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Hereditary Angioedema Due to C1-Inhibitor Deficiency – From a Genetic Point of View

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Editorial

Hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) is an autosomal dominant disease characterized by acute edematous swelling of the skin and/or of the mucosa. Gastrointestinal involvement can cause severe abdominal pain, whereas edema of the upper airways may cause suffocation – these conditions may even be fatal without proper treatment [1,2]. C1-INH-HAE results from the functional deficiency of the *C1*-inhibitor (C1-INH) protein, which plays an important role in the regulation of the classical and of the lectin complement pathways, as well as in controlling the coagulation, the fibrinolytic and the kinin-kallikrein cascades [3-5]. C1-INH (a member of the serpin family of plasma proteins, which inhibits proteases by forming covalent bonds with them) is the sole inhibitor of the activated proteases C1r and C1s [6]. Further, it is a major regulator of activated coagulation factor XII and of plasma kallikrein, which limits the generation of the vasoactive peptide bradykinin. In C1-INH deficiency, this regulatory step is impaired, which in turn results in an episodic increase of bradykinin production and in the consequent increase in vascular permeability and in edema formation [7-9].

Two types of C1-INH-HAE are distinguished according to C1-INH activity and protein/antigen concentration. In C1-INH-HAE type I seen in 85% of all patients, the serum concentration of C1-INH is less than 35% of normal, whereas in C1-INH-HAE type II, the serum level of the C1-INH protein is normal or even elevated; however, a large percent of the secreted protein is non-functional. Clinically, the two types are indistinguishable from each other [10]. As C1-INH antigen levels are low in type I, but are normal in type II, a functional C1-INH assay must be carried out to confirm the diagnosis of C1-INH-HAE type II.

The C1-INH gene (*SERPING1*, *OMIM#606860*; *GenBank NM_000062.2*) extends over a 17-kb genomic region located on chromosome *11q12-q13.1* and consists of 8 exons [11]. Up to now, more than 400 different mutations, scattered over the entire gene, have been reported and collected in a specific, online HAE database, HAEdb (http://hae.enzim.hu/) [12] and in the HGMD (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php). According to the latter, 197 missense/nonsense mutations, 43 variations affecting the splice site, 3 regulatory alterations, 113 small deletions, 35 small insertions, 12 small indels, 48 large deletions, and 5 large insertions have been published up to now. As many as 25% of all unrelated cases with C1-INH-HAE result from de novo mutations of the *SERPING1* gene [13]; this high ratio can be attributed to different mechanisms including the high incidence of repetitive elements and *CpG* sites. Introns 3, 4, 6 and 7 are rich in Alu repeat sequences [14], which represent 'hotspots' for non-homologous recombination events that may cause partial deletions or duplications of the gene [15]. Such large rearrangements of the *SERPING1* gene account for approximately 20% of genetic defects in C1-INH-HAE patients [16,17]. *CpG* sites are prone to spontaneous deamination and therefore, represent mutational hotspots. A frequent target for recurrent amino acid substitutions is the CpG dinucleotide in the first two positions of codon 466, which encodes the central arginine (*CGC*) of the reactive center of C1-INH. Missense mutations of this amino acid lead to the generation of a dysfunctional protein and thus, to C1-INH-HAE type II [18]. In about 5 per cent of cases, no mutation can be detected in the coding region of the *SERPING1* gene [19,20]. In these patients, the underlying variation may affect an intronic or an untranslated region, and possibly modify protein expression.

Much progress has been achieved in the elucidation of the pathomechanism and of the genetic background of C1-INH-HAE since 1963, when Donaldson & Evans described quantitative and/or qualitative C1-INH deficiency in the background of this condition [21]. However, the mechanism behind the variable disease phenotype, a typical feature of C1-INH-HAE, has not been identified yet. Some carriers of the *SERPING1* mutation may remain symptom-free throughout their lives (as much as 14% of the patients according to the study of Roche et al. [22]). Other patients experience subcutaneous edema, which follows a milder course; whereas others again suffer frequent, life-threatening attacks [9].

In View of the Foregoing, What is the Significance of Genetic Testing in C1-INH-HAE?

