The Genetics of Hereditary Angioedema: the Iceberg Slowly Emerges

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ABSTRACT

Three separate forms of hereditary angioedema (HAE) attributable to bradykinin accumulation have been identified. The prototype HAE is caused by a genetic deficiency C1 inhibitor (C1-INH-HAE) and is characterized by remarkable allelic heterogeneity in that, related with the disease, over 450 different mutations of various types in the SERPING1 gene, encoding for the C1-inhibitor, have been detected up to now. Beyond this, 2 new clinically indistinguishable forms of HAE with normal C1-INH activity have been recently described, one attributed to alterations in the F12 gene encoding for coagulation factor XII (FXII-HAE), and another of unknown origin (U-HAE). The linkage between most SERPING1 mutations and C1-INH-HAE has been deduced from their consequences on the structure/function properties of C1-inhibitor, a fact raising doubts about the causality of all mutations (especially the missense mutations) detectable in the coding region of the SERPING1 gene. Moreover, in about 5% of C1-INH-HAE cases, no SERPING1 alterations can be detected even after meticulous analysis of the coding region of the gene. On the other hand, accumulating evidence indicates a possible relationship between C1-INH-HAE phenotype and certain SERPING mutation types and/or functional alterations on the genes involved in the function or degradation of bradykinin, like the F12 gene. Finally, it is presumed that the mutagenic liability and/or the epigenetic regulation of SERPING1 might be responsible for alterations induced by environmental factors that justify the variable course of the disease in the different life periods of the same patient. Thus, nowadays, the genetics of the disorder presents with a challenging complexity, the clarification of which, is expected not only to shed light on the pathogenetic mechanisms of HAEs but also to lead to a more effective individualized treatment and prevention of attacks.

INTRODUCTION

Hereditary angioedema (HAE) is a relatively rare, potentially life-threatening genetic disorder manifested by recurrent incapacitating attacks of edema, spontaneously developing in any body location.\(^1,2\) The inheritance of the disorder as an autosomal dominant trait with incomplete penetrance was described for a first time in 1917.\(^3\) In 1963, Donaldson and Evans studied three HAE families and discovered that the disease was caused by a genetic deficiency of the C1-inhibitor (C1-INH).\(^4\) Following the discovery and sequencing of the SERPING1 gene encoding for C1-INH,\(^5,6,7,8\) it was confirmed that the disease occurs in individuals heterozygous for mutations of this gene,\(^9,10\) except in few cases carrying homozygous defects, mainly due to consanguineous parents.\(^11,12,13\)

Subsequently, elegant studies with genetically modified mice demonstrated that edema formation in C1-INH-dependent HAE forms is the result of pathologic activation of the kinin-kallikrein system,\(^14\) and established that bradykinin, through binding with its tissue receptors, is the principal mediator of HAE symptoms.\(^15,16\) Thus and despite the fact that the genetics of SERPING1 was appearing increasingly complex, up to the turn of the century HAE was attributed absolutely to the genetic C1-INH deficiency (C1-INH-HAE).

Two clinically indistinguishable types of C1-INH-HAE had been described, due to different type and location of SERPING1 defects. Type 1 C1-INH-HAE accounts for 80-85% of all cases and is defined by reduced plasma C1-INH concentrations (around 5% to 30% of expected values), leading to low C1-INH function. Type 1 C1-INH-HAE mutations occur throughout the gene, resulting in the production of truncated or misfolded proteins that are not secreted efficiently. In type 2 C1-INH-HAE (15-20% of cases), the mutations are typically single amino acid substitutions, located mainly at exon 8 that contains the critical hinge region and reactive center of C1-INH. These mutations lead to the production of a dysfunctional C1-INH protein that results in low C1-INH function despite normal levels of antigenic C1-INH. Typically, in both C1-INH-HAE types, C4 plasma levels are also decreased.\(^2\)

In 2000, however, a new form of HAE with normal C1-INH activity was described that was clinically indistinguishable from the up to then known disease types.\(^7,18\) This type of HAE affects predominantly women and depends on or is worsened by increased levels of endogenous (e.g., pregnancy) or exogenous estrogen (e.g., oral contraceptives or hormone replacement therapy). Thereafter, carriage of mutations in the F12 gene encoding for coagulation factor XII (FXII, Hageman factor) was revealed in some of these patients and was considered responsible for this HAE type (FXII-HAE).\(^19\)

