UAlg FMCB

UNIVERSIDADE DO ALGARVE

FACULDADE DE MEDICINA E CIÊNCIAS BIOMÉDICAS

# Hereditary Angioedema: Clinical features and enzyme polymorphisms in Hereditary angioedema with F12 mutation Spanish families; Management of Pregnancy and Delivery in Patients with Hereditary Angioedema with C1 Inhibitor Deficiency.

**TESE DE DOUTORAMENTO**

**MACARENA PIÑERO SAAVEDRA**

**FARO, 2022**

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Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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***A** mis padres, María y Rafael,*

***quienes sembraron la semilla,***
***a la Dra Teresa González-Quevedo,***
***quien regó y guío el tallo,***
***Y a la Dra Mª Antonia São Braz,***
***quien me acompañó a recolectar el fruto….***

***Del médico que he sido, soy y seré***

*"Last but not least, I wanna thank me*
*I wanna thank me for believing in me*
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*I wanna thank me for, for never quitting"*

*Snoop Dogg*

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# Abstract

Hereditary angioedema (HAE) is a rare disease encompassed within bradykinin-mediated angioedema. It is divided into two main groups: HAE with C1 inhibitor deficiency (HAE-C1INH), and HAE with normal C1 inhibitor. Within the latter, there are several subtypes based on genetic alterations. The most frequent form presents mutations in Factor XII (HAE-FXII). This work is divided into two sections. On the one hand, we have made a clinical and genetic description of a population of HAE-FXII patients and we have investigated the potential role of angiotensin-converting enzyme and aminopeptidase-P polymorphisms in the expression of the disease. Our findings indicate that certain HAE-FXII populations may not necessarily have different symptom patterns to patients with HAE-C1INH. Involvement of locations other than the skin is common and could even be the main expression of disease. The significant delay in HAE-FXII diagnosis suggests a possible lack of awareness in the medical community about this life-threatening disease. The enzyme polymorphisms studied were not a major determinant of disease expression in our population. On the other hand, we described the effect of pregnancy and deliveries on symptoms of HAE-C1INH. To this end, a nationwide cooperative study was carried out in Spain, involving 5 HAE reference centres. We can conclude that pregnancy appears to have a variable influence on the clinical expression of HAE-C1INH, and may differ from one patient to another and to a lesser extent, from one pregnancy to the next. Attacks tend to occur more frequently but they do not appear to increase in severity. pdhC1INH prophylaxis should be administered prior to caesarean delivery and is also highly recommended for vaginal delivery in patients with additional risks factors or severe HAE-C1INH symptoms during pregnancy or previous deliveries. pdhC1INH (2000 U) should always be available in the delivery room and during hospitalization.



**Keywords**: Hereditary angioedema (HAE), Factor XII of coagulation (FXII), C1 inhibitor (C1INH), angiotensin-converting enzyme (ACE), aminopeptidase P (APP), pregnancy

# Resumo

O angioedema se define como a aparição abrupta dum edema em áreas de derme profunda ou tecido subcutâneo, afetando a pele ou mucosas, de qualquer localização. É o resultado de um processo fisiopatológico subjacente de aumento local de permeabilidade capilar, envolvendo a libertação local ou sistémica de um ou vários mediadores vasoativos, principalmente histamina ou bradicinina. Em base a estes mediadores, o angioedema pode ser classificado como: angioedema induzido pela ativação mastocitária, angioedema induzido pela bradicinina, ou angioedema idiopático. O angioedema mediado por bradicinina, como o seu nome indica, está produzido por um aumento nos níveis de bradicinina, principal componente do sistema de contato o sistema calicreina-cinina. Não presenta um componente alérgico ou inflamatório associado. Este tipo de angioedema pode apresentasse em forma adquirida ou hereditária. Esta última será o motivo do nosso estudo.

