HAE with normal C1-INH
(HAE type III, HAE-III)

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Mainz 1985

- Observation of a family
- Seven women (no man!) had relapsing skin swellings and abdominal pain attacks. Two of them also tongue swellings and laryngeal edema.
- All these 7 women had normal C1-INH and normal C4
Ten patients with normal C1-INH and normal C4 had the symptoms of HAE (relapsing skin swellings, abdominal pain attacks, and laryngeal edema).

The came from 10 families.

Each of these 10 patients had at least one relative in his family with the same symptoms.

Totally, they had further 26 affected family members.

So we had a total of 36 patients with hereditary angioedema and normal C1-INH in plasma!
• All 36 patients were females!

• 36 affected females
• 0 affected males

• $M : F = 0 : 36$
Hereditary Angioedema

• A. Hereditary angioedema due to a genetic deficiency of functional active C1 esterase inhibitor (type I; type II)

• B. A new type: Hereditary angioedema with normal C1 esterase inhibitor (HAE type III)

Hereditary angioedema with normal C1-inhibitor

Autosomal dominant inheritance

Hereditary angioedema with normal C1-inhibitor

Autosomal dominant inheritance

Hereditary angioedema with normal C1-inhibitor

Incomplete penetrance

Figure 6: Pedigree of a new HAE family showing transmission of disease to children from an unaffected female

Hereditary Angioedema with normal C1-INH (Type III)

- Clinical description of the disease in the year 2000 (Bork et al., Lancet: 10 families with 36 patients)

- Several months later, in the same year: One similar family (Binkley & Davis, JACI)
Hereditary Angioedema Type III

Many (but not all!) women observed an influence of estrogens:

Induction or worsening of the clinical symptoms of the disease by
a) Oral anticonceptives
b) Hormone replacement therapy (also with estrogens alone)
c) Pregnancy

138 Patients from 43 Families with Hereditary Angioedema and Normal C1-INH

<table>
<thead>
<tr>
<th>Number of clinically affected members per family:</th>
<th>Number of families:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20</td>
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<tr>
<td>3</td>
<td>10</td>
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<td>4</td>
<td>3</td>
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<td>5</td>
<td>3</td>
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<td>6</td>
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<td>7</td>
<td>2</td>
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<tr>
<td>8</td>
<td>2</td>
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<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>
Death cases in HAE type III

• Four patients died by asphyxiation:
  • One in her 16th year of life (first episode of upper airway obstruction).
  • One in her 36th year of life (10th episode of upper airway obstruction).
  • One in her 38th year of life (8th episode of upper airway obstruction).
  • One in her 48th year of life (numerous episodes of upper airway obstruction before).

Hereditary angioedema with normal C1 inhibitor

Angioedema of the lips and tongue in a woman aged 35 years

Hereditary Angioedema Type III

Laboratory results:
C1-INH activity: normal/subnormal
C1-INH protein: normal
C4 concentration: normal
C1q: normal
No mutation in the C1-INH gene
In 2006: Diagnosis of Hereditary Angioedema Type III

A. Recurrent angioedema in one or more organs
B. No hives/urticaria
C. More than one family member affected
D. Laboratory results: normal C1-INH and C4

HAE with normal C1-INH (=HAE type III) versus HAE-C1-INH

- Patients have normal C1-INH protein and activity.
- Mainly women are clinically affected.
- The number of children already affected before the age of 10 years is low. Clinical symptoms start in adulthood in more patients than in HAE-C1-INH.
- There are more disease-free intervals during the course of the disease.
- Symptoms are less frequent compared with HAE-C1-INH.
- Facial swellings, mainly lip swellings, are relatively more frequent.
HAE with normal C1-INH (=HAE type III) versus HAE-C1-INH (I)

- The tongue is considerably more often affected: Recurrent tongue swelling is observed in many patients and is a cardinal symptom of the condition.
  - Many patients have only skin swellings.
  - Many patients have only recurrent skin swellings and tongue swellings.
  - Abdominal attacks are less frequent.
  - Suffocation may be preceded and caused by a tongue swelling.
  - There is no erythema marginatum as is highly characteristic of HAE-C1-INH.
Further developments (I)
Genetics 1
Hereditary Angioedema with normal C1-INH

- 2001: Initiation of a microsatellite linkage study of the whole genom in the 4 largest of the 10 families from Mainz in the Max Delbrück center in Berlin (co-operation Bork/Hennies).

