Genetics of hereditary angioedema

Elemente de genetică în angioedemul ereditar

Katalin Csép[1*], Dumitru Moldovan[2,1]

1 University of Medicine and Pharmacy of Tîrgu-Mureş, Genetics Department
2 University of Medicine and Pharmacy of Tîrgu-Mureş, Mureş County Hospital, Internal Medicine Clinic, Allergology and Clinical Immunology Department

Abstract

Hereditary angioedema is a rare monogenic disorder caused by the mutation of the C1INH (C1 esterase inhibitor) gene with a consecutive quantitative or qualitative defect of the coded serpin that has a major role in preventing the unnecessary activation of the complement system. The deficiency of the activated C1 esterase inhibitor leads to episodic localized subcutaneous or potentially lethal submucosal edema. This autosomal dominantly inherited disorder is characterized by genetic heterogeneity, frequent de novo mutations, incomplete penetrance and other less common genetic mechanisms with direct implications in the clinical management and may represent challenging situations for the clinician. Mutation analysis, due the allele heterogeneity and frequent de novo mutations, is done mainly for research purposes and needs a complex testing strategy. Although it is not necessary for confirming the diagnosis, it may provide important information to our general understanding of the disease, optimal management of the affected families and development of novel therapeutic agents.

Keywords: *hereditary, angioedema, genetics*

Rezumat

Angioedemul ereditar este o boală ereditară monogenică rară cauzată de mutaţia genei C1INH (inhibitorul C1 esterazei) şi deficitul calitativ şi cantitativ al serpinei codificate care are un rol major în prevenirea activării excesive a sistemului de complement. Deficienţa inhibitorului activat de C1 esterază produce edem subcutanat localizat sau submucos potenţial letal. Această afecţiune autozomal dominantă este caracterizată de heterogenitate genetică, mutaţii de novo frecvente, penetranţă incompletă şi alte mecanisme genetice mai puţin obişnuite interpretarea cărora poate reprezenta o adevărată provocare pentru clinician. Din cauza heterogenităţii alelice şi a frecvenţei mare a mutaţiilor de novo, analiza mutaţiilor se realizează mai mult în scop de cercetare şi necesită investigaţii complexe. Deşi nu este necesar pentru confirmarea diagnosticului, poate contribui cu informaţii importante la cunoaşterea bolii, managementul optim al familiilor afectate şi dezvoltarea unor strategii terapeutice noi.

Cuvinte cheie: *angioedem, ereditar, genetică*

[*] **Corresponding author:** Katalin Csep, University of Medicine and Pharmacy Tîrgu-Mures, Genetics Department 38 Gh. Marinescu Str., Tîrgu-Mureş, Romania, Tel: +40 265 215 551 184 Fax: +40 265 210 407, E-mail: genetica.umftgmro@gmail.com

Introduction

Hereditary angioedema (HAE, OMIM #106100) is a rare genetic disorder caused by a quantitative or qualitative defect of the plasma protein C1 esterase inhibitor (C1-INH) determined by the mutation of the coding gene (C1INH). It should be noted though, that other forms of inherited angioedema which are not associated with mutations of the C1INH gene have also been identified (1). C1-INH deficiency causes the uncontrolled complement and contact systems' activation that will lead to the release of vasoactive mediators, mainly bradykinin. Clinically, this will determine recurrent episodes of acute edema at the level of the skin or the mucous membrane of the respiratory and/or digestive tract (2).

HAE is estimated to affect 1 in 50,000 – 150,000 persons, with no known interracial differences. Both sexes are affected, but the estrogen-dependent type III is predominant in women. Though it is an inherited disorder, perinatal angioedema is extremely rare, and the clinical onset is usually set in the first or second decade of life. There are two main phenotypic variants described. Type I is defined by the low antigenic and functional plasma levels of a normal C1-INH, while type II is characterized by normal or elevated levels of a dysfunctional C1-INH (low function but normal or increased antigenic level). Recently, an estrogen-dependent variant has been identified (2, 3).

