Genetic Causes of Hereditary Angioedema

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1888: Osler describes a hereditary form of angio-neurotic edema

1963: Donaldson and Evans observed a biochemical defect in patients: C1 esterase inhibitor was lacking in blood
Hereditary Angioneurotic Edema: Two Genetic Variants

Abstract. Serums of patients with hereditary angioneurotic edema lack inhibitory activity against the esterase derived from the first component of complement. In one group of patients this lack appears to result from failure to synthesize the esterase inhibitor of the first component of complement, whereas in another group of patients an abnormal, nonfunctional protein is synthesized.

Rosen et al., Science, 1965
Formal genetic aspects of HAE

Bygum et al., *Allergy*, 2011

- Autosomal dominant inheritance (50% chance for each child to inherit the disease)
- Variable expressivity within families
1888: Osler describes a hereditary form of angio-neurotic edema

1963: Donaldson and Evans observed a biochemical defect in patients: C1 esterase inhibitor was lacking in blood

1970s / early 1980s: Genetic linkage studies did not lead to identification of the genomic position of the C1 inhibitor gene (too few available genetic markers; not all chromosomes covered)
The history of hereditary angioedema....
from a geneticist's point of view

Human inhibitor of the first component of complement, C1: Characterization of cDNA clones and localization of the gene to chromosome 11
(protease inhibitors/serpins)

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Communicated by Ruth Sager, December 31, 1985

ABSTRACT C1 inhibitor is a heavily glycosylated plasma protein that regulates the activity of the first component of complement (C1) by inactivation of the serine protease subcomponents, C1r and C1s. C1 inhibitor cDNA clones have been isolated, and one of these (pCIINH1, 950 base pairs) has been partially sequenced. Sequence analysis demonstrates that the C1 inhibitor is a member of the serpin "superfamily" of protease inhibitors. In the region sequenced, C1 inhibitor has 22% identity with antithrombin III, 26% with α1-antitrypsin and α1-antichymotrypsin, and 18% with human angiotensinogen. C1 inhibitor has a larger amino-terminal extension than do the other plasma protease inhibitors. In addition, inspection of residues that are invariant among the other protease inhibitors shows that C1 inhibitor differs at 14 of 41 of these positions. Thus, it appears that C1 inhibitor diverged from the group relatively early in evolution, although probably after the divergence of angiotensinogen. Southern blot analysis of BamHI-digested DNA from normal individuals and from rodent–human somatic cell hybrid cell lines (that contain a limited but varied human chromosome complement) was used to localize the human C1 inhibitor gene to chromosome 11. The structural basis of C1 inhibitor function, to define its evolutionary relationship to other protease inhibitors, and to analyze the molecular genetic basis of hereditary angioneurotic edema, we have begun to examine the C1 inhibitor gene. In this report, we have characterized a C1 inhibitor cDNA clone that represents approximately half the coding sequence of the protein, and we have compared the sequence of this clone with the analogous regions of other protease inhibitors. In addition, we provide evidence that the human C1 inhibitor gene is on chromosome 11.
The history of hereditary angioedema.... from a geneticist’s point of view

- 1888: Osler describes a hereditary form of angio-neurotic edema

- 1963: Donaldson and Evans observed a biochemical defect in patients: C1 esterase inhibitor was lacking in blood

- 1970s / early1980s: Genetic linkage studies did not lead to identification of the genomic position of the C1 inhibitor gene (too few available genetic markers; not all chromosomes covered)

- 1986: Davis et al. assign the gene for C1 inhibitor to chromosome 11 (in human-rodent somatic cell hybrids)

- 1989: cytogenetic localization of the C1 inhibitor gene to 11q12-q13.1 using in-situ hybridization (Theriault et al.)
C1 Inhibitor gene (**SERPING1**)

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**(ch37) Chromosome 11: 56,873,659 - 57,873,658**
Genomic structure of the C1 Inhibitor gene (SERPING1)

- gene spans approx. 17 kb
- 8 exons
- 17 Alu-repeats

Agostoni et al., JACI, 2004
Mutation analysis in the common types of HAE (I and II) since >20 years

-HAE type I: 85% of all patients; low plasma levels of C1INH (<<50%)
Often large deletions spanning one or more exons
Small in/dels leading to frameshift mutations
Single nucleotide mutations (missense, nonsense)

-HAE type II: 15% of all patients; normal or elevated levels of C1INH;
mutations cause low C1INH activity

⇒More than 200 mutations reported, scattered along the entire gene
pronounced allelic heterogeneity!
HAE mutation database

Statistics of the HAEdb

This page is generated by the database engine, which means that the numbers represent the actual data.