Efforts, however, towards uncovering the underlying genetic defect(s) in cases of HAE were not always
successful. In about 5% of C1-INH-HAE cases, no SERPING1 alterations can be detected even after meticulous analysis of the coding region of the gene.\textsuperscript{20, 21, 22} On the other hand, about 70% of patients with clinical history suggestive of HAE but with normal C1-INH function have no mutation in the causative genes associated with HAE (SERPING1, F12) (unknown HAE, U-HAE).\textsuperscript{23, 24} In a number of U-HAE cases, the positive family history is accompanied by deficiency of one or several kininases (carboxypeptidase N [CPN], aminopeptidase P [APP], angiotensin I-converting enzyme [ACE]) occasionally associated with defects in encoding genes.\textsuperscript{25} Moreover, evidence accumulated during recent years indicates that defects in genes encoding proteins involved in kinin generation or catabolism other than C1-INH and FXII may be involved in the pathogenesis of HAE and modify its clinical expression.\textsuperscript{26, 27} Thus, near a century after the recognition of the hereditary nature of HAE, the genetics of the disorder presents with a challenging complexity that has been attempted to be presented in this review.

**DEFECTS IN THE SERPING1 GENE**

C1-INH-HAE is characterized by a remarkable allelic heterogeneity in that over 450 different SERPING1 mutations related with the disease have been detected up to now, according to the Human Gene Mutation Database (Online Mendelian Inheritance in Man – OMIM ID 106100)\textsuperscript{28} and a database specific to this disease (HAEdb. hae.enzim.hu).\textsuperscript{29} These mutations are scattered over all the 8 exons and exon/intron boundaries of the SERPING1 gene [serpin peptidase inhibitor, clade G (C1 inhibitor), member 1] (OMIM # 606860; GenBank NM_000062.2) that extends over a 17159 bp genomic region located on chromosome 11q12-q13.1 and presents an unusual promoter with no TATA sequence but a TdT-like initiator and a polypurine-polypyrimidine tract.\textsuperscript{7} A large variety of SERPING1 mutation types have been observed in C1-INH-HAE patients (Table 1), including gene alterations that represent extremely rare mutagenesis events. Such an alteration is the recently described substitution of two consecutive nucleotides TC to AA resulting in a termination codon (F225X).\textsuperscript{30}

As it is mentioned in detail above, the relative position of SERPING1 mutations within the gene dictates the type of C1-INH-HAE. However, \textasciitilde{}20-25% of all unrelated C1-INH-HAE cases have no apparent family history of C1-INH-HAE (i.e. sporadic) and present de novo SERPING1 mutations with the same mutational spectrum as the familial cases.\textsuperscript{31}

It is worthy to be noticed that SERPING1 exhibits also a kind of phenotypic heterogeneity. Beyond mutations associated with C1-INH-HAE, the gene harbors a number of single nucleotide polymorphisms (SNPs), which have been associated with other diseases. One of the few common missense variants in the coding region, the polymorphism rs4926 [c.1438G>A, p.V480M], is associated with nasal carriage of *Staphylococcus aureus*,\textsuperscript{32} while six common intronic SNPs (especially the rs2511989) are strongly associated with age-related macular degeneration in Caucasians.\textsuperscript{33} Amongst them, the rs2511988 [c.1030-20A>G], located into intron 6, upstream of the 3’ splice site of exon 7, has been proposed more likely to have functional consequences.\textsuperscript{34} However, such an association was not confirmed in Chinese,\textsuperscript{35} and Japanese populations.\textsuperscript{36} Similarly, a SERPING1 mutation (replacement of Ala443 with Val) affecting complement regulation but preserving kallikrein inhibitory activity has been described in one family. None of the members of this family had angioedema but they had presented with systemic lupus erythematosus possibly because of the acquired deficiency in C2 and C4.\textsuperscript{37}

Its pronounced allelic heterogeneity makes the SERPING1 gene a prime example of mutagenic liability. Investigation of SERPING1 mutations has revealed several key features about the DNA itself as well as protein structure-function relationships that explain this