The final diagnosis is made in the presence of a major clinical and lab abnormality; therefore, neither family history nor mutation analysis is mandatory for confirming HAE. The high frequency of de novo mutations and genetic heterogeneity underscore the importance of DNA testing, so mutation analysis is carried out mainly for research purposes. However, genetic testing can contribute with useful information to the clinical management in the cases of negative family history or in patients with borderline levels of C1q (4-6).

Inheritance

HAE is a genetic disorder, described and identified as an inherited disease more than a hundred years ago (1888), when Sir William Osler published a family tree in which individuals were affected in five consecutive generations.

It is a monogenic disease caused by various mutations of the C1INH gene, and the pattern of inheritance is autosomal dominant. However, many unusual phenomena characteristic for dominant disorders make genetic counseling challenging and autosomal recessive inheritance is suspected in certain mutations (c.2103C>T, 606860.0013) (1, 8).

Dominant disorders are clinically manifested in patients heterozygous or - less frequently - homozygous for the mutant allele. In heterozygotes, the normal allele determines half the level of the gene product observed in healthy recessive homozygotes. However, in HAE, plasma C1-INH levels range between 5-30% of normal levels, far from the 50% expected for an autosomal dominant defect. (7, 8) A couple of studies have demonstrated there is both a down-regulated expression of the normal mRNA and an increased C1-INH consumption in HAE patients that explain the lower-than-expected plasma level of C1-INH in heterozygotic patients (9).

Homozygosis is rarely described, and because of the low frequency of the mutation in the general population, it appears more frequently after consanguineous marriage. Because of the lack of such cases published in the literature, for a long time it was assumed that the homozygous genotype was a lethal combination. Recently, however, two instances of Homozygosis have been described, one in the promoter region of the gene and one in a coding region (8, 10, 11).

The family history is positive in 50-80% of the cases; however, due to the high frequency of spontaneous mutations and other, less frequent phenomena like incomplete penetrance, it may be negative. The reported spontaneous mutation rate is approximately 25% (12). It is presumed that the existence of several

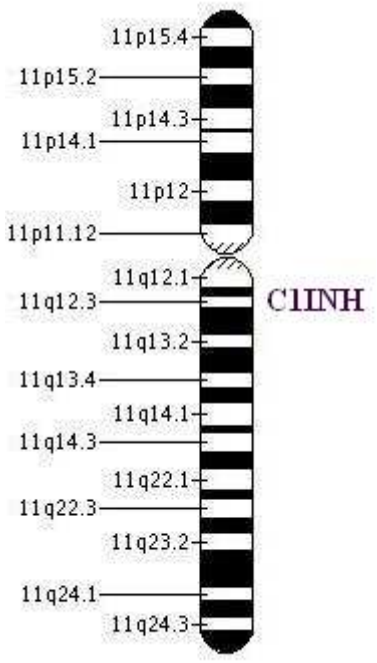

Figure 1. The C1-INH/SERPING1 gene mapped to 11q11-q13.1

Alu repeats in the C1INH gene predisposes to large rearrangements and thus the high rate of de novo mutations (13). Due to incomplete penetrance, 5% of the adult patients with HAE are identified after their child is diagnosed, and though carrying and transmitting the mutation to their children, they remain asymptomatic throughout their lives.

Mosaicism in the gonads in both the mother and the father has also been described (*606860.0014, TYR199TER and c.3_73del, p.N1fsX34) and, due to the different recurrence risk, needs special attention (1, 8).

The C1-INH/SERPING1 gene mutations

The gene product C1-INH is a multifunctional serine protease inhibitor, which plays an important role in controlling the complement system – the classical and alternate pathways, the contact system, the coagulation and fibrinolytic cascade. It is produced mainly in the parenchymal cells of the liver, as a 478 amino acids-long single chain of 71 kd; further proteolytic processing does not occur. Monocytes-macrophages, microglial cells, fibroblasts, endothelial cells and megakaryocytes may also contribute to its synthesis (8). With approximately 30% carbohydrate, C1-INH is one of the most highly glycosylated serum protein (1).

The disease is caused by the mutation of the gene coding for C1-INH (C1I/C1INH/SERPING1, *606860; GenBank NM_000062.1) localized at 11q11-q13.1 (*Figure 1*). It is 17 kb long and contains 8 exons and 7 introns, and it presents an unusual promoter with no TATA sequence but a TdT-like initiator and a polypurine-polypyrimidine tract (8).