Current version: 2005.2.0. (evolution of the database)

Last entry submitted: 2012-08-15

Number of entries by mutation type:
- Number of micro mutations: 239
- Number of gross mutations: 45

Distribution of the mutations by the owners:
- admin: 68
- AllergyLab-FMRP: 1
- crouet_lab: 3
- sevilla_lab: 4
- tordai-lab: 17
- tosi-lab: 56
- trascasa-lab: 44
- Wuerzburg: 92

Number of laboratories contributed: 9

Number of e-mail addresses in the maillist: 34

http://hae.enzim.hu/
Kalmar et al., *Hum Mutation*, 2005
High rate of *de novo* mutations in the C1 Inhibitor gene

-20-25% of patients have no apparent family history of AE (i.e. sporadic)

-They present *de novo* mutations in the C1 Inhibitor gene (same mutation spectrum as the familial cases)
Mutational hotspot at C1 Inhibitor gene (*SERPING1*)

- C1 inhibitor gene is located very close to the centromer
- Centromeric regions are considered the most rapidly evolving compartments in the eucaryotic genome

⇒ Higher mutation rates (especially at CpG nucleotides) and repetitive sequences (unequal crossing over!) seem to be responsible
Alu-repeats mediate non-allelic homologous recombination (NAHR)

Mutation analysis of the C1 Inhibitor gene (*SERPING1*) in HAE patients

- High degree of allelic heterogeneity requires comprehensive mutation screening in each new patient

- Traditional methods: Southern Blotting, SSCA, DHPLC, ...... (labour-intensive, time-consuming)

- We are now establishing: MLPA and (next-generation) sequencing
HAE type I and II – unsolved questions

- Type I: loss of one allele should leave 50% C1INH serum levels; in fact, only 10-20% serum levels are seen. What is the reason?

- C1INH genotype and HAE phenotype correlate poorly

- What is the basis for intrafamilial (=same mutation) phenotypic variability? Modifier genes / environment?
HAE with normal CINH (HAE III) – a novel (and rare) type of hereditary angioedema

The Lancet, July 2000

Hereditary angioedema with normal C1-inhibitor activity in women

Konrad Bork, Sven-Erik Bamstedt, Petra Koch, Heiko Traupe

Summary

Background Hereditary angioedema (HAE) is a well defined autosomal dominant disease (Mendelian inheritance in Man #108100) that results from an inherited deficiency of C1 (the activated first component of complement) inhibitor function. We report an unusual variant of HAE with normal biochemical C1-inhibitor function, occurring only in women.

Methods We screened 574 patients with recurrent angioedema of the skin for presence of HAE. 283 patients were selected, in whom angioedema was associated with abdominal pain attacks or recurrent life-threatening episodes of upper-airway obstruction, or both, rather than with urticaria. We measured C1-inhibitor concentration and functional activity as well as complement C4 concentration and took pedigrees to characterise patients.

Findings 94 HAE cases with C1-inhibitor deficiency, positive family history, or both were identified. Biochemical testing showed that 54 patients from 49 families had a functional C1-inhibitor deficiency. 11 of these patients had no affected family members (probably representing de-novo mutations). Ten women with HAE, from ten families, had normal C1-inhibitor protein concentrations and function, and normal C4 concentration. A more detailed study of these families identified another 26 affected members, who were also all women. Of those women, 14 could be studied and also had normal C1-inhibitor concentration and function. The disease was seen in successive generations, and in offspring of affected mothers, the sex ratio (M/F) was shifted to 1/1-5.

Interpretation HAE with normal C1-inhibitor concentration and function represents a unique disease entity affecting only in women. The formal genetics of this entity are suggestive of an X-linked dominant mode of inheritance. For this disorder we propose the term hereditary angioedema type III (HAE III).


Introduction

Recurrent angioedema of the skin is a commonly diagnosed clinical symptom that can be seen in various clinical entities. Some types of angioedema of the skin are associated with episodes of upper-airway obstruction that might be life-threatening. Asphyxiation by laryngeal oedema is well known in hereditary angioedema (HAE) due to C1-inhibitor (deficiency) and in recurrent angioedema induced by angiotensin-converting-enzyme (ACE) inhibitors. Therefore, the exact type of angioedema must be identified in each patient.

