected, exclusion of mutation in *SERPING1* gene may also help to establish the diagnosis.

DETECTION OF HAE-CAUSATIVE VARIANTS C1-INH-HAE genotyping

Statement 3. *SERPING1 genotyping should cover all exonic, 5'- and 3'-untranslated regions and exon-intron boundaries for*

single-nucleotide variants, multinucleotide variants, and large defects.

Sanger sequencing is generally the most cost-effective and preferred technique when undertaking genetic screening of small, single genes such as *SERPING1*. However, high-throughput technologies are now available for massive DNA sequencing and are being successfully applied to *SERPING1* screening.⁵⁴ Whole-exome sequencing panels allow for the identification of point and frameshift mutations or copy number variation in a rapid and straightforward manner. Considering the high density of interspersed Alu elements in the *SERPING1* locus and its tendency to undergo nonhomologous end-joining processes leading to gross deletions or insertions, the detection of large defects is required using additional approaches, such as Southern blot, long-range PCR, or the cost-effective, criterion standard Multiplex Ligation-dependent Probe Amplification. Further developments for the specific estimation of *SERPING1* exonic doses have been recently published, allowing a precise characterization of the deleted or inserted DNA fragment by multiplex exon quantification.⁵⁵

Irrespective of the laboratory techniques used for *SERPING1* genotyping, the analysis should cover all exonic, 5'- and 3'-untranslated regions and exon-intron splice junctions for single-nucleotide variants, multinucleotide variants, and large defects. The detection of missense mutations early in the screening process should not prevent a complete screening of the gene because copresence (cis/trans) of a second truly pathogenic variant cannot be excluded.

Genotyping of newly affected members of HAE families with known pathogenic *SERPING1* mutations where the biochemical diagnosis has been confirmed does not add to their clinical management. However, affected parents are usually interested to know whether their asymptomatic children carry the responsible mutation, or less often request prenatal or preimplantation diagnosis. In these cases, an analysis targeted to the variant under consideration is sufficient and more cost-effective.

In case of the detection of a known pathogenic mutation reported by a reputable source in a public database (eg, ClinVar and Human Gene Mutation Database), reporting is straightforward. The same approach should not be applied to previously unreported variants. As far as the pathogenicity assessment of these variants is concerned, supporting evidence is required, such as population data, *in silico* predictions, and strict cosegregation with the disease provided that large pedigrees are available. Nevertheless, considering the low polymorphic nature of this gene, novel sequence variants are likely associated with deleterious features causing loss of secretion and/or function, which allows for the classification of most nonsense, splicing, small frameshift mutations, and large defects. Missense mutations and changes affecting untranslated sequences in the 5' or 3' ends require additional evidence of pathogenicity, according to ACMG recommendations (see below). Variants affecting canonical splicing or nearby sequences can be classified as pathogenic but might have to be proved as such at the RNA level in the remaining cases.²² Acknowledging that not all laboratories have the facilities to apply advanced molecular biology techniques (cloning, RNA and functional studies, intronic region genotyping), affiliated or reference laboratories may be used.

Statement 4. *Segregation study of novel SERPING1 variants with the disease in multiple family members can provide key evidence*

of their pathogenicity and penetrance, and should be performed where possible.

In cases of the detection of novel *SERPING1* variants, their segregation with the disease in multiple affected family members can be weighted as moderate or strong evidence of pathogenicity, depending on the structure and informativity of the family. That case may also be mentioned when a novel variant, not found in healthy populations according to international databases, is detected in multiple unrelated affected individuals. This would also support evidence of pathogenicity even in the absence of a family segregation study. This situation is quite rare; however, it may occur when a larger set of patients is analyzed simultaneously, especially in previously unstudied populations. Similarly, lack of segregation of a variant with a phenotype provides strong evidence of tolerance. At least 1 affected and 1 unaffected first-degree relative, preferably both parents, must be examined to uncover the segregation of *SERPING1* variants. An analysis targeted to the variant under consideration is sufficient for the examination of the family members. Careful clinical evaluation is required to rule out mild symptoms of reportedly unaffected individuals or to ensure that asymptomatic family members are truly unaffected. Also, family relationships need to be confirmed to exclude nonbiological relationships (eg, nonpaternity).

nl-C1-INH-HAE genotyping

Search for *F12*, *PLG*, and *ANGPT1* gene variants is the only diagnostic approach in the setting of nl-C1-INH-HAE given the lack of biochemical diagnostic testing (see Statement 2).