Mutation screening was carried out in Spanish, Italian, Hungarian and other populations. The studies revealed pathogenic and non-pathogenic mutations, polymorphisms and rare variants in practically every sequence of the gene (14-17).

HAE is characterized by important genetic heterogeneity: almost 300 mutations in different regions of the C1-INH gene caused by various mechanisms have been described until now. Pathological mutations can occur in the exons or in the introns or in the regions controlling gene expression, and are produced by various mechanisms. If the mutation results in the loss of production of the C1-INH protein, type I of HAE develops. If the mutation results in the generation of a non-functional mutant protein, type II of HAE develops. Mutations causing type II are typically found within the active site or regions involved in the folding and presentation of the active site, causing a malfunction of the protein (18). Allele heterogenity can be both iso- and heteroallelic. The mechanism of the mutation can be point mutations – mis-sense or non-sense substitution or frameshift mutation, splice site errors, deletions or insertion of smaller or longer sequences – micro-deletions/insertions associated or not with frame-shift, large rearrangements - deletions or duplications, CNVs (copy number vari-

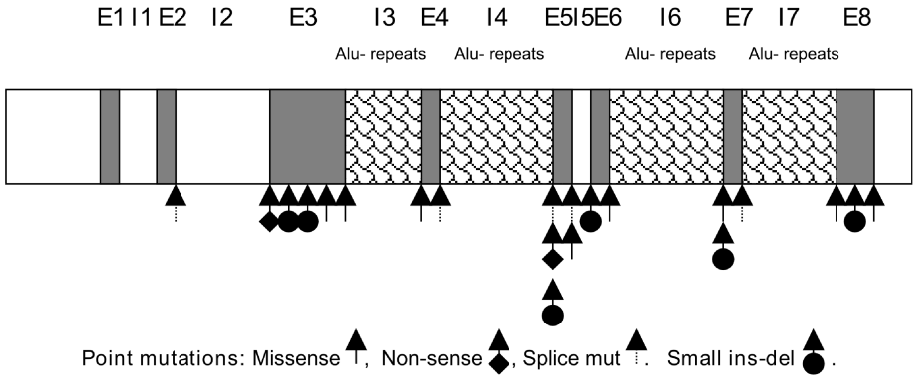

Figure 2. Distribution and type of the most frequent mutations in the C1INH gene

ation) (*Figure 2*) (8). There are 17 Alu repeats that occur throughout the gene, associated with an increased frequency of gross mutations. Deletions and duplications caused by these repeats account for approximately 15-20 % of the mutations in the C1-INH gene (13).

C1-INH mutations are published in well-known databases like MIM and the on-line version OMIM (MIM – Mendelian Inheritance in Man). Recently, comprehensive, frequently updated and interactive databases of C1INH mutations have also been published (19, 20). In the Hungarian database accessed at http://hae.enzim.hu/, mutations are classified in 2 large groups: gross mutations involving DNA fragments of up to 41 kb, and micro-mutations encompassing all non-gross mutations. With the last entry submitted on 2008-07-18, the number of registered micro-mutations and gross mutations is 238 and 45, respectively. Regarding the mechanism of the mutations reported, the following distribution was found: mis-sense mutations - 36%, frameshift mutations – 23.8%, gross mutations - 15%, non-sense mutations with truncated protein - 8.8%, splice-site mutations - 8,8%, small indels without frameshift - 4,8%, mutations in the promoter region -1,4% and others (19).

Though mutations can be localized anywhere – both in exons or introns and non-coding control regions, it seems that there are certain mutational "hot spots" that apparently code for amino acid sequences important for the protein function. There is an unusually high frequency of mutations located in exon 8 which contains the critical hinge region and reactive center of C1-INH, and it was confirmed by several study groups that exon 4 is particularly prone to rearrangements (17, 21).

The estrogen-sensitive or dependent form of HAE (previously type III, or type IV as suggested by Kranke et al.) presenting with identical simptoms associated with normal C1-INH concentration and function seems to be also a monogenic form mapped to 5q33-qter (#610618). It was suggested that the mode of inheritance could be autosomal dominant or X-linked dominant, and there is evidence that it can be caused by mutations in the gene coding for the coagulation factor XII (22, 23).