Diagnostic issues

Genetic testing is not necessary in the first place to confirm the diagnosis of type I or type II C1-INH-HAE [23]. However, it can prove helpful in cases where the laboratory diagnosis based on complement tests is ambiguous, for instance due to the early-life variability of C1-INH concentration in childhood [24]. Furthermore, molecular genetic analysis of *SERPING1* by direct sequencing and/or Southern blotting, quantitative PCR, MLPA (multiplex ligation-dependent probe amplification) is an effective means for confirming the clinical diagnosis and identifying mutation carriers early on, before any clinical manifestation becomes apparent [25]. It is, therefore, a useful tool for improving the adequacy of patient care.

Prenatal diagnostics

Prenatal diagnostics enables parents carrying genetic risks to avoid passing severe, or life threatening heritable diseases to their offspring. The negative findings may allay their concerns and encourage them to have unaffected children. Prenatal diagnostics is not routine in C1-INH-HAE patients, as the mutation of the *SERPING1* itself may not be a valid indication for terminating pregnancy, because it can cause

only a non-fatal, manageable disease in the child. The severity of the disease cannot be predicted in advance, as even the same *SERPING1* mutation may be associated with largely different phenotypes [26].

Remarkably, genetic studies have made it possible for the first time to establish the diagnosis before implantation, and this allowed the birth of a healthy boy from a patient with C1-INH-HAE [27].

Mapping genotype-phenotype correlations

The great variability in the natural course of C1-INH-HAE among families with *SERPING1* mutations as well as the observation that in some cases members of the same family share an apparently similar disease phenotype, has raised the question whether there is a relationship between the type of mutations and the phenotype of the disease. Answering this is difficult because of the low incidence of the disorder and of the great number of mutations in its background.

It was generally accepted for decades that there is no or only little correlation between the type of *SERPING1* mutations and disease phenotype [25,28,29]. Most of the mutations in type *I* irrespectively of their types (large deletions, nonsense, frame-shift, splice site mutations) cause the complete loss of protein secretion from one allele and all mutations result in the secretion of a nonfunctional protein in type II (causing an indistinguishable phenotype from the former), but there are some type I mutations (missense, small in-frame insertions/deletions) with not fully characterized functional effects. It is rational to hypothesize that among these latter, some may result in less harmful consequences that may be associated with a milder phenotype (later disease onset, and/or less severe/frequent attacks) as suggested by some recent studies.

A study by Bors et al. [30] in 106 patients with C1-INH-HAE showed that missense mutations of the *SERPING1* gene are associated with less severe disease. This was indicated by the lower annual attack frequency and the smaller dose requirements of C1-INH concentrate in missense mutation carriers, compared with the remainder of the study population [30]. Xu et al. analyzed the possible genotype-phenotype correlations from a different point of view in 48 patients with C1-INH-HAE. They showed that the antigenic level of C1-INH was significantly lower in carriers of mutations with proven disease-causing potential (nonsense, frameshift, and mutations on Arg466) than in patients with possibly deleterious mutations (in-frame and missense not including those on Arg466). Despite these results, there was no difference between the two groups in a severity score based on the frequency and location of the attacks, as well as on the use of emergency treatment, or on the functional activity of C1-INH [31]. In another study of 45 patients with C1-INH-HAE, Bafunno et al. [32] found no correlation between the different types of *SERPING1* mutations (9 missense, 5 nonsense, 6 frameshift, 1 small deletion and 1 splicing defect), and the severity score (based on clinical manifestation, age at disease onset, and the need for long-term prophylaxis) [32]. In the largest study ever in this field, Speletas et al. showed in 265 C1-INH-HAE patients (including the study population of Bors et al. [30]) from 4 different countries that the presence of *SERPING1* missense mutations may be associated with a less severe clinical course. This was suggested by the significantly later onset of the disease in missense mutation carriers than in patients with all other *SERPING1* defects (nonsense, splice defects, frameshift, small insertions/deletions) [33].

Furthermore, some cases with the rare, homozygous C1-INH deficiency have been reported that represent another aspect of genotype-phenotype correlations. In these, the edematous symptoms of fluctuating severity affected only homozygous, but not heterozygous carriers. When compared with other heterozygous mutations, this interesting observation suggests a possible relationship between certain mutation types and disease severity. A study by Verpy et al. [16] showed that a homozygous mutation in the promoter of *SERPING1* is associated with low C1-INH levels and a severe clinical course of C1-INH-HAE. On the other hand, family members heterozygous for this mutation had C1-INH levels within the normal range (but usually close to its lower limit), and did not experience angioedema attacks [16]. In another study, a symptomatic carrier of a homozygous Ile462Ser mutation exhibited lower-than-normal C1-INH levels and reduced functional activity, whereas the heterozygous relatives were symptom-free [34]. Similarly, a patient with a homozygous Arg400Cys substitution had severe symptoms, but his heterozygous relatives were asymptomatic [35].