Statement 5. *Exon 9 of F12 is the only region of the gene that is recommended to be analyzed as a routine molecular diagnosis of FXII-HAE.*

In nl-C1-INH-HAE, the first gene to be investigated is *F12*. Although there are no consistent epidemiological data of FXII-HAE prevalence,⁵⁶ specific mutations in *F12* are the most commonly identified in nl-C1-INH-HAE.⁵⁷⁻⁵⁹ Because the 4 mutations in *F12* described to cause FXII-HAE are located within the proline-rich region encoded by exon 9,^{27,31,60} this is the only region from *F12* recommended to be analyzed for routine molecular diagnosis in nl-C1-INH-HAE. The sequencing of exon 9 of *F12* by the Sanger method is the criterion standard and can be performed from a small amount of DNA, as previously described.^{57,61,62} Sequencing both forward and reverse strands of exon 9 is recommended owing to the polymorphism c.1018+19delG (rs35966430) in intron 9 (allele frequency = 0.6173; ExAc database), which may hamper the analysis. More than 40 mutations described throughout the gene are associated with *F12* deficiency in the HGMD,¹⁵ indicating that alterations outside exon 9 are unlikely to be responsible for HAE. However, it must be noted that the FXII proline-rich region spans from p.296 to p.349; that is, its last 9 amino acids are encoded in exon 10. Thus, analyzing only the exon 9 possible mutations in nucleotides encoding p.341-p.349, located in exon 10, will be disregarded. Because of the incomplete penetrance observed in FXII-HAE, family history is not a requirement for the molecular investigation.

To date, for the *PLG* gene only 1 mutation (p.Lys330Glu) associated with nl-C1-INH-HAE has been identified. Once a pathogenic variant is described, it can be searched with a large array of methods. PCR-based methods without or with Sanger sequencing are widely available, accurate and precise, and use

relatively cheap reagents. Otherwise, the pathogenic *PLG* mutation could be detected by the use of automated sequencing-based methods in combination with the analysis of the other 2 genes related to nl-C1-INH-HAE.

Few loss-of-function mutations have been identified in the *ANGPT1* gene. Because it is reasonable that different variants may occur that impair the functionality of the angiotensin-1, the whole gene should be analyzed and automated sequencing-based methods are suggested.

VARIANT PATHOGENICITY CURATION

About a third of *SERPING1* variants are not protein-truncating or misfolding missense mutations, most of which lack strong evidence regarding their pathogenicity. At the same time, only half of the nearly 600 HAE-associated *SERPING1* variants found in the literature are reported in public databases, such as HAEdb and ClinVar, with not uncommon discordances among them. Moreover, information regarding variants of HAE-related genes that have been proved benign is missing from public databases. Bearing also in mind the high mutagenicity of the *SERPING1* gene and the dramatic increase in variants identified after the implementation of high-throughput DNA sequencing, their interpretation is a challenging process and highlights the necessity for a standardized pipeline of variant pathogenicity curation.

Nomenclature

Statement 6. *Human Genome Variation Society nomenclature should be used in the reports.*

In accordance to ACMG-Association for Molecular Pathology (AMP) guidelines, “uniform nomenclature, informed by a set of standardized criteria, is recommended to ensure the unambiguous designation of a variant and enable effective sharing and downstream use of genomic information.”⁶³ The use of the Human Genome Variation Society nomenclature⁶⁴ is recommended, using available online tools.⁶⁵ Clinical reports should include sequence reference (eg, NM_000062.2) derived from the National Center for Biotechnology Information RefSeq database⁶⁶ to ensure unambiguous naming of the variant at the DNA level, as well as to provide the coding (“c.”) and protein (“p.”) nomenclature to assist in functional interpretations. In the case of *SERPING1* genotyping, where a historical alternate nomenclature has been used,¹⁶ HGVS nomenclature should be used with the additional notation of the historical naming being optional.

Variant submission to ClinVar

Statement 7. *Clinical laboratories and researchers should submit all detected variants to ClinVar, including the clinical assertions and evidence used for variant classification.*

Data sharing through a centralized open-source database is essential to achieve accurate and consistent interpretation of variants identified by genetic testing. Launched in 2013, the publicly accessible ClinVar database is an international, submission-driven archive of variant-condition interpretations hosted by the National Center for Biotechnology Information. It is a key partner of the Clinical Genome Resource,⁶⁷ whose goals include the development of community resources to standardize genomic variant interpretation and facilitate the sharing of genomic data.^{68,69}

To this aim, clinical laboratories and researchers involved in genotyping patients with angioedema should submit all detected variants to ClinVar, including clinical assertions (pathogenic, variant of uncertain significance [VUS], and benign) and evidence used for variant classification. Novel variants proven benign/likely benign in accordance to ACMG-AMP guidelines should be also submitted.