Practical aspects: mutation analysis, genotype-phenotype correlation and genetic counseling

Due to the genetic heterogeneity and the inconsistent genotype-phenotype correlation, neither the confirmation of the final diagnosis nor the initiation of the therapeutic intervention requires the recognition of the existent mutation.

As the mutation mechanisms are very variable from point mutations to small indels with or without frameshift and large rearrangements - deletions and duplications, and frequently previously undescribed mutations have to be identified, different methods are used to clarify the type and location of the mutation. Molecular diagnosis is further confronted with

the high frequency of newly produced mutations. The golden standard for mutation detection in HAE is sequencing, carried out generally by the automated enzymatic method.

Though several different mutations have been associated with HAE, only a few of them recur in more than one family, so targeted search of known mutations by simple and widely accessible methods like PCR-RFLP is not applicable. Designing diagnostic strategies in the C1INH mutation analysis remains so far an unsolved task. Focusing on specific regions provides a significant amount of data but is not exhaustive and sufficient.

All these factors explain why various strategies are applied, and sometimes the use of a combination of techniques is required.

Furthermore, in such cases of genetic heterogeneity, the newly identified mutations must be carefully interpreted before a final result is obtained. Co-segregation in the family of the mutation with the disease must be demonstrated and a polymorphism must be excluded.

Methods used to scan for point mutations or small deletions or insertions are the simple, inexpensive and highly sensitive single-strand conformation analysis, hetero-duplex DNA analysis, denaturing gradient gel electrophoresis. They must be followed by the sequencing of the modified region to determine the exact mutation. Chemical cleavage of mismatches is adequate for detecting most of the point mutations and can be further improved by fluorescence assisted mismatch analysis. In certain cases these methods fail. Analysis of large genomic DNA rearrangements - deletions and insertions - can be searched for by RT-PCR and Southern blotting using restriction enzymes in single or multiple digestions (BamHI, BclI, BglII, EcoRI, HindIII, SacI, SalI, PvuI, PstI, etc.) and then the identified region must be further sequenced (24-27).

Genetic heterogeneity and variable clinical manifestations raises the question of existing genotype - phenotype correlations. Unfortunately, as frequently seen in other monogenic disorders, these relationships are inconsistent. However, these relationships are worth to be studied as theoretically they could have major predictive prognostic value and also implications in choosing the adequate treatment and developing novel therapeutic agents targeting the actual molecular mechanism and making individualized therapy on a molecular level possible.

If the mutation results in the loss of production of C1-INH protein, type I of the disease develops. If the mutation results in the generation of a non-functional mutant protein, type II develops (3, 4, 6). In spite of these lab anomalies, the clinical phenotypes in the two forms are indistinguishable. Type I is very heterogeneous and mutations are distributed over the entire gene: they include large deletions or insertions probably in correlation with the high density of Alu repeats in the introns and point mutations that lead to a failure of protein synthesis or secretion most frequently by a non-sense mutation and synthesis of a truncated protein (13).

In type II, usually a mis-sense substitution can be identified, mainly within the coding region for the reactive loop of the C1-INH located in exon 8 (17, 21). Mutations at Arg444 (the P1 residue) are estimated to be present in up to 70% of type II HAE patients (28).

It must also be noted that non-pathological genetic variants of the gene occurring in the presence of completely normal C1-INH level and function have also been described (8). On the other hand, both other genetic and also environmental factors might modulate the disease, probably in a complex manner, with combinations of the aggravating factors specific for the individual. Several more or less important non-genetic disease modifying factors have been identified so far, like sex hormones or infections.

The clarification of certain genetic modifying factors is only at the beginning. It was demonstrated that both increased C1-INH catabolism and decreased expression of the normal C1- INH allele is present in patients contributing

to the lower than expected amounts of functionally active C1-INH. This may be explained by a dominant negative effect in certain mutations or other non-allelic gene-gene but also gene-environment interactions. The relationship between copy number of genes - C4A and C4B and the clinical course of HAE was studied. It was found that a high copy number of the C4B gene can be a protective factor against disease severity; therefore the authors consider its assessment in HAE to be justified (29).