<table>
<thead>
<tr>
<th>Type of defect</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>Missense mutations</td>
<td>34.2%</td>
</tr>
<tr>
<td>Nonsense mutations</td>
<td>7.1%</td>
</tr>
<tr>
<td>Frameshift alterations (including small deletions or insertions)</td>
<td>31.2%</td>
</tr>
<tr>
<td>Splice-site defects</td>
<td>9.4%</td>
</tr>
<tr>
<td>Regulatory mutations</td>
<td>1.1%</td>
</tr>
<tr>
<td>Large gene rearrangements (exon(s) deletions or insertions)</td>
<td>17.0%</td>
</tr>
</tbody>
</table>

Table 1. The types of SERPING1 defects in patients with C1-INH-HAE registered in HAEdb.\textsuperscript{29}
phenomenon.\textsuperscript{38, 39} SERPING1 location very close to the centromere is expected to be a reason for its high mutation rate, since centromeric regions are considered the most rapidly evolving compartments in the eukaryotic genome. Another feature of the SERPING1 gene to which its high mutation spectrum has been attributed, is the high incidence of DNA repetitive elements. The 7 introns of the gene contain 17 Alu repeat sequences\textsuperscript{40} which represent ‘hotspots’ for non-homologous recombination events that may cause partial deletions or duplications of the gene.\textsuperscript{40} Large deletions/duplications caused by Alu repeats occur throughout the gene. These damages account for 15–20\% of all cases of C1-INH-HAE and no protein product is expected due to the major gene disruption.

An additional structural feature of the SERPING1 gene potentially responsible for the higher than average mutation rates is the high frequency of CpG sites. Like Alu elements, CpG sites represent mutational ‘hotspots’ as they are prone to spontaneous deamination. The CpG dinucleotide in the first two positions of codon 466 encoding the central arginine (CGC) of the reactive center of C1-INH seems to be a frequent target for recurrent amino acid substitutions.\textsuperscript{41} Moreover, the region encoding the reactive center, like in some other serpins, contains both primary and secondary structure DNA polymerase pause sites, which enhance its rates of mutation and evolution. Despite the fact that the resulted mutations do not affect the production of C1-INH, they lead to the generation of a dysfunctional protein and thus, to C1-INH-HAE type 2. Only one mutation located away from the reactive center, has been observed to result in C1-INH-HAE type 2. It is a deletion of Lys251 that creates a potential new glycosilation site.\textsuperscript{42} The carbohydrate group Asn250 could interfere with the reactive center region or simply lead to an essentially irreversible alteration in this region.

As a result of the large number of private mutations and the unavailability of relatives, appropriate functional or segregation studies in large kindreds to prove the linkage between specific mutations and the disease have been carried out only on a fraction of SERPING1 alterations.\textsuperscript{43} Thus, the pathogenetic role of SERPING1 alterations has been usually deduced from evidence that they disrupt the protein product. Such evidence is obvious with large rearrangements, small deletions/duplications, nonsense mutations, splicing defects or deletions of few amino acids but not with missense mutations, representing approximately 30–40\% of the cases. So, the mechanism by which missense mutations lead to protein defect has been usually be demonstrated after the identification of profound structural alterations affecting functionally relevant parts of the protein as a result of a particular amino acid change.\textsuperscript{21, 44} This is not, however, the truth with all cases of missense mutations reported as causal. For example, when missense mutations deposited in HAEdb were submitted in bioinformatics analysis, at least one of them was found benign making its causative effect disputable. The reanalysis of SERPING1 in this case uncovered an additional genetic damage that was deleterious for the protein function (nonsense mutation), obviously representing the causative alteration.\textsuperscript{20} This fact raises doubts about the causality of all indiscriminately the mutations detectable in the coding region of the SERPING1 gene.

Moreover, the finding that in about 5\% of C1-INH-HAE no mutation can be detected in the coding region of SERPING1\textsuperscript{20, 21, 22} indicates that, in some patients, a causative defect modifying C1-INH expression may be located in an intronic or an untranslated region of the gene. Actually, such functional intronic alterations have been recently reported.\textsuperscript{45, 46, 47} In any case, the comprehensive analysis of SERPING1 must be affirmed before attributing the disorder to the possibly deleterious effect of a detected missense mutation or before reporting the result of a genetic analysis as negative.