O angioedema hereditário (HAE) é uma doença rara e infradiagnosticada. Caracteriza-se por episódios de angioedema da pele e membranas mucosas, abdómen, bem como episódios faríngeos e laríngeos que podem levar à morte por asfixia. He dividido em dois grupos: (1) HAE por déficit de C1 Inibidor (HAE-C1INH), onde podemos encontrar um déficit quantitativo ou qualitativo de C1 inibidor –principal regulador do sistema de contato-; (2) HAE com C1 Inibidor normal (HAE-nC1INH): este grupo engloba aqueles doentes diagnosticados com HAE, sem déficit de C1INH associado. Até a data, 9 mutações têm sido identificadas como responsáveis: 4 localizadas no gene *F12* (HAE-FXII), y uma respetivamente nos genes *PLG, ANGPT, KNG, MYOF* e *HS3ST6*. Em ambos casos, sendo uma doença rara, a prevalência é baixa. Os dados que temos hoje baseiam-se em séries de casos e revisões sistemáticas, uma vez que ainda não existem grandes estudos prospectivos ou registos internacionais que forneçam dados agregados. Por conseguinte, existem ainda lacunas no conhecimento sobre patofisiologia, aspectos clínicos, tratamentos e epidemiologia.

Este trabalho apresenta duas secções. A primeira secção está dedicada a descrição das características de uma população espanhola portadora de mutação *F12,* assim como o estudo do papel potencial na expressão da doença dos

polimorfismos da enzima convertidora de angiotensina (ECA) e da aminopeptidase P (APP), principais enzimas degradadoras de bradicinina. Para este fim, foi realizado um estudo descritivo, prospectivo, observacional na "Unidade de Referência em Angioedema" do Hospital Universitário Virgen del Rocío (Sevilha), entre janeiro de 2009 e abril de 2015. Foram incluídos um total de 57 indivíduos pertencentes a 9 famílias.

Todos os nossos pacientes tiveram a mutação p.Thr309Lys, até à data descrita como a mutação mais frequente encontrada no HAE-nC1INH. Uma vez que encontramos doentes sem historial familiar previamente conhecido e com mutações, recomendamos a procura de mutações *F12* em mulheres com ataques de angioedema sem urticária associada e com níveis normais de C1 inibidor. Desde um ponto de vista clínico, o nosso trabalho permitiu várias contribuições relevantes: é o primeiro estudo onde se observa uma ocorrência significativa de sintomas abdominais, geralmente associados a HAE-C1INH, que podem muitas vezes ser subestimados ou mal diagnosticados; encontramos também uma taxa mais elevada de sintomas faríngeos/laríngeos do que em relatórios anteriores. Isto demonstra que praticamente todos os doentes com HAE-FXII podem estar em risco de ter um ataque com risco de vida; descrevemos pela primeira vez na literatura a existência de sintomas prodrômicos; outros triggers que não fossem estados hiperestrogénicos, como stress e traumatismos, foram descritos. Não existem tratamento aprovados para HAE-FXII, pelo que o uso foi off-label. Na ausência de ensaios clínicos específicos, a notificação da utilização destes medicamentos off-label é a única ferramenta que nos permite hoje orientar a base do tratamento nestes doentes. O tratamento a curto prazo com concentrado de C1 inibidor e Icatibant assim como profilaxia a longo prazo com ácido tranexâmico foram úteis.

Ainda não foi encontrada nenhuma causa para explicar a grande variedade de expressão clínica entre os indivíduos diagnosticados com HAE, tanto na forma clássica, HAE-C1INH, como HAE-nC1INH. As conjecturas centram-se numa produção exagerada de bradicinina ou numa diminuição da sua metabolização. Portanto, a hipótese de que pode haver outras alterações genéticas que possam influenciar esta expressão clínica foi posta em cima da mesa várias vezes. Neste contexto, postulou-se que a co-presentação de polimorfismos associados a uma diminuição da atividade enzimática da ECA e da APP, as principais enzimas

degradantes da bradicinina, poderia influenciar a gravidade desta doença. A combinação do alelo I e do alelo A, relacionados com uma menor função enzimática, foi detectada em 17% dos pacientes. Os polimorfismos analisados não foram um determinante importante da expressão da doença na nossa população.

O objetivo da segunda secção deste projeto era descrever o efeito da gravidez e do parto nos sintomas do HAE-C1INH e rever a necessidade e segurança dos tratamentos disponíveis durante o período do estudo. Realizámos um estudo retrospectivo de doentes com HAE-C1INH que engravidaram antes ou depois do diagnóstico de HAE-C1INH. Um total de 61 pacientes foram recrutados em 5 hospitais de referência em HAE em Espanha. Foi avaliado um total de 125 gravidezes a termo, 14 abortos e 4 interrupções voluntárias da gravidez. A experiência reportada anteriormente limita-se a casos e 3 séries, sendo o nosso trabalho o maior publicado até à data. Este trabalho evidencia que a gravidez tem uma influência variável na expressão clínica do HAE. Como resultados a salientar, não foram observadas diferenças entre as doentes que tinham um diagnóstico anterior e as que não o tinham. Na grande maioria das doentes com mais de uma gravidez, não se verificaram grandes variações entre as gravidezes. Ao contrário de publicações anteriores, não foram observadas diferenças entre trimestres na nossa série. Os ataques ocorrem com mais frequência, mas não aumentam em gravidade. Embora o parto vaginal e mesmo as cesarianas sejam geralmente bem toleradas, a profilaxia pdhC1INH deve ser administrada antes do parto por cesariana e é também recomendada antes do parto vaginal se houver fatores de risco adicionais. Não se observaram abortos espontâneos relacionados com a doença de base. Na ausência de ensaios clínicos, a utilização de pdhC1INH como profilaxia de longa duração em grávidas demonstrou ser segura.

Em conclusão, a nossa investigação tem contribuído ao conhecimento desta doença rara, desde o ponto de vista epidemiológico, clínico, de tratamento y genético.

**Palavras-chave**: angioedema hereditário (HAE), Fator XII (FXII), C1 inibidor (C1INH), encima convertidora de angiotensina (ACE), aminopeptidase P (APP), gravidez.

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# List of Abbreviations, Acronyms and Symbols

| | |
|---|---|
| ºC | degre célsius |
| µl | microliter |
| µM | micro molar |
| ng | nanogram |
| bp | base pair |
| 3’ | 3 prime |
| 5’ | 5 prime |
| F | forward |
| R | reverse |

| | |
|---|---|
| AAs | attenuated androgens |
| AAE | acquired angioedema |
| AAE-ARB | acquired angioedema due to angiotensin II receptor blocker |
| AAE-C1INH | acquired angioedema due to C1 inhibitor deficiency |
| AAE-ACEI | acquired angioedema due to angiotensin converting enzyme inhibitor |
| AAE- InH | idiopathic non histaminergic angioedema |
| AAE-RAASI | acquired angioedema due to use of renin-angiotensin-aldosterone system inhibitors |
| ACE | angiotensin converting enzyme |
| ACEI | angiotensin converting enzyme inhibitor |
| AE | angioedema |
| AE-MC | mast cell mediated – induced angioedema |
| AE-BK | bradykinin induced angioedema |
| ANGPT | angiopoietin 1 |
| APP | amonipeptidase P |
| ARB | angiotensin II receptor blocker |
| BK | bradykinin |
| BK1R | bradykinin type 1 receptor |
| BK2R | bradykinin type 2 receptor |

| | |
|---|---|
| C1INH | C1 inhibitor esterase |
| C1q | complement component C1q |
| C4 | complement component 4 |
| CK-1 | cytokeratin-1 receptor |
| Cl2Mg | magnesium chloride |
| COX-1 | cyclooxygenase 1 |
| CPN | carboxipeptidase N |
| ddH2O | double-distilled water |
| dNTP | deoxynucleotide triphosphate |
| DPPIV | dipeptidyl peptidase IV |
| DPPIVI | dipeptidyl peptidase IV inhibitor |
| EDTA | ethylenediamine tetraacetic acid |
| FXI | factor XI of coagulation |
| FXII | factor XII of coagulation |
| FXIIa | activated factor XII of coagulation |
| gC1qR | receptor for the globular heads of complement component C1q |
| HAE | hereditary angioedema |
| HAE- ANGPT | hereditary angioedema with normal C1 inhibitor due to mutations in *angiopoetin-1* gene. |
| HAE-C1INH | hereditary angioedema due to C1 inhibitor deficiency |
| HAE-C1INH-1 | hereditary angioedema due to C1 inhibitor deficiency type 1 |
| HAE-C1INH-2 | hereditary angioedema due to C1 inhibitor deficiency type 2 |
| HAE-FXII | hereditary angioedema with normal C1 inhibitor due to mutations in *F12* gene. |
| HAE-HS3ST6 | hereditary angioedema with normal C1 inhibitor due to mutations in heparan sulfate glucosamine 3-O-sulfotransferase |
| HAE-KNG | hereditary angioedema with normal C1 inhibitor due to mutations in *kininogen 1* gene. |
| HAE-MYOF | hereditary angioedema with normal C1 inhibitor due to mutations in *MYOF* gene |

| | |
|---|---|
| HAE-nC1INH | hereditary angioedema with normal C1 inhibitor |
| HAE-PLG | hereditary angioedema with normal C1 inhibitor due to mutations in *plasminogen* gene. |
| HAE-UNK | hereditary angioedema due to unknown cause |
| HK | high-molecular-weight kininogen |
| HS | heparin sulfate |
| HS3ST | heparan sulfate glucosamine 3-O-sulfotransferase Sulfotransferase |
| HSP90 | heat shock protein 90 |
| Ig | immunoglobulin |
| KNG | kininogen 1 |
| KKS | kallikrein-kinin system |
| LK | low-molecular-weight kininogen |
| LT | leukotrienes |
| LTP | long-term prophylaxis |
| Lys-BK | Kallidin |
| MBL | mannose-binding lectin |
| MYOF | myoferlin |
| NEP | neutral endopeptidase |
| NSAIDs | non-steroidal anti-inflammatories |
| PCR | polymerase chain reaction |
| pdhC1INH | Plasma-derived human C1 esterase inhibitor concentrate |
| PG | prostaglandins |
| PK | prekallikrein |
| PKa | kallikrein |
| PLG | plasminogen |
| PRCP | prolylcarboxypeptidase |
| RAAS | renin-angiotensin-aldosterone system |
| RAASI | renin-angiotensin-aldosterone system inhibitor |
| RCL | reactive centre loop |
| STP | short-term prophylaxis |
| TIE2 | angiopoetin receptor |
| TK | tissue kallikrein |
| tPA | tissue plasminogen activator |

| | |
|---|---|
| TXA | thromboxanes |
| u-PAR | urokinase plasminogen activator receptor |
| VEFG | vascular endothelial growth factor |
| VEGFR | vascular endothelial growth factor receptor |
| XPNPEP2 | X-Prolyl Aminopeptidase 2 gene |

# Chapter 1

# General Introduction

## 1.1 Concept of Angioedema

Angioedema (AE) is defined as an abrupt, transient swelling of defined areas of deep epidermis or subcutaneous tissue (skin, mucous membranes, or both) (Greaves & Lawlor, 1991). The swelling is nonpitting, erythematous or skin-colored, variable duration and location. Local pain or warmth may be associated symptoms, but is infrequently associated with pruritus (Kaplan & Greaves, 2005; Grigoriadou & Longhurst, 2009; Bork, 2014).

AE is the result of an underlying pathophysiologic process of locally increased capillary permeability, involving the local or systemic release of one or several vasoactive mediators, primarily histamine or bradykinin (BK) (Maurer & Magerl, 2021). Although the biochemical cascades produced by BK and histamine are different, both result in rather similar clinical signs and symptoms.

## 1.2 Classification of Angioedema

AE can be classified in multiple ways, as there is no clear consensus. One of the classic ways of classifying AE is on the basis of its relation to the responsible vasoactive mediator, as well as the mechanisms or defects by which it is produced. Depending on its physiopathology, AE can be divided into three main groups (table 1) (Cicardi et al, 2014; Kaplan & Greaves, 2005):

(a) Mast Cell mediated –induced AE (AE-MC) (classically called histaminergic AE or allergic AE) – mediated by substances released in the degranulation of mast cells and/or basophils-.

(b) Bradykinin - induced AE (AE-BK) - mediated by elevation of BK-.

(c) Idiopathic angioedema: there is a third group of as yet unknown mechanism.

**Table 1.1. Classification of angioedema**

<table>
<tr><td rowspan="15">AE</td><td rowspan="6">Mast cell mediated – induced AE</td><td>Immunological factors</td><td>IgE mediated</td><td>Food, drugs, insects</td></tr>
<tr><td></td><td>Auto-immune</td><td>Auto-immune urticaria</td></tr>
<tr><td rowspan="4">Non immunological factors</td><td>LT/PG imbalance</td><td>NSAIDs-induced or NSAIDs-exacerbated</td></tr>
<tr><td>Other drugs</td><td>Neuromuscular blocking agents, iodine contrast media, opioids….</td></tr>
<tr><td colspan="2">Physical, psychological and environmental factors</td></tr>
<tr><td colspan="2">Histamine releasing food</td></tr>
<tr><td colspan="3">idiopathic (good response to antihistamines, corticosteroids or adrenaline)</td></tr>
<tr><td rowspan="13">Bradykinin induced AE</td><td rowspan="4">Acquired</td><td>C1INH deficiency</td><td>Lymphoproliferative disorder</td></tr>
<tr><td></td><td>Auto-immune disorder</td></tr>
<tr><td>RAASI drugs</td><td>ACEI, DPPIVI</td></tr>
<tr><td colspan="2">Idiopathic (no response to antihistamines, corticosteroids or adrenaline)</td></tr>
<tr><td rowspan="9">Hereditary</td><td rowspan="2">C1INH deficiency</td><td>Type I (quantitative)</td></tr>
<tr><td>Type II (qualitative)</td></tr>
<tr><td rowspan="7">With C1INH normal</td><td>Mutation FXII</td></tr>
<tr><td>Mutation PLG</td></tr>
<tr><td>Mutation KNG1</td></tr>
<tr><td>Mutation ANGPT</td></tr>
<tr><td>Mutation MYOF</td></tr>
<tr><td>Mutation HS3ST6</td></tr>
<tr><td>Unknown</td></tr>
<tr><td colspan="3">Idiopathic</td></tr>
</table>

AE: angioedema; Ig: immunoglobulin; NSAIDs: non-steroidal anti-inflammatories; LT: leukotrienes; PG: prostaglandins; RAASI: renin-angiotensin-aldosterone system inhibitor; C1INH: C1 inhibitor esterase; ACEI: angiotensin converting enzyme inhibitor; DPPIVI: dipeptidyl peptidase IV inhibitor; FXII: factor XII; PLG: plasminogen; ANGPT: angiopoietin 1; KNG1: kininogen 1; MYOF: myoferlin; HS3ST6: Heparan sulfate glucosamine 3-O-sulfotransferase. (Modified from Giavina-Bianchi et al., 2017 & Cicardi et al., 2014).

### 1.2.1 Mast Cell mediated – induced angioedema

AE-MC is the most common form of AE (Zingale et al., 2006), and presents the same physiopathology mechanism as chronic urticaria (Kaplan & Graves, 2009; Zuberbier et al., 2022). It is caused by the release of vasoactive substances by mast cells and/or basophils: **histamine** - as the main molecule implicated -, prostaglandins, leukotrienes, cytokines and chemokines (Kaplan & Greaves, 2009; Castells, 2006). This liberation can be induced by immunological factors (Ig-E mediated hypersensitivity reactions or auto-immune disorders) or non-immunological factors –pseudoallergic AE- (drugs, histamine releasing food, physical, psychological or environmental factors) (Kaplan, 2008). AE-MC is characterized by a short duration (<24h), usually is accompanied by urticaria, and typically presents a good response to treatment with antihistamines, corticosteroids and/or adrenaline (Zuberbier et al., 2022).

Pseudoallergic AE is the result of an inhibition of cyclooxygenase 1 (COX-1), enzyme that metabolizes arachidonic acid to prostaglandins (PG), thromboxanes (TXA) and prostacyclin. Administration of drugs that inhibit COX-1 results in increased formation of proinflammatory cysteinyl leukotrienes (LT), leading to AE in some individuals (Kaplan, 2008). Immunological mechanism is not usually involved. AE appears, usually accompanied of urticaria, 1-6h after the drug ingestion. Some patients can present respiratory symptoms associated, and usually reverts spontaneously before 24h (Kowalsky et al., 2011).

### 1.2.2 Bradykinin - induced angioedema

As its name implies, AE-BK is produced by increased **BK** levels, major component of kallikrein-kinin system (KKS). Unlike AE-MC, AE-BK angioedema doesn’t have any allergic or inflammatory component associated (Bas et al., 2007; Zuraw et al., 2013). AE-BK can occur either on an acquired (AAE) or hereditary (HAE) form. Both forms share some clinical features and treatment options.

*1.2.2.1 Acquired forms of bradykinin - induced angioedema*

The acquired form is divided in 3 groups:

- Acquired angioedema with C1 inhibitor deficiency (AAE-C1INH) (Cugno et al., 2008; Gobert et al., 2020; Shi & Wang, 2021): first described by Caldwell in 1972, it is characterized by low levels of C1 inhibitor esterase (C1INH) due to hyper-activation on complement system. This low levels are the result of a hyper-consumption of C1INH or its auto-antibody – mediated inactivation. It is usually associated with lymphoproliferative disorders or autoimmune diseases.

- Angioedema due to renin-angiotensin-aldosterone inhibitor (AAE-RAASI): AE is a well-known side effect of angiotensin enzyme converter inhibitors (ACEI) (AAE-ACEI), affecting 0'1-0'7% of patients (Stone & Brown, 2017, Montinaro & Cicardi, 2020). It has been also described cases of angioedema due to gliptins - dipeptidyl peptidase IV inhibitor (DPPIVI) - (Cassano et al., 2021), angiotensin II receptor blocker (ARB) (AAE-ARB) (Abdi et al., 2002) and renin inhibitor Aliskiren (Toh et al., 2012).

- Idiopathic non histaminergic angioedema (AAE-InH): this group includes all non-hereditary forms after exclusion of mast cell -induced origin (no response to antihistamines, corticosteroids or adrenaline), intake of drugs or C1INH deficiency.

*1.2.2.2 Hereditary forms of bradykinin-induced angioedema*

It is possible to identify different forms of HAE (Agostini et al., 2004; Maurer et al., 2022):

- Hereditary angioedema with C1 inhibitor deficiency (HAE-C1INH): this was the first described form of HAE. HAE type 1 (HAE-C1INH-1), characterized by a quantitative deficit of C1INH, and HAE type 2 (HAE-C1INH-2), characterized by a qualitative deficit of C1INH.

- Hereditary angioedema with normal C1 inhibitor (HAE-nC1INH): is subclassified in function of the genetic alteration founded:

1.- Mutations in *F12* gene (HAE-FXII)

2.- Mutations in *angiopoetin-1* (*ANGPT)* gene (HAE- ANGPT)

3.- Mutations in *Plasminogen (PLG)* gene (HAE-PLG)

4.- Mutations in *Kininogen1 (KNG)* gene (HAE- KNG1)

5.- Mutations in *Myoferlin (MYOF)* gene (HAE- MYOF)

6.- Mutations in *Heparan sulfate glucosamine 3-O-sulfotransferase (HS3ST6)* gene (HAE-HS3ST6)

- Unknown hereditary angioedema (HAE-UNK) embrace those hereditary forms which genetic disorder remains unseen.

## 1.3 Physiopathology of Bradykinin- Induced Angioedema

Diseases encompassed by AE-BK have as a common denominator a predisposition to self-limiting episodes of increased vascular permeability secondary to high BK levels (Davis, 2005; Busse & Buckland, 2013; Kaplan, 2014; Walford & Zuraw, 2014; de Maat et al., 2021).

BK (a nanopeptide released from kininogens by kallikreins) is part of the KKS or contact system, formed by a family of vasoactive peptides, which are involved in several functions in the organism (Long et al, 2016). BK is one of the main molecules in charge of vascular smooth muscle regulation, vascular dilation and tissues permeability. In addition, BK shows an important antihypertensive, antithrombogenic, antiproliferative and antifibrinogenic effect (Maurer et al., 2011). C1INH, component of complement system, is the main protease inhibitor of KKS, and therefore of permeability.

Early studies described both the activation of the complement system and the KKS, so both systems were initially postulated as candidates to mediate non-histaminergic angioedema (Laurell et al, 1976; Berrettini et al., 1986; Bühler et al., 1995; Cugno et al., 1996; Nielsen et al., 1996). Initial investigations with plasma derived from HAE-C1INH patients showed no evidence of kinin formation as a consequence of an activation of the classical complement cascade (Fields et al., 1983). However, over the years, several studies had strongly supported the involvement of KKS and BK, as its main mediator, in increasing vascular permeability: (1) in 1969, Juhlin and Michaëlsson demonstrated that when plasma kallikrein (PKa) was administered intradermally to patients with HAE, there was an increased permeability response. In addition, elevated PKa levels were found in induced blisters produced in the skin of these patients (Curd et al., 1980); (2) in the same year, Talamo et al observed elevated BK levels during attack in HAE patients. However, it was not until 1983 that Fields et al postulated that BK was responsible for the increased permeability, based on the destruction of kinins in plasma of HAE patients mediated by the enzyme carboxypeptidase B, but not by trypsin. This was interpreted as a consistent explanation of the BK formation in such patients; (3) during angioedema acute attacks in patients with HAE, decreased levels of both Prekallikrein (PK) and High-molecular-weight kininogen (HK) were observed, and HK was widely cleaved, (Schapira et al.,1983; Cugno et al., 1996); (4) in 1994, Shoemaker et al. demonstrated that BK could be generated in plasma from HAE patients in vitro. Two later, elevated BK levels were found in HAE patients during acute episodes of angioedema but not during remission periods of the disease (Cugno et al., 1996; Nussberger et al., 1998). Lately, the same study group demonstrated, in these patients, that there was a clear elevation in plasma BK levels in the swollen forearm compared with the unaffected arm (Nussberger et al., 1999); (5) the involvement of BK was also support by the discovery of some mutations in the gene encoding C1INH, which lead to significant loss of activity in its inhibitory activity to C1r and C1s, but maintained normal inhibitory activity on kallikrein (PKa) and Factor XII of coagulation (FXII), with no evidence of angioedema in the carriers (Zahedi et al., 1995, 1997); (6) more recently, in an animal model, it was shown that mice modified to be C1INH deficient had a significant increase in vascular permeability compared to unmodified (wild-type) mice, which was reversible with intravenous administrations of human C1INH. This study also

supported the discovery that the activity of BK occurred through its interaction at the BK type 2 receptor (BK2R) (Han et al., 2002).

These studies laid the groundwork for the current acceptance of BK as the main mediator responsible for this type of angioedema (Kaplan and Joseph, 2016, 2017; Marceau et al., 2020; de Maat et al., 2021).

### 1.3.1 Physiological bradykinin - forming cascade and inactivation of bradykinin

#### 1.3.1.1 *Bradykinin - forming cascade*

BK is formed from two kininogens, circulating proteins which contain the BK sequence (Lee-lundberg et al., 2005): HK and Low-molecular-weight kininogen (LK). Both molecules are formed by a heavy and a light chain with the BK sequence in between, distributed in several domains (figure 1.1) (Colman & Schmaier, 1997): domains D1 to D3 compound the heavy chain and D4 domain contains the 21 amino acids long BK sequence. Light chain is composed by domains D5 and D6 in HK and D5 in LK. In HK, D6 binds to PK and FXI while D3, D4 and D5 bind to the receptor complex on endothelial cells.