It should be noted that the majority of studies on mutations in HAE that demonstrated poor genotype-phenotype correlations have been cross-sectional; to improve our understanding about such relationships, family based studies have been designed, but no breakthrough result was obtained (30).

As HAE is a monogenic disorder, family counseling plays a fundamental role in the management of the affected families. When the first case in the family is diagnosed, other family members need to be tested. Children are usually asymptomatic at birth, however close clinical and laboratory follow-up with regular testing is indicated. Early testing may assist relatives in taking informed decisions about family planning. Prenatal screening is not yet feasible, though if the mutation in the family is known this can be looked for. Patients and their families need to be educated about the disease, its inheritance and possibilities of management. As part of this activity, on a national level, the Romanian Network for Hereditary Angioedema was created (www.haenet.ro) (31). Molecular mapping of HAE in some of the Romanian patients has revealed mis-sense mutations and splicing defects besides certain variants and a mutation in the 5'-UTR region. Expanding the study to further family members and families may reveal further insights to the genotype-phenotype relationships and gene-gene interactions, while comparing data obtained in other countries of the region may clarify if there is a geographical difference in the genetic background of the disease (32).

Collecting data in national and international patient registries has been initiated and it can provide in the future the necessary statistical data to understand such rare disorders (33).

References

1. Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Number: #106100. Last edited: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=106100
2. Moldovan D., Mihály E., Popescu F.D, Szöllösi G.A., Dobreanu M. Hereditary C1-inhibitor esterase deficiency: a rather well defined entity. Revista Română de Medicină de Laborator 2009; 2:41-48
3. Cicardi M., Bergamaschini L., Cugno M. et al. Pathogenetic and clinical aspects of C1 inhibitor deficiency. Immunobiology. 1998; 199: 366-76
4. Agostoni A, Aygören-Pürsün E, Binkley KE, Blanch A, Bork K, Bouillet L, et al. Hereditary and acquired angioedema: problems and progress: proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. J Allergy Clin Immunol. 2004 Sep;114(3 Suppl):S51-131
5. Gompels MM, Lock RJ, Abinun M, Bethune CA, Davies G, Grattan C, et al. C1 inhibitor deficiency: consensus document. Clinical & Experimental Immunology. 2005; 139(3), 379-394
6. Bowen T., Cicardi M., Bork K, Zuraw B., Frank M., et al. Hereditary angiodema: a current state-of-the-art review, VII: Canadian Hungarian 2007 International Consensus Algorithm for the Diagnosis, Therapy, and Management of Hereditary Angioedema. Ann Allergy Asthma Immunol. 2008;100(Suppl 2):S30–S40
7. Tosi M. Molecular genetics of C1 inhibitor. Immunobiology. 1998;199:358–365
8. Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Number: *606860. Last edited: 1/26/2009. http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606860
9. Pappalardo E, Zingale LC, Cicardi M. C1 inhibitor gene expression in patients with hereditary angioedema: Quantitative evaluation by means of real-time RT-PCR. J Allergy Clin Immunol. 2004;114:638-464
10. Frank MM, Atkinson JP. Homozygous C1 inhibitor deficiency: The conclusion of a long search. J Allergy Clin Immunol. 2006;118(6):1327-1329
11. Blanch A, Roche O, Urrutia I, Gamboa P, Fontan G, Lopez-Trascasa M. First case of homozygous C1-inhibitor deficiency. J Allergy Clin Immunol. 2006;118:1330-1335.
12. Pappalardo E, Cicardi M, Duponchel Ch, Carugati A, Choquet S, Agostoni A, et al. Frequent de novo mutations and exon deletions in the C1 inhibitor gene of patients with angioedema. J Allergy Clin Immunol 2000;106:

1147-1154
13. Stoppa-Lyonnet D, Carter P, Meo T, Tosi M. Clusters of intragenic Alu repeats predispose the human C1 inhibitor locus to deleterious rearrangements. Proc Natl Acad Sci USA 1990;87:1551–1555
14. Kalmár L, Bors A, Farkas H, Vas S, Fandl B, Varga L, et al. Mutation screening of the C1 inhibitor gene among Hungarian patients with hereditary angioedema. Hum Mutat 2003(6): 498
15. Blanch A, Roche O, López-Granados E, Fontán G, López-Trascasa M. Detection of C1 inhibitor (SERPING1/C1NH) mutations in exon 8 in patients with hereditary angioedema: evidence for 10 novel mutations. Hum Mutat 2002(5): 405-406
16. Bowen B, Hawk JJ, Sibunka S, Hovick S, Weiler JM. A review of the reported defects in the human C1 esterase inhibitor gene producing hereditary angioedema including four new mutations. Clin Immunol. 2001(2): 157-163
17. Roche O, Blanch A, Duponchel C, Fontán G, Tosi M, López-Trascasa M. Hereditary angioedema: the mutation spectrum of SERPING1/C1NH in a large Spanish cohort. Hum Mutat 2005(2): 135-144
18. Bowen B, Hawk JJ, Sibunka S, Hovick S, Weiler JM. A review of the reported defects in the human C1 esterase inhibitor gene producing hereditary angioedema including four new mutations. Clin Immunol. 2001;98:157–163
19. Kalmar L, Hegedus T, Farkas H, Nagy M, Tordai A. HAEdb: a novel interactive, locus-specific mutation database for the C1 inhibitor gene. Hum Mutat. 2005;25(1):1-5
20. Piirilä H., Väliaho J., Vihinen M. Immunodeficiency mutation databases (IDbases) Hum Mutat. 2006 Sep 26;27(12):1200-1208. http://bioinf.uta.fi/SERPING1base/
21. Pappalardo E, Caccia S, Suffritti C, Tordai A, Zingale LC, Cicardi M. Mutation screening of C1 inhibitor gene in 108 unrelated families with hereditary angioedema: functional and structural correlates. Mol Immunol. 2008;45(13):3536-3544
22. Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Number: #610618. Last edited: 9/14/2007. http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=610618
23. Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. Biochem Biophys Res Commun. 2006;343: 1286-1289
24. Verpy E, Biasotto M, Brai M, Misiano G, Meo T, Tosi M. Exhaustive mutation scanning by fluorescence-assisted mismatch analysis discloses new genotypephenotype correlations in angiodema. Am J Hum Genet 1996;59:308–319
25. Weiler CR, Van Dellen RG. Genetic Test Indications and Interpretations in Patients With Hereditary Angioedema. Mayo Clin Proc. 2006;81(7):958-972
26. Zuraw BL, Herschbach J. Detection of C1 inhibitor mutations in patients with hereditary angioedema. J Allergy Clin Immunol. 2000;105:541–546
27. Duponchel C, Di Rocco C, Cicardi M, Tosi M. Rapid detection by fluorescent multiplex PCR of exon deletions and duplications in the C1 inhibitor gene of hereditary angioedema patients. Hum Mutat. 2001(1);17:61–70
28. Kang HR, Yim EY, Oh SY, Chang YS, Kim YK, Cho SH, et al. Normal C1 inhibitor mRNA expression level in type I hereditary angioedema patients: newly found C1 inhibitor gene mutations. Allergy 2006(2): 260-4
29. Blaskó B, Széplaki G, Varga L, Ronai Z, Prohászka Z, Sasvari-Szekely M, et al. Relationship between copy number of genes (C4A, C4B) encoding the fourth component of complement and the clinical course of hereditary angioedema (HAE). Mol Immunol. 2007 r;44(10):2667-2674
30. S-A Cumming, D J Halsall, P W Ewan, D A Lomas. The effect of sequence variations within the coding region of the C1 inhibitor gene on disease expression and protein function in families with hereditary angio-oedema J Med Genet 2003;40:e114
31. Moldovan D. Angioedemul ereditar: de la un caz la o rețea națională. Revista Medic.ro. 2007;31:85-88
32. Germenis A. The molecular basis of HAE: the Romanian and Greek experience. J Rom Soc Allergol Immunol 2009;6:3, 87
33. Mallbris L, Nordenfelt P, Björkander J, Lindfors A, Werner S, Wahlgren CF. The establishment and utility of Sweha-Reg: a Swedish population-based registry to understand hereditary angioedema. BMC Dermatology 2007;7.6