In every day clinical practice, the molecular analysis of SERPING1 is beginning with the amplification of all (8) exons and the exon-intron boundaries by PCR and the detection of mutations by direct sequencing. If no mutation could be found by sequencing, or in case a detected missense mutation would be proven benign for protein function by bioinformatic tools, further analysis for the identification of large gene rearrangements (exon deletions or duplications) is performed. To this purpose, two different techniques are widely used, namely the long -range PCR and the multiplex ligation-dependent probe amplification (MLPA). In long-range PCR, segments of the SERPING1 gene encompassing at least 7–15 KB are amplified using common oligonucleotide primers, specific Taq polymerase mixes and long-time PCR conditions.\textsuperscript{20, 21, 51} MLPA is a high throughput, sensitive technique for detecting copy number variations in genomic sequences,\textsuperscript{48} usually performed by commercially available kits (SALSA MLPA probemix P243-A2 SERPING1 kit, MRC-Holland, Amsterdam, The Netherlands). Targeted analysis by simple and widely accessible methods, like polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP), is not applicable in SERPING1 molecular studies. Such molecular approaches could be useful in the molecular studies of families with known SERPING1 mutations, when the latter modify the DNA sequence in order to be recognized by restriction enzymes.

**GENOTYPE-PHENOTYPE CORRELATIONS IN C1-INH-HAE**

The clinical expression of C1-INH-HAE is characterized by a great variability. Features of the disease, like the age at disease onset, the frequency and the triggers of
attacks, the severity and localization of edema, the prodromal signs and symptoms and its natural course vary broadly even amongst members of the same family sharing the same mutation.\textsuperscript{2,49,50} Some patients suffer frequent, life-threatening attacks, others experience a mild disease, whereas as much as 14% of carriers of \textit{SERPING1} mutation may remain symptom-free throughout their lives.\textsuperscript{51,52} As a result, about 5% of the adult C1-INH-HAE patients are identified after their child is diagnosed, and though carrying and transmitting the mutation to their children, they may remain asymptomatic throughout their lives. However, the factors influencing this heterogeneous clinical manifestation of C1-INH-HAE represent one of the oldest unsolved problems of the disease.

Early after the initial detection of \textit{SERPING1} mutations and their consequences on the complex structure of C1-INH, it was assumed that certain genetic alterations might result in specific disease phenotypes. The very first evidence for such a genotype/phenotype correlation was derived from studies indicating that the presence of one mutated allele affects mRNA transcription and protein secretion, which leads to downregulation of the normal allele and, finally, to C1-INH plasma levels ranging from 10% to 30% of normal.\textsuperscript{53,54,55} A correlation, however, between C1-INH levels and the clinical phenotype of the disease is difficult to be confirmed because, irrespectively of the type of \textit{SERPING1} mutation, almost all heterozygotes present with plasma C1-INH concentration lower than the expected 50% by mechanisms that remain as yet unknown. Reports of homozygous C1-INH-HAE patients, which suffered a severe disease form, while their heterozygous family members were symptom-free,\textsuperscript{11,12,56} offered further evidence supporting the possible relationship between certain mutation types and disease phenotype.

During the following decades, the increasing number of mutations combined with the low incidence of the disease did not allow a strong correlation between certain \textit{SERPING1} mutations and the clinical phenotype of C1-INH-HAE to be confirmed. Thus, it was generally accepted that there is no or only little correlation between the type of \textit{SERPING1} mutations and disease phenotype.\textsuperscript{57,58} Subsequent studies attempted to correlate the type of \textit{SERPING1} mutations with the severity of the disease but they reached to conflicting results.\textsuperscript{26,59,60} Interestingly, however, in the largest study ever in this field, Speletas et al.\textsuperscript{26} examined 265 C1-INH-HAE patients from 4 European countries (including the study cohort of the previous studies\textsuperscript{60}) and showed that patients carrying \textit{SERPING1} missense mutations have a significantly lower probability of manifesting HAE attacks before the 10th year of age than those with all other \textit{SERPING1} defects (regulatory, nonsense, splice defects, frameshift, large insertions/deletions). Bearing in mind that early onset of C1-INH-HAE symptoms is predictive of high severity of the disease course,\textsuperscript{61,62} carriage of missense mutations may represent an index of a less severe HAE-C1-INH clinical course.