In many patients, angioedema is associated with urticaria. If resolving urticaria occurs simultaneously or otherwise with angioedema, the two disorders are assumed to be symptoms of the same disease. This assumption is valid in chronic idiopathic urticaria and angioedema; IgE-mediated reactions to foods, drugs, insect toxins, and other substances; serum sickness; urticaria and angioedema induced by aspirin and non-steroidal anti-inflammatory drugs, azo dyes, and benzochromes; and reactions caused by substances that induce direct histamine release from mast cells.

Recurrent angioedema in patients without urticaria can be due to several pathogenetic mechanisms. Most frequently, recurrent angioedema is part of the above entities, in many patients as the combination of urticaria and angioedema, but in some patients as recurrent angioedema alone. Furthermore, recurrent angioedema without urticaria can result from inherited or acquired C1-inhibitor deficiency, or be induced by ACE inhibitors. HAE due to C1-inhibitor deficiency is not associated with urticaria, whereas ACE inhibitors may cause recurrent angioedema alone, or in association with urticaria. In recurrent angioedema C1-inhibitor deficiency, anti-histamines and corticosteroids are not effective. Other types of angioedema without urticaria are rare and include local angioedema secondary to physical stress, such as vibratory angioedema. Some patients with recurrent angioedema have clinical symptoms that cannot be ascribed to one of these disorders. This review
Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema

Karen E. Binkley, MD, FRCP,* and Alvin Davis III, MD†
Toronto, Ontario, Canada, and Boston, Mass

Background: Two genetic forms of hereditary angioedema (HAE) are currently recognized. Both are transmitted in an autosomal dominant manner and are characterized by recurrent episodes of localized angioedema. Involvement of the gut leads to episodes of severe abdominal pain, and laryngeal involvement can lead to airway obstruction and even death. One type results from heterozygosity for a nonexpressed Cl inhibitor allele, and the other results from heterozygosity for a nonfunctional Cl inhibitor allele.

Objective: This report identifies a third type of HAE, with a unique estrogen-dependent phenotype.

Methods: Detailed medical histories were obtained from family members, and a pedigree was constructed to ascertain the mode of inheritance. Determination of serum complement factors, Cl inhibitor protein, Cl inhibitor function, coagulation factor XII, plasma prekallikrein, high molecular weight kininogen, and selected DNA sequences were performed in affected members by using standard assays.

Results: Episodes of angioedema were clinically indistinguishable from those associated with previously described forms of HAE; however, these occurred only during pregnancy or the use of exogenous estrogens. Patients were otherwise asymptomatic, except for one patient who had acetaminophen-acetaminophen-steroidal anti-inflammatory drug-related angioedema later in life. History was available for members spanning 4 generations, and affected individuals were identified in 3 generations. Of 46 family members, phenotype could be determined in 43 members. Seven were affected, and 6 were not. One male of undetermined phenotype was an obligate carrier. The unique estrogen-dependent nature of the phenotype means that the status of several members in the third and fourth generation remains unknown. The disorder appears to be transmitted in an autosomal dominant fashion, although other modes of inheritance cannot be excluded entirely. Cl inhibitor protein, C1 inhibitor function, C2, C4, C1q, coagulation factor XII, prekallikrein, and high molecular weight kininogen were normal in 3 affected family members during asymptomatic periods. DNA sequencing revealed no abnormality in 3 patients in the coding region of the gene encoding Cl inhibitor or in the S′ flanking regions of the genes encoding C1 inhibitor and factor XII.

Conclusions: This family appears to have a novel form of inherited angioedema that does not result from Cl inhibitor deficiency or dysfunction. The phenotype is uniquely estrogen dependent. Implications for diagnosis and treatment are discussed. Further studies are required to define the exact nature of the genetic abnormality involved. (J Allergy Clin Immunol 2000;106:546–50.)