Statement 8. *The constitution of an approved by ClinGen HAE Variant Curation Expert Panel is recommended to help evaluate the pathogenicity of individual genetic variants submitted to ClinVar, to design and validate gene-optimized rules for variant classification, and to submit what has been reported in existing centralized (HAEdb) and internal databases.*

Submitters can find all relevant information in the Help session of the ClinVar submission portal.⁷⁰ Registration of their organization is initially required and following ClinVar approval, laboratories can proceed with variant submission, in real-time or retrospectively, for single cases or large-scale studies. As more information becomes available allowing the interpretation of new sequence variants, it is also recommended that the laboratories amend previous reports and provide updated results to the ClinVar database.

Submitted variants require standardized annotation (HGVS nomenclature or chromosomal coordinate change), associated condition, interpretation of clinical and/or functional significance (including benign variants), interpretation criteria (ACMG-AMP), collection method, allele origin (germline or somatic), and individual affected status. A wide range of additional information can be of utility, such as variant (number of variant observations, cosegregation, functional data) and patient-relevant data (family history, ethnicity and/or geographic origin, sex, age of onset, C1-INH levels and function, C1q and C4 concentration, etc).

A specific goal of ClinGen is to develop expert panels in different clinical domains to evaluate the clinical validity of gene-disease relationships and pathogenicity of individual genetic variants submitted to ClinVar. The constitution of such an approved by ClinGen HAE Variant Curation Expert Panel would be extremely useful not only in novel *SERPING1* variant interpretation but even more in the curation of newly discovered HAE-related genes. To this aim, this expert panel would design and validate gene-optimized rules for variant classification, and by their use could gain the responsibility to submit to ClinVar HAE-associated variants that have been reported in existing centralized (HAEdb) and internal databases along with the relevant supporting evidence of their pathogenicity.

Adaptation of the ACMG-AMP variant classification framework

Statement 9. *Laboratories performing and reporting results of HAE genetic testing are encouraged to incorporate the ACMG-AMP guidelines as they have been adapted here.*

In 2015, the ACMG and the AMP released a widely adopted landmark guidance document for variant interpretation.⁶³ These guidelines describe the process for classifying variants into 5 categories—pathogenic, likely pathogenic, VUS, likely benign, and benign—on the basis of criteria using multiple types of evidence (eg, population data, computational and predictive data, functional data, and phenotype/family history data). However, the ACMG-AMP guidelines do not take into consideration gene-

TABLE II. ACMG-AMP criteria for variant classification supporting pathogenicity (in **bold**): very strong (PVS1); strong (PS1-4); moderate (PM1-6); supporting (PP1-5) or supporting tolerance (plain text): stand-alone (BA1); strong (BS1-4); supporting (BP1-6)*

Databases/literature	Population data	Allele frequency >5%	BA1
		Allele frequency greater than expected for disorder	BS1
		Absent from controls	PM2
	Disease association	Observed in a healthy adult individual	BS2
		Reputable source recently variant as benign	BP6
		Reputable source recently variant as pathogenic	PP5
		Prevalence in affected increased than in controls	PS4
		Patient's phenotype/family history highly specific for gene†	PP4
		Found in case with an alternate disease cause	BP5
		Weighted as PP1-PS4 with increased segregation data	PP1-PS4
Segregation	Yes		
	No		BS4
	<i>De novo</i>	Paternity & maternity confirmed	PS2
		Paternity & maternity not confirmed	PM6
Annotation	Null variant	Nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, large defects	PVS1
	Synonymous/intronic	Not conserved AND no impact in splicing	BP7
		Multiple computational tools show splicing alteration	PP3
	In-frame INDELS	In a repetitive region without a known function	BP3
		In a nonrepeat region or stop-loss variant	PM4
	Missense	Same amino acid change as another pathogenic variant	PS1
		Novel at the same position as another pathogenic variant	PM5
		Located in mutational hot spot/functional domain	PM1
		In a gene with low rate of benign variation†	PP2
		Functional studies show damaging effect Yes	PS3
		Functional studies show damaging effect No	BS3
	Allelic data	Multiple computational tools show damaging effect Yes	PP3
		Multiple computational tools show damaging effect No	BP4
		Dominant Cis/Trans with pathogenic variant	BP2

*Criteria BP1 (variant in a gene where only truncating variants cause disease) and PM3 (variant in trans with another pathogenic variant in recessive mode of inheritance) are not applicable in the case of *SERPING1*.