The intrafamilial heterogeneity of C1-INH-HAE prompted further studies aiming to investigate the effect possibly exerted on disease phenotype by common \textit{SERPING1} polymorphisms and/or by alterations in genes encoding other proteins involved in its pathogenesis. In this context, a strong correlation was found by Bors et al.\textsuperscript{26} between the severity of the disease and the $F12$-$46C/T$ polymorphism (rs1801020). This result was recently confirmed by Speletas et al.\textsuperscript{26} after the analysis of 258 C1-INH-HAE patients from 113 unrelated European families (including the study cohort of Bors et al.).\textsuperscript{26} In this study, the carriage of $F12$-$46C/T$ polymorphism was found strongly associated with a 7-year delay in disease onset, and significantly but negatively associated with the need for long-term treatment, both independently of the type of \textit{SERPING1} mutations. The $F12$-$46C/T$ polymorphism (c.-4C>T, rs1801020) is located in the promoter region of the $F12$ gene, 4 nucleotides before the initiation codon (ATG, Methionine), creating a new initiation codon (ATG) for transcription of the mRNA and a frameshift that produces a truncated protein. The T allele destroys the Kozak’s consensus sequence (GCCAGGCCATGG) for translation initiation signaling and prevents proper recognition of the translation initiation site.\textsuperscript{63,64} Thus, the above finding could be attributed to the effect that the polymorphism has on the synthesis of FXII and, secondarily, on the production of bradykinin.

The interesting finding of Blasko et al.\textsuperscript{65} that the biannual attack rate is lower in patients carrying 3 or 4 copies of C4B gene encoding the B isotype of the C4 protein, has not been confirmed as yet. Conflicting are the reported results regarding the association of the C allele of a common \textit{SERPING1} polymorphism (c.-21T>C, rs28362944) with the C1-INH-HAE severity.\textsuperscript{66,67} However, Duponchel et al.\textsuperscript{68} showed that transfections with the mimigene construct carrying the C variant at position c.-21 consistently yielded a weak product lacking exon 2 in the transfected cell lines, suggesting that this allele might contribute to the lower expression of the normal protein and, hence, to the occurrence of more severe disease.

Finally, no correlation was found between the severity of C1-INH-HAE and the p.V480M polymorphism (c.1438G>A, rs4926) of the \textit{SERPING1} gene,\textsuperscript{65,70} the functional polymorphisms in the genes of B1 and B2 bradykinin receptors (DBKR1, DBKR2), in the ACE gene or in the gene of mannose-binding lectin (\textit{MBL2}).\textsuperscript{71,72,73}

Another interesting feature of C1-INH-HAE is that the proportion of the various types of disease-related gene alterations is different between different geographical
regions. For example, missense mutations and small deletions/insertions represent 30% and 28%, respectively, of all SERPING1 alterations observed in Hungarian patients, while the corresponding percentages among Romanian patients are 50% and 14%.\(^{20}\) Moreover, it is well known that the disease may follow a variable course in the different life periods of the same patient. These findings indicate the possible implication of environmental factors either in the mutagenesis or in the epigenetic regulation of the SERPING1 gene.

**OTHER GENETIC CAUSES OF HEREDITARY ANGIOEDEMA**

Amongst HAE patients suffering from estrogen-dependent (or estrogen-associated) inherited angioedema with normal plasma levels of fully functional C1-INH and without causal mutations at the SERPING1 locus, a subset of about 300 reported cases have been attributed to alterations in the F12 gene (OMIM #610619).\(^{74}\) The F12 gene has been mapped to 5q33-qter and is comprised 13 introns and 14 exons covering 12 kb.\(^{75}\) The F12 promoter contains at position -44/-31 a palindrome similar, but not identical, to an estrogen-responsive element (ERE) together with 4 hemisite EREs between positions -1314 and -608.\(^{76}\) The presence of these elements underlies the mechanism by which estrogens enhance FXII concentrations in plasma, and possibly is associated with not only the FXII-HAE but, to a different degree, with all HAE types.