Key words: Hereditary angioedema

FIG 1. Pedigree of the affected family.
Locating the gene(s) responsible for HAE III - linkage analysis

- 4 pedigrees with multiple affected women with HAE III diagnosed and recruited by Konrad Bork (Mainz)
Linkage analysis in 4 German HAEIII families – Possible locations of the disease gene (H.C. Hennies)

Note: none of the suggested loci meets the threshold for significant linkage (HLOD > 3.0)
Linkage analysis in 4 German HAEIII families – Possible locations of the disease gene (H.C. Hennies)
To the Editor:

Binkley and Davis recently identified a third family of Italian origin. Clinical presentation was similar except for the unique occurrence in women, not men, of sexual precocity due to estrogen. More particularly, C1-inhibitor (C1INH) deficiency (a known cause for angioedema) was excluded in this family. This familial pattern was confirmed simultaneously by Bork et al in women from a family previously called “HAE type III.” Bork et al suggested X-linked transmission because the condition was restricted to women; no affected offspring from affected women. In contrast, the current case was transmitted in an autosomal dominant fashion. This is the first autosomal dominant family with HAE type III, in which X-linked dominant transmission can be excluded.
Locating the gene(s) responsible for HAE III - linkage analysis

- Chrom. region 5q35 co-segregates with HAE III in the French family

Chromosomal region 5q35 contains the gene for blood coagulation factor XII (F12)
Identification of c.1032C->A (p.Thr328Lys) in patients with HAE III

Activity of FXII in patients with Thr328Lys mutation – evidence for increased enzymatic activity

Relative FXII amidolytic activity in plasma from HAE III and healthy individuals (control), determined using the FXIIa-specific chromogenic substrate S-2303.

Most likely pathomechanism: Thr328Lys is a gain-of-function mutation that increases enzymatic activity of FXII. Pathological FXII activity may trigger excessive bradykinin generation, which results in an increase of endothelial permeability and vascular leakage.

S-2303 turnover in plasma of a patient with HAE type III (amidolytic activity markedly increased), and in a patient with HAE type III from FXII-unlinked family F10 (FXII activity normal).

Evidence for locus heterogeneity in HAE III

- 3 of 4 German pedigrees have FXII Thr328Lys mutation, as well as the French family
- One German family not linked to the FXII locus! => responsible for difficulties to find the FXII locus
Thr328Lys mutation carriers in 3 German and 1 French family have a common founder (approx. 11th century)

French family with HAE III co-segregates FXII Thr328Lys mutation in affected women and men

Disease severity class (as defined by Cicardi & Zingale in Agostoni et al., JACI 2004):
4 or 5 in men versus 1 or 2 in women. Several male carriers (<50 yrs.) were symptomless

One other single nucleotide mutation described in \textit{FXII} gene

BRIEF COMMUNICATION

A novel mutation in the coagulation factor 12 gene in subjects with hereditary angioedema and normal C1-inhibitor

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Abstract In hereditary angioedema with normal C1-inhibitor two different missense mutations of codon p.Thr328* in the coagulation factor 12 gene have been reported in some families. In this study a novel factor 12 gene mutation, the deletion of 72 base pairs (bp) (c.971_1018 +24delT2*), was identified in a family of Turkish origin, in two sisters with recurrent skin swellings and abdominal pain attacks and in their symptom-free father. This deletion caused a loss of 48 bp of exon 9 (coding amino acids 324* to 340*) in addition to 24 bp of intron 9, including the authentic donor splice site of exon 9. The large deletion of 72 bp was located in the same F12 gene region as the missense mutations p.Thr328Lys* and p.Thr328Arg* reported previously. Our findings confirm the association between F12 gene mutations modifying the proline-rich region of the FXII protein and hereditary angioedema with normal C1-inhibitor.

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Functional consequences of 72 bp del. in FXII?

FXII wildtype protein – 615 AA

FXII truncated protein – up to 324 AA normal, then frameshift introducing 10 other AA, then stop
So far, we screened more than 70 families from different countries (Germany, France, Italy, Spain, Sweden, Belgium, USA, Brazil, Argentine) for mutations in FXII.

Only 10-30% can be explained by the Thr328Lys mutation (and Thr328Arg).

Majority of HAE III patients with FXII mutation origin from Middle Europe (in particular Spain and France).

Mutation carriers investigated so far go back to a common founder.

The Thr328Lys mutation is highly penetrant but variably expressed in women, has very low penetrance in males. So far unexplained.

Mutations in FXII other than Thr328Lys seem to be extremely rare, (Gain-of-function model may be an explanation).
Genetic analysis of Factor XII and bradykinin catabolic enzymes in a family with estrogen-dependent inherited angioedema

Qing Ling Duan, PhD, Karen Binkley, MD, FRCPC, and Guy A. Rouleau, MD, PhD, FRCPC, OQ

Background: Recent studies reported a gain-of-function mutation in the gene encoding coagulation Factor XII (F12) among 5 German and French families with estrogen-associated angioedema who share a common ancestor. The role of this factor, additional pathways that might contribute to increased bradykinin levels, or both remain to be determined in other families with estrogen-dependent or estrogen-associated inherited angioedema.