- Results: No significant results. No major linkage signals for the chromosome 11 (C1-INH) or chromosome 5 (fXII); minor signals in chromosome 6 and 16
• Due to the poor results of the genome scan we focussed on candidate genes:

• Our **functional hypothesis**:
  • abnormal factor XII molecule may lead to inappropriate activation of the kinin-forming cascade
• 20 affected unrelated patients from 20 families were investigated, one of each family.

• 6 of these 20 patients showed a mutation in the factor XII gene ($F12$)

• The mutations were located in exon 9 of $F12$. 
• We found 2 different mutations in the coagulation factor XII gene (on chromosome 5):

• Both were **missense mutations** (Thr309Lys, Thr309Arg)

• Both were located exactly in the same region!

• 5 patients had mutation 1 (lysin); one patient mutation 2 (arginin)
Missense mutations of the Thr309 residue of factor XII in hereditary angioedema with normal C1 inhibitor

• All family members of the corresponding 6 families were tested.

• 22 affected women of those 6 families showed the mutations (and 8 unaffected men). 145 healthy controls: no mutations.
Co-segregation of missense mutations of the Thr309 residue with disease phenotype

Family 003 (T309K)

I

II

III

IV

Co-segregation of missense mutations of the Thr309 residue with disease phenotype

• **Results 1:**
  • 23 affected women; all carry the mutation
  • 0 woman, who had angioedema but did not carry the mutation.

• **Results 2:**
  • Co-segregation of disease (clinical symptoms) and the mutation in the pedigrees (in women only!) (exception: a few clinically not affected transmitters)

• **Conclusion 1:**
  • The clinical disease of this type of recurrent angioedema is associated with the mutations in \( F12 \).

• **Conclusion 2:**
  • This makes it highly probable that these mutations in \( F12 \) have something to do with the cause of the disease.
Coagulation Factor XII gene

- Relative "stable" gene:
- Rarely new mutations
- Limited number of mutations known until now (about 40)
- Both HAE mutations in $F12$ were not known before.
HAE with mutation in *F12* (HAE-III-FXII)

- **Published until now:**
- **Original description:** Dewald & Bork, BBRC 2006 (6 families) (5 Lys, 1 Arg)

- **Families published later on (I, 2006 to 2009):**
  - Cichon et al., Am J Hum Genet 2006 (1 family) (Lys)
  - Bouillet et al., Brit J Dermatol 2007 (1 family) (Lys)
  - Fiz Matias et al., Rev Esp Anestesiol Reanim 2007 (1 family) (Lys)
  - Martin et al., JACI 2007 (1 family) (Lys)
  - Bell et al., Pathology 2008 (1 family) (Lys)
  - Prieto et al., JAICI 2009 (1 family) (Lys)
  - Duan et al., JACI 2009 (1 family) (Lys)
  - Bork et al., JACI 2009 (13 families [7 new families, 6 Lys, 1 Arg])
  - Hentges et al., JACI 2009 (1 family) (Lys)
  - Nagy et al., J Dermatol Science 2009 (1 family) (Lys)
HAE with mutation in the \textit{F12} gene (HAE-III-FXII)

- **Published until now:**
  - **Original description:** Dewald & Bork, BBRC 2006 (6 families) (5 Lys, 1 Arg)

- **Families published later on (II., 2010 to 2012):**
  - Picone et al., Obstetr Gynecol Int 2010 (1 family) (Lys)
  - Vitrat-Hincky et al., Allergy 2010 (3 families) (Lys)
  - Bork et al., Clin Immunol 2011 (1 family) (Del)
  - Brazil: Poster AAAAI 2012), (1 family) (Lys)
  - Marcos et al., Ann Allergy Asthma Immunol 2012 (13 families) (Lys)
HAE with mutations in \textit{F12}