†Criteria PP2 and PP4 apply by default in case of genotyping patients with C1-INH-HAE.

specific factors, such as disease-specific incidence and prevalence rates.

To use the ACMG-AMP framework in HAE, we developed a disease-specific customization (Table II) with the aim of improving consistency especially for *SERPING1* variant interpretation and expert curation of reported *SERPING1* variants. Thus, each variant derived from the HAE genotyping algorithm has to be assessed with respect to its presence in public, internal, and disease-specific databases, population data, computational predictions, *in vivo* and *in vitro* test results, segregation evidence, and allelic and variant-specific information (Figure 1). The above-mentioned available evidence must be weighted and ACMG criteria supporting pathogenicity (very strong PVS1, strong PS1-4, moderate PM1-6, or supporting PP1-5) or tolerance (stand-alone BA1, strong BS1-4, or supporting BP1-6) must be applied, resulting in variant classification in 1 of the 5 categories (benign, likely benign, VUS, likely pathogenic, pathogenic).

Thereafter, the variant classification and supporting evidence have to be submitted to ClinVar, reviewed by an HAE expert panel committee for cross-evaluation and approval, and reported to the referral clinician. When new evidence (public or patient specific) alters the initial variant assessment, classification must be repeated, and updated results should be shared with all involved parties (clinician, patient, ClinVar records, HAE expert

panel). The laboratory conducting the genetic testing may be required to exclude the copresence of other pathogenic defects in case of uncertainty, for example, when a variant initially reported as causative is evaluated by another reputable source as benign, likely benign, or VUS.

Thus, all laboratories performing and reporting the results of HAE genetic testing are encouraged to incorporate the ACMG-AMP workflow as they have been adapted here, train their personnel in the use of genetic resources and tools for the evaluation of evidence, and assign trained experts for the clinical and technical validation of sequence variation. Openly available online tools can be used, enabling an achievable record of the specific evidence type (eg, Genetic Variant Interpretation Tool⁷¹, and Varsome⁷²) and strength (eg, IntrVar⁷³) used for determining pathogenicity.

How to treat secondary findings

Secondary (incidental) findings unrelated to the indications for ordering an analysis may result in HAE genotyping as an outcome of sequencing specific regions of relevant genes or as a consequence of high-throughput next-generation sequencing (exomes or panels of genes). For example, in the process of genotyping nl-C1-INH-HAE, the detection of a novel variant in *F12*, *PLG*, or *ANGPT1* genes or a rare variant in another gene related to bradykinin metabolism and predicted *in silico* as