Objective: The purpose of this study was to determine whether mutations in F12 and polymorphisms in the genes encoding aminopeptidase P (APP) and angiotensin I–converting enzyme (ACE), which have been associated with increased bradykinin levels, contribute to estrogen-dependent inherited angioedema in a large family of Italian origin.

Methods: We screened the coding regions of F12 and the gene encoding membrane-bound APP (XPNPEP2), for genetic variants in the 3 affected female subjects. In addition, we genotyped this family for the insertion/deletion polymorphism in the ACE gene, which accounts for variable ACE levels.

Results: The 3 affected female subjects all have the threonine-to-lysine (Thr328Lys) mutation, which is associated with higher Factor XII activity. In addition, they have at least one A allele of rs3788853 at the XPNPEP2 locus, which is associated with lower APP activity, and at least one I allele in ACE, which is associated with reduced ACE activity.

Conclusion: A missense mutation in F12 is present in the 3 affected female subjects of this family with estrogen-dependent angioedema. Mutations in APP might contribute to estrogen-dependent or estrogen-associated inherited angioedema and explain some of the observed heterogeneity. (J Allergy Clin Immunol) 106:100; http://www.ncbi.nlm.nih.gov/omim).

Key words: Estrogen-dependent inherited angioedema, bradykinin, coagulation Factor XII, angiotensin I–converting enzyme, aminopeptidase P

Classic forms of hereditary angioedema (HAE; types I and II) are autosomal-dominant disorders resulting from decreased levels or function of the inhibitor for the first component of the complement pathway (C1-INH).1,2 These manifest as localized swelling of parts of the face, upper respiratory tract, gastrointestinal tract, genitalia, hands, and/or feet (Online Mendelian Inheritance of Man [OMIM] 106100; http://www.ncbi.nlm.nih.gov/omim).3 Inadequate C1-INH fails to restrict the activity of coagulation Factor XII (Hageman factor) and kallikrein, leading to increased production of bradykinin, a potent vasodilator and an important mediator of angioedema.3,4 Additional forms of HAE and sporadic angioedema, which are clinically indistinguishable from classic HAE, have also been reported among individuals with normal C1-INH concentrations and function. These include cases of unknown cause (idiopathic angioedema),5,6 drug-induced angioedema (eg, angiotensin I–converting enzyme [ACE] inhibitors7 and plasminogen activators8) and angioedema that affects only women.9,10 The latter forms are known as estrogen-dependen-
APP and ACE polymorphisms as modifiers for HAEIII?

Duan et al., JACI 2009
Searching for the molecular causes of HAE with normal C1INH.....
where do we stand?

- Patients with family history of angioedema
  - Hereditary angioedema with normal C1 INH (HAE type III)
    - with FXII mutation (10-30% of the clear hereditary cases)
      » Estrogen dependent – mainly women affected
      » Non estrogen dependent
    - without FXII mutation (70-90%, genetically unexplained)
FXII-unlinked families collected within the ERARE-HAEIII consortium

Recruited by Marco Cicardi / Andrea Zanichelli
Searching for unknown gene mutations for HAEIII in the „exome“

- 21,000 genes with 185,000 exons in the human genome (the “exome”)
- these protein coding regions constitute only about 1% of the genome, translates to about 30 megabases (Mb) in length
- it is estimated that protein coding regions constitute about 85% of the disease-causing mutations
Exome sequencing – from sequencing raw data to a disease-causing mutation

Sequencing machines
- Solexa
- SOLiD
- 454

Raw short sequences

One sequencer run generates several 100,000 fragments that represent all exons several times

Mapping and alignment

Which sequence fragment belongs to which gene/exon?

Identify all genetic variants present

Identify all genetic variants (mismatches with reference genome) and characterize

Identify the so far unknown mutation

Potential disease-causing mutation is identified among potential polymorphisms
EU-project (E-RARE programme)
Genetics, Pathophysiology, and Therapy of Hereditary Angioedema Type III

2008 - 2012

The European Partners

- **France**
  Grenoble (C. Drouet)
  Angers (L. Martin)

- **Italy**
  Milan (M. Cicardi)

- **Germany**
  Bonn (S. Cichon, C. Stieber)
  Cologne (H. C. Hennies)

- **Sweden**
  Stockholm (T. Renné)
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