- Published until now:

- \textbf{41 families} with a mutation in the gene coding for the coagulation factor XII
- Among them:
  - 38 families with a Thr309Lys mutation (all authors)
  - 2 families with a Thr309Arg mutation (Mainz series)
  - 1 family with a 72bp deletion mutation (Mainz series)
HAE with mutations in \textit{F12}

- **Published until now:**
  - 41 families with a mutation in the factor 12 gene
  - Among them:
    - Germany: 14 families
    - France: 7 families
    - Spain: 15 families
    - UK: 1 family
    - Italy/Canada: 1 family
    - Luxemburg: 1 family
    - Australia: 1 family (\(?; 1\) patient)
    - Brazil: 1 family (Poster AAAAI 2012)
- **Not yet published:**
  - US: 2 families
Conclusion:

- Mutations in a new angioedema gene:
- coagulation factor XII gene on chromosome 5 (telomeric 5q region)
- (no C1-INH gene mutations in chromosome 11q as in classical HAE !)
Further developments (II)
Function of FXII in HAE-III-FXII
Pathomechanism of HAE-III-FXII:

How could the mutations work?
Analysis of FXII activity in plasma from patients with HAE type III. 

a, Relative FXII amidolytic activity in plasma from HAE type III and healthy individuals (control), determined using the FXIIa-specific chromogenic substrate S-2303. Substrate turnover was measured photometrically via absorbance at a 405-nm wavelength. Data are presented in box-and-whisker plots showing the median (dark line in the box), 25th–75th percentiles (box), and 5th–95th percentiles (whiskers). 

b, Time course of S-2303 turnover in plasma of a representative patient with HAE type III (“French fam, IV:01”) from the French family, in the absence or presence of the FXIIa inhibitor PCK (2 mM) and in a patient with HAE type III (“F10, IV:11”) from German family F10. For comparison, FXII substrate cleavage in a plasma sample from a healthy control is plotted. Whereas FXII amidolytic activity is markedly increased because of the p.Thr328Lys mutation in the French patient, FXII activity is normal in the German patient.

Kallikrein-like activity measured by chromogenic substrate S-2302 without and with activation by dextran sulfate. With SBTI, addition of SBTI, which inhibits KK, FXIa and plasmin but not FXIIa; with PCK, addition of H–D–Pro–Phe–Arg–CMK, which inhibits KK, FXIIa and other trypsin-like proteases; HAE-FXII, six patients with Thr309Lys mutation; HP, six healthy probands. F, factor; HAE, hereditary angioedema; HP, healthy probands; KK, kallikrein; PCK, H–D–Pro–Phe–Arg–CMK; SBTI, trypsin inhibitor from soybean.
Bork K et al. Kallikrein-kinin system and fibrinolysis in hereditary angioedema due to F12 gene mutation
Pathomechanism of HAE-III-FXII:

Unknown up to now.

Cichon et al., 2006: Increased function of $F12$ (gain-of-function mutation): A 4-fold increase of function of FXII

Bork et al., 2009: Normal FXII activity during the interval between 2 attacks.
Hereditary Angioedema with normal C1-INH (HAE Type III)

1. Hereditary angioedema with normal (or subnormal) C1-INH with mutations in the factor 12 gene (HAE-III-FXII)

2. Hereditary angioedema with an unknown genetic cause (normal C1-INH activity in plasma, no causative mutation in the gene coding for C1-INH, and neither one of the known F12 gene mutations (p.Thr309Lys or p.Thr309Arg)) (HAE-III-unknown).
Further developments (III)
Characterization of HAE-III-FXII
Patients with HAE-III-FXII (Mainz 2009)