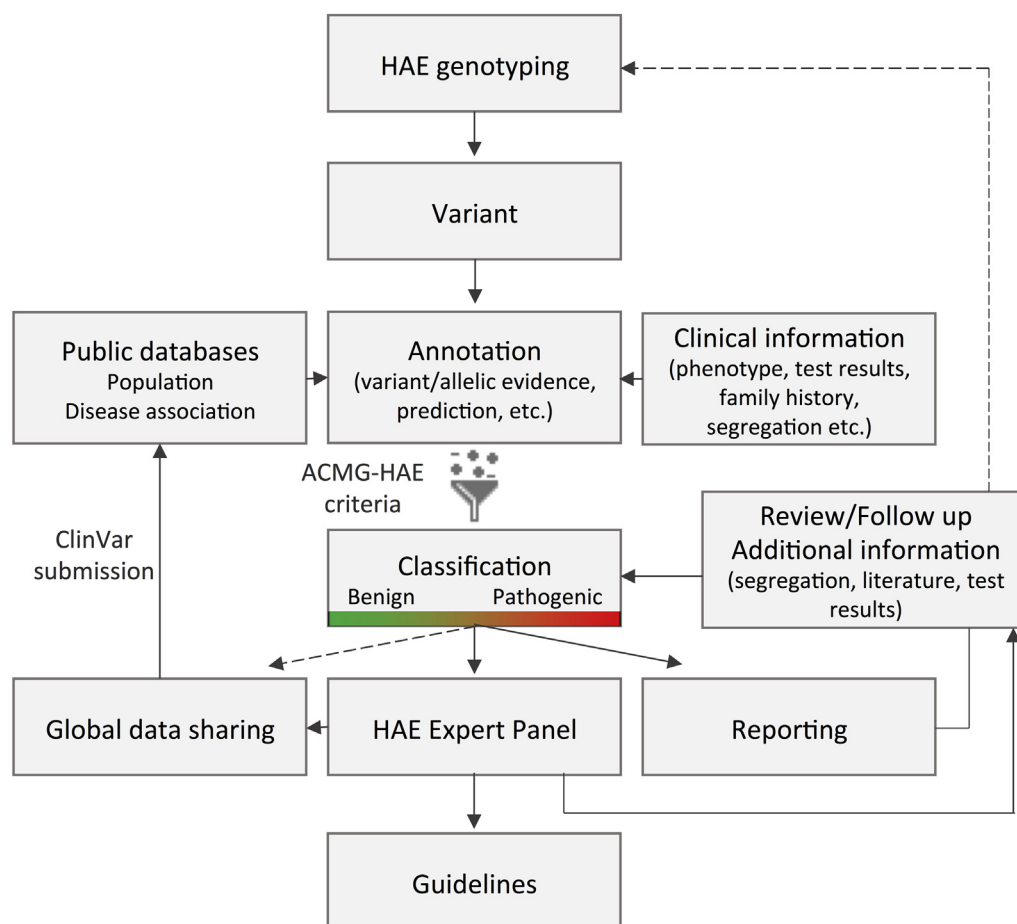


FIGURE 1. Variant assessment workflow.

deleterious may raise suspicions regarding its clinical significance. These variants should not be reported to clinicians unless appropriately substantiated. Incidentally detected HAE-related genes/variants should be evaluated by the above-mentioned ClinGen HAE Curation Expert Panel according to ACMG guidelines.

Secondary findings unrelated to HAE phenotype but with medical utility to the ordering physician or patient can be reported according to ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing.^{7,4}

Genotyping Patients with HAE in the clinic

Statement 10. *There is an urgent need to establish and implement clinical practice guidelines, standardized procedures for gene testing and external quality assessment programs to provide a framework for best laboratory practice and reporting on the genetic diagnosis of HAEs.*

Genetic testing for HAE is a practical and feasible way to validate clinical diagnosis and is defined as a medical test, most often carried out on a blood sample, that looks for changes in a person's genetic material. Unlike phenotypic testing, the genotypes are unequivocal, with no borderline values. Accordingly, the reliability of the result is totally accepted by referring doctors. Because of the potentially important clinical and familial implications of results of genetic analyses, laboratories must undertake

stringent internal quality measures in identifying critical steps in the preanalytical phase, the analytical phase, and the post-analytical phase of genetic testing.

Preanalytical phase. Clinicians should provide necessary information about the test requested, the specimen type, demographic data required for probable reporting to ClinVar (see above) as well as an appropriate patient's informed consent. Moreover, indication for testing must be provided, including whether a family history of HAE is known and, if available, the family-specific mutation. If possible, the type of HAE, at least whether it is about C1-INH-HAE or nl-C1-INH-HAE, should be clarified.

Analytical phase. Because there is a lack of validated testing methods and standardized operating procedures for the detection of mutations in HAE genes, each laboratory should have standardized operating procedures and testing requirements for HAE genes mutation analysis and result reporting.

Studies systematically comparing the sensitivity, specificity, and reproducibility of the different techniques for genotyping patients with HAE are lacking and the concordance between different diagnostic methods is also largely unknown. Therefore, there is an urgent need to establish and implement clinical practice guidelines and standardized procedures for gene testing in patients with HAE. Similarly, guidelines using the standard

definition should be proposed to provide a framework for best laboratory practice and reporting on the genetic diagnosis of HAEs as agreed by the above-mentioned ClinGen HAE Curation Expert Panel. These guidelines should be intended to be used primarily by molecular geneticists and by other health professionals involved in the care of these patients.

External quality assessment programs should be designed aiming to ensure optimal accuracy and proficiency in gene testing in HAE across all laboratories. External quality assessment can alert laboratories to problems and shortcomings and help to improve, where necessary, their laboratory procedures. It should therefore become an integral part of quality assessment in the laboratory, thus contributing to maintaining confidence in the reliability of genetic testing among patients and health professionals.