Several loss-of-function mutations in the F12 gene associated with FXII deficiency have been described.\(^{77}\) Beyond these, four F12 alterations have been identified so far which, according to their co-segregation patterns, were assumed to be causal for HAE with normal C1-INH function. Firstly, two distinct missense mutations located on the exon 9 have been described, resulting in threonine-to-lysine (Thr328Lys) and threonine-to-arginine (Thr328Arg) substitutions. Moreover, a large deletion of 72 bp (c.971_1018 + 24del72) located at the exon 9/intron 9 border was identified in two unrelated families of Turkish origin that is involved in the proline-rich region of the FXII in which the above two substitutions are located.\(^{78,79}\) More recently, a duplication of 18 bp (c.892_909dup) causing the repeated presence of 6 amino acids (p.298-303) in the same region of factor XII was also described in a Hungarian family.\(^{80}\)

The Thr328Lys substitution has been identified in clinically affected and symptom-free individuals from numerous families,\(^{81}\) whereas the Thr328Arg replacement has been found only in two German families.\(^{81}\) After the discovery of these mutations, it was suggested that they confer a putative gain of FXII function leading to upregulation of contact system activation and increased bradykinin formation. Actually, patients with FXII-HAE who are carriers of the Thr328Lys mutation present with an increased amidolytic FXII activity, but with normal plasma levels of the protein.\(^{82}\) On the contrary, however, Bork et al.\(^{83}\) have shown that there is no difference between FXII-HAE patients with the Thr328Lys mutation and their healthy probands neither in FXII surface activation by silicon dioxide nor in kallikrein-like activity with and without activation by dextran sulfate, indicating that the Thr309Lys mutation does not cause a gain-of-function of FXII. Thus, the role of the F12 mutations in the generation of FXII-HAE and the underlying pathophysiology of this HAE type remains still poorly understood, although its response to an antagonist of bradykinin receptor type 2 (icatibant)\(^{34}\) supports the assertion that contact pathway dysregulation is involved with bradykinin being the primary mediator.

To this point, it has to be mentioned that in a few patients with FXII-HAE, genetic polymorphisms in bradykinin degradation enzymes APP and ACE have been described.\(^{85,86}\) Insertion/deletion polymorphisms of the ACE gene are responsible for 50% of the variability in ACE serum levels.\(^{87}\) with the insertion of the allele (I) associated with reduced ACE transcription and decreased bradykinin degradation.\(^{88}\) Similarly, the A allele at the SNP rs3788853 locus, located 5’ of XPNPEP2, which codes for membrane-bound APP, has been commonly detected in FXII-HAE patients. This allele is associated with decreased APP activity, decreased bradykinin degradation, and angioedema induced by ACE inhibitors.\(^{89,90}\) Supporting evidence towards the contribution of these polymorphisms to the clinical phenotype of FXII-HAE has been recently provided by the results of Charignon et al.\(^{91}\) indicating that ACE and CPN exhibit a significant inverse relationship with FXII-HAE severity.

Finally, a form of HAE has been described once in the literature associated with CPN deficiency.\(^{92}\) The proband of the affected family displayed an 11-year history of angioedema occurring about once weekly with attacks lasting 24 hours. The CPN activity was as low as 20% of normal levels. Several family members were clinically affected with some combination of angioedema or chronic urticaria, as well as hay fever or asthma, and had slightly depressed serum CPN, suggesting autosomal recessive inheritance. More than two decades after the publication of this case, Cao and Hegele\(^{93}\) sequenced CPN1 gene which encodes the catalytic subunit of CPN, in the archival genomic DNA of the proband and identified a frameshift mutation in exon 1 due to a single G insertion at nucleotide 385 (385fsInsG), and a missense mutation in exon 3 that predicted substitution of aspartic acid for the wild-type conserved glycine at amino acid 178 (G178D) to which the CPN deficiency could be attributed. Interestingly, the CPN1 harbors five nonsynonymous mutations and several genomic alterations (especially in 3’ UTR-region), associations and functional consequences of which are still unknown.
DIAGNOSTIC ISSUES

Genetic testing is not included in the first-line diagnostic approach of patients with angioedema, since the vast majority of angioedema cases can be diagnosed on the basis of family history, clinical picture and complement tests (antigenic and functional levels of C1-INH, C1q and C4). However, these may not always indicate the form of angioedema, so genetic testing can be proven a valuable tool for improving diagnostic accuracy and, finally, the adequacy of patient care.