• 53 Families with HAE type III
• 13 Families (24.5%) with mutations in the \( F12 \) (HAE-III-FXII)
• In these 13 families: 53 patients with clinical symptoms
• In the following, experience in 35/53 patients (all women)

Distribution of edema episodes in various organs and skin regions in 35 patients with HAE-III-FXII on a per-patient and a per-episode basis

<table>
<thead>
<tr>
<th>Attack sites</th>
<th>Number and percentage of patients</th>
<th>Number and percentage of attacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin (total)</td>
<td>35 (100%)</td>
<td>4070 (62.2%)</td>
</tr>
<tr>
<td>Face</td>
<td>35 (100%)</td>
<td>1506 (37.0%)</td>
</tr>
<tr>
<td>Extremities</td>
<td>19 (54.3%)</td>
<td>2530 (62.2%)</td>
</tr>
<tr>
<td>Genitals</td>
<td>5 (14.2%)</td>
<td>27 (0.7%)</td>
</tr>
<tr>
<td>Neck</td>
<td>3 (8.6%)</td>
<td>7 (0.2%)</td>
</tr>
<tr>
<td>Stomach/gut</td>
<td>20 (57.1%)</td>
<td>2267 (34.6%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>14 (40.0%)</td>
<td>86 (1.3%)</td>
</tr>
<tr>
<td>Larynx</td>
<td>12 (34.3%)</td>
<td>74 (1.1%)</td>
</tr>
<tr>
<td>Uvula</td>
<td>11 (31.4%)</td>
<td>50 (0.8%)</td>
</tr>
<tr>
<td>Attacks (total)</td>
<td>35 (100%)</td>
<td>6547 (100%)</td>
</tr>
</tbody>
</table>

Age at onset in HAE-III-FXII

HAE-III-FXII, Trigger Factors

1. Estrogens
   - Oral contraceptives
   - Pregnancy
   - Hormonal replacement therapy

2. ACE inhibitors, Angiotensin II receptor blockers

3. Others (triggers for acute attacks)
   - Trauma
   - Pressure
   - Emotional stress
   - Menstruation
   - Ovulation
   - Infectious disease
• A striking clinical feature of HAE-III-FXII: predominance in women.

• Assumption: Estrogens play an important role in the regulation of the phenotypic expression of the disease.
Influence of oral contraceptives, pregnancies, and hormone replacement therapy on HAE-III-FXII in 35 women

<table>
<thead>
<tr>
<th></th>
<th>Intake of OC n [%]</th>
<th>Pregnancy n [%]</th>
<th>Receiving HRT n [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction of the first clinical symptoms of HAE-III-FXII</td>
<td>17 [63.0]</td>
<td>3 [12]</td>
<td>0 [0]</td>
</tr>
<tr>
<td>Exacerbation of the preexisting symptomatic HAE-III-FXII</td>
<td>8 [29.6]</td>
<td>7 [28]</td>
<td>3 [42.9]</td>
</tr>
<tr>
<td>No influence</td>
<td>2 [7.4]</td>
<td>10 [40]</td>
<td>4 [57.1]</td>
</tr>
<tr>
<td>Improvement of symptoms</td>
<td>0 [0]</td>
<td>5 [20]</td>
<td>0 [0]</td>
</tr>
<tr>
<td>Total</td>
<td>27 [100]</td>
<td>25 [100]</td>
<td>7 [100]</td>
</tr>
</tbody>
</table>
Further developments (IV)
Genetics 2
• A Turkish family: 2 of 6 family members: HAE type III

• Two sisters: Clinical symptoms of HAE type III

• The parents, a brother and a son of one of the 2 sisters: unaffected

• The two sisters and their father: a mutation in $F12$

Gel electrophoresis of the PCR products
Raw data of sequence

WT-Kontrolle:

G-30480:

G-30481:
genomische DNA im Bereich Exon 9+10 (72bp Deletion unterstrichen):

| ACCTCCGGCCCCAGGGCTCCGGCTCCGGCCGGCTCTAAACGGGCCCCGTTGGTGCTGA |
|----------------|----------------|----------------|
| CAGGAACCGGACAACGACATCCGGCCGGTGGTCTTGCTTGGTGCTGACCCTGAACCCG |
| CTGGGAGTACTGGACCTGGCACGTGCGAGACCCCACCCAGGCGCCGCTCCGACC |
| GGTGTCCTCTAGGGCTTTATGTCCGCACTCATGCCGCCGCTCCGAGCCGAGCCG |
| GGGCCAGGAGCCGAGCCGCCGGGGGCTAGATTCCGGCAGGCCGCCGGGCTCTC |
| CGTCCTCAGCCCTCGTCTCCACAGCCCTGCGCCGGAAGCGGGGACAGCCGCCTTCCT |
| GACCAAGGAGCAGCGCACATGAGCTGCAGGCGCACAGCGCTCCGCCAGATCCTGTTTCATGAC |
| CCGCTGCTGTTGGCGGCCGCTGGTGCCCCTACGCCGGGCGCACCCTACATGCCGCCGCTGTA |
| CTGGGCCCAGATTCTTCGCGCCGCCAGCCTCATTGCGCCCCTCTGCTGGTCTGAGCCG |
| TCACTGCCCTGCGAGGACCCGCGAGTAGATCCCGCCGAGGCCGCCGCCGGCTCTC |
Hereditary angioedema with normal C1 inhibitor (HAE type III)

- Not a missense mutation (1 base pair) like the mutations known until now

- A large deletion (72 base pairs)

- This large deletion comprises the same gene location as the missense mutations (prolin-rich region)
A. exon 9

320* 321* 322* 323* 324* 325* 326* 327* 328* 329* 330* 331* 332* 333*
P A P P P K P Q P T T T T T P P

B. exon 9  intron 9

320* 321* 322* 323* 324* del 72bp
P A P P P K
C C G G C A C C G C C G A g g a g c c g a g c c g g c c g g c g a g c t a g
Hereditary angioedema with normal C1 inhibitor (HAE type III)

This is the third mutation in $F12$ which is associated with HAE-FXII.
In 2012: Diagnosis of Hereditary Angioedema Type III

A. Recurrent angioedema in one or more organs
B. No hives/urticaria
C. More than one family member affected
D. Laboratory results: normal C1-INH and C4
E. Mutation in $F12$: HAE-III-FXII (1. subtype of HAE type III)
F. No mutation in $F12$: HAE-III-unknown (2. subtype of HAE type III)
Limitations in the diagnosis of HAE Type III (I)

I. Concerning “C. More than one family member affected“

This is not quite true: in a few women the HAE-III mutation „Lys“ was found although they were the only affected family members. So they were single cases of HAE-III-XII.
Limitations in the diagnosis of HAE Type III (II)

II. Single cases of HAE-III-unknown cannot be diagnosed at present, since the genetic alteration is still unknown.
Name of Angioedema

- Hereditary angioedema with normal C1 inhibitor
  - Subtype A: Hereditary angioedema with normal C1 inhibitor and factor XII gene mutations
  - Subtype B: Hereditary angioedema with normal C1 inhibitor and unknown cause
Clinical diagnosis

• Family history of AE
• Mostly women affected in subtype with FXII mutation
• Onset most common in the second and third decade of life
• Skin swellings, abdominal attacks, tongue swelling, laryngeal attacks
• Potential trigger factors: estrogens (oral contraceptives, hormonal replacement therapy, pregnancy, ACE-I)
Laboratory Diagnosis

- Normal C1-INH activity
- Normal C1-INH concentration
- Normal C4
- Genotyping for F12
Therapeutic Approach

- Avoidance of estrogen medication (oral contraceptives, hormonal replacement therapy) and ACE-I
- Consider for on-demand therapy (experience only in single cases until now)
  - C1-INH
  - Icatibant
  - ecallantide
- Consider for prophylaxis therapy (experience only in single cases until now)
  - Progestins
  - Tranexamic acid
  - Danazol in post-menopausal women