The role of additional mutations/polymorphisms with a possible influence on the symptoms of C1-INH-HAE

In some cases, there is a large variability in the phenotype of C1-INH-HAE (frequency, and severity of symptoms) – even within the same family, where the family members share the same mutation. The above mentioned observation - that family members with the same *SERPING1* mutation exhibit different phenotypes – prompted further studies into the polymorphisms of the genes encoding proteins involved in the pathomechanism of C1-INH-HAE.

Investigations of a common sequence variation within the *SERPING1* gene (Val480Met) showed that this has no detectable effect on protein stability, plasma levels, or the manifestations of C1-INH-HAE [20,36]. On the other hand, Cumming et al. demonstrated that the C allele of a common polymorphism (c.-21T/C) within *SERPING1* is associated with a more severe disease course [37]. However, a subsequent study by Bygum et al. [38] could not confirm this finding [38]. An in vitro study by Duponchel et al. showed that transfections with the minigene construct carrying the C variant at position c.-21 consistently yielded a weak product lacking exon 2 in the cell lines to which it was applied. This suggests that this allele might contribute, at RNA level, to the lower expression of the normal protein and the occurrence of more severe forms of angioedema [39].

Several studies investigated possible relationships between the functional polymorphisms in the genes of B1 and B2 bradykinin receptors (*BDKR1, BDKR2*), angiotensin-converting enzyme (ACE; an enzyme that breaks down bradykinin), or mannose-binding lectin (*MBL2*; a protein of the lectin pathway; its complex with *MASPs* is inactivated by C1-INH) and the severity of C1-INH-HAE; however, found no such correlation [40-42]. Further, Blasko et al. [43] showed a relationship between the clinical course of C1-INH-HAE and the copy number of the *C4B* gene encoding the *B* isotype of the *C4* protein: bi-yearly attack rate was lower in patients carrying 3 or 4 copies of *C4B* gene [43]. The study by Bors et al. [30] in 106 patients with C1-INH-HAE showed that symptoms appeared at a much younger age in patients homozygous for the rare allele of the 46T>C polymorphism in the gene of factor XII (a protein inhibited by C1-INH that has an essential role in bradykinin release) than in the remainder of the study population [30].

In summary, due to the greatly variable symptoms in C1-INH-HAE, several studies have been performed to establish a possible correlation between the genotype and the phenotype of the disease. However, the findings of these are difficult to compare, because of the diverse grouping of various mutations, of the different polymorphisms

analyzed, and of the different methodology used to evaluate the severity of C1-INH-HAE. Further, an exact and objective method for the latter purpose is not yet available. Moreover, as C1-INH-HAE is a rare disease (with an incidence of 1:10,000 to 1:50,000), exploring genotype-phenotype correlations is rather difficult. Any meaningful study into the latter would require conducting a large population trial with international collaboration. Speletas et al. were the first to publish a multicenter study evaluating the relationship between genotype and phenotype [33] but it is to be hoped that additional studies will be conducted in ever larger patient populations to investigate ever more markers of disease severity. In addition, beside the genetic background - including the presence of disease-causing mutations or polymorphisms of the *SERPING1* gene as well as of other genes, which may modify the manifestations of the disease – the response of any given patient to various trigger factors known to provoke edematous attacks may be quite diverse. Therefore, the severity and course of C1-INH-HAE are characterized by high variability not only among families or among the members of the same family. The disease may follow a variable cause also in the different life periods of the individual, during which environmental factors (certain medicinal products, mental stress, physical trauma and weather changes) have been observed to have a role that may also hamper the elucidation of the background effects of heredity on disease phenotype [44].

Conclusion

C1-INH deficiency is a genetically heterogeneous disorder with a rather variable phenotype. Understanding the underlying genotype-phenotype relationship would be most helpful in predicting disease severity – this could assist in choosing the appropriate treatment or in the use of prenatal diagnostics. Moreover, it could enhance our understanding of the molecular mechanisms of C1-INH function by analyzing structure-function correlations. For this purpose, large-scale studies should be conducted in international collaboration. In addition, it would be expedient to characterize disease severity in individual patients by taking into account the repeated measurements of standardized markers (frequency, severity, acute treatment requirement) on multiple occasions over a longer period.

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