In general, genetic testing is useful in case with high clinical suspicion of HAE where the complement tests are inconclusive. Such typical examples are doubtful cases necessitating differentiation between the hereditary and the acquired form of angioedema. More interesting is the confirmation of diagnosis upon the first presentation of the disease in children with a positive family history of C1-INH-HAE. Patients with early symptom onset have more severe disease than those with later onset, and a differential diagnosis demanding gastrointestinal edema is a common presenting symptom in children with C1-INH-HAE. Notwithstanding that the clinical appearance of the disease is very unusual before the second year of age, the problem is that up to this age antigenic and functional C1-INH levels are not reliable indicators of C1-INH-HAE as their reference ranges are extended downward with respect to the adult reference ranges. Should, therefore, the confirmation of C1-INH-HAE diagnosis is needed before the second year of age, genetic analysis must be performed. Moreover, bearing in mind that missense SERPING1 mutations and the carriage of the F12-46C/T functional polymorphism are significantly associated with the age at C1-INH-HAE onset, genetic analysis of the offspring of an affected parent might contribute to a more reliable counseling of them.

As far as FXII-HAE is considered, detection of F12 mutations is the only test to date that confirms its diagnosis. Identification of pre-symptomatic individuals in pedigrees with an established diagnosis of FXII-HAE should be a priority in order exogenous estrogens (e.g. oral contraceptives in young women) and the possibility of fatal attacks to be avoided.

Genetic testing for a specific mutation after chorionic villous sampling or amniocentesis could be helpful in prenatal diagnosis of C1-INH-HAE or FXII-HAE. In established pregnancies, it could be considered only in cases of an affected parent carrying a known genetic defect in SERPING1 or F12 gene. As far as all genetic defects of the SERPING1 gene detected in HAE patients may be not pathogenic, prenatal diagnosis must be considered only when the parent’s mutation is undoubtedly disease-causing. Given that SERPING1 or F12 defects may result in a nonfatal manageable disease in the offspring, the severity of which might be significantly different than that of its parent as well as that advances in therapy have significantly improved the quality of life of patients, prenatal diagnosis should be decided by the parents following an appropriate counselling and the evaluation of benefits and risks. Taking into account that no SERPING1 mutation can be detected in 8-10% of cases as well as that U-HAE cases represent a proportion up to 70% of estrogen-associated HAEs, preimplantation genetic diagnosis might be more attractive than traditional prenatal diagnosis in families with C1-INH-HAE or FXII-HAE in that it may allow the selection of healthy embryos.

FUTURE DIRECTION

The emerging picture is that HAEs represent a family of diverse disorders of kinin (mainly bradykinin) metabolism, with a much greater genetic complexity than that initially, after the discovery of SERPING1 gene, was considered. Bearing this in mind, future research has to elucidate a series of interrelated aspects. First, the genetic damage underlying U-HAE as well as C1-INH-HAE cases where no SERPING1 alterations can be detected by conventional approaches remain to be identified. Contemporary genetic approaches, like genome-wide association studies or next-generation sequencing, could give reliable answers to these as well to some of the following questions. Second, it has to be clarified whether the presence of all SERPING1 alterations considered so far responsible for C1-INH-HAE, are isolated or are expressed in tandem with either functional alterations on the genes involved in the function or degradation of bradykinin, or display SERPING1 polymorphisms and mutations with as yet unknown functionality. Third, the controversial area of genotype/phenotype correlations must be elucidated, especially since supporting evidence has been recently provided. To this point, epidemiologically powerful studies on large enough patients’ cohorts as well as standardization of the method used for the evaluation of the disease severity are required. Finally, the presumable effect of environmental factors on the disease phenotype necessitates epigenetic studies to be performed. Clarifying these aspects is expected not only to shed light on the pathogenetic mechanisms of HAEs but also to lead to a more effective individualized treatment and prevention of attacks.

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