Postanalytical phase. Information resulting from a genetic test may inform clinical decision making and can influence attitudes and actions of patients and their relatives. The reporting of molecular genetic tests for heritable conditions by the laboratory issuing the report to the health care provider is complex because interpretation of the test result frequently relies on patient- and family-specific information. Thus, clinicians expect test results that can provide guidance for integrating the findings into patient care.

The laboratory should include key elements into the report of the genetic test, such as patient identifiers, description of the test method including coverage, detection rate, and relevant family-specific information, such as suggestions for further testing, recommendations to identify and counsel other family members at risk for having a disease-associated mutation, or referral to a genetic counselor or other medical doctor with definite expertise. Patients, their relatives as well as clinicians may not have sufficient expertise to interpret and assess risk conferred by individual genetic variants. Thus, it is mandatory to develop and make available education and information resources useful to clinicians, laboratory professionals, patients, and family members.

Genetic counseling

Statement 11. *It is recommended to implement genetic counseling in collaboration with experts specialized in human genetics and in the management of HAE. This includes the pedigree-analysis and counseling with regard to family planning. The latter comprises explanation of the a priori risk for a planned child to be affected with HAE, the identification of medicinal products appropriate for the treatment of HAE before conception, the possibilities of fertilization and preimplantation-prenatal diagnostics, the risk of interventions and their prevention, along with neonatal screening. Genetic counseling needs to be placed within the legal framework of each country.*

The recent advances in identifying genetic causes for HAE have triggered an increased demand for genetic testing. We strongly recommend that genetic testing should occur in combination with genetic counseling to inform patients about the possibilities and limitations of the genetic testing and the consequences the genetic information will have for the patient and other family members. It is the aim of genetic counseling to help the patients make informed decisions for or against genetic testing.

During genetic counseling, careful pedigree-analysis should be performed by screening both the symptomatic and the symptom-free family members. The patient should be informed about the type of inheritance, the concept of penetrance of individual

mutations, as well as genetic and clinical heterogeneity. The patient should be counseled in questions of family planning. Preconception planning is occasionally recommended because some of the medicinal products appropriate as long-term treatments for HAE (eg, attenuated androgens) may cause masculinization of the female fetus and hence, their discontinuation is advised before contemplated conception. If conception is achieved by technical means (ie, by salpingography, assisted reproductive therapies, artificial insemination, and *in vitro* fertilization), the risks of high-dose estrogen therapy should be discussed. The possibility that the intervention itself may trigger an HAE episode should also be considered along with the means for the prevention of this complication.⁷⁵

Preimplantation (selection of mutation-free oocytes and spermatozoa, *in vitro* fertilization) and prenatal diagnosis (amniocentesis or chorionic villus sampling) for therapeutic abortion may be considered—in accordance with the regulations applicable in the given country—if a mutation has been detected in a family. However, a prediction of the disease severity is often difficult due to the presence of individual differences in disease severity for the same mutation.⁵¹ This should be taken into account by the patients when considering far-reaching decisions such as termination of pregnancy.

If the DNA mutation underlying the C1-INH deficiency in a family with HAE is known, there is the possibility to perform a DNA test in asymptomatic newborns or infants. If not, C1-INH/complement testing may be performed from cord blood or peripheral blood.⁵² If C1-INH-HAE is excluded in an affected family, it is recommended to consider mutational analysis of other known HAE-causing genes, *F12*, *PLG*, and *ANGPT1*. Known (ie, previously described) mutations identified in these genes are readily usable in the respective family, for example, for presymptomatic testing. More challenging will be the identification of so far unknown variants in these genes. They need to be evaluated for their potential pathogenicity by bioinformatic tools, and it is possible that the clinical significance and thus their value for genetic testing in the respective family may remain unclear.

CONCLUSIONS

The incorporation of new genetic findings from HAE research into clinical practice will be an ongoing challenge, as we expect the discovery of additional genes relevant to HAE. After the round table discussion on genetics of HAE of the 11th C1-INH Deficiency and Angioedema Workshop, HAE cosegregating with a novel kininogen 1 gene mutation changing the N-terminal cleavage site of bradykinin was published.⁷⁶

This consensus document aims to define a structure to aid clinicians and geneticists improve and harmonize molecular diagnostics for not only the current known genes for HAE but those yet to be discovered to optimize patient care.

Amendment

According to a mutation update published after the acceptance of the manuscript for publication, the number of reported nonpolymorphic *SERPING1* variants is now 748 among which the percentage of *de novo* mutations is as low as 5.6%.⁷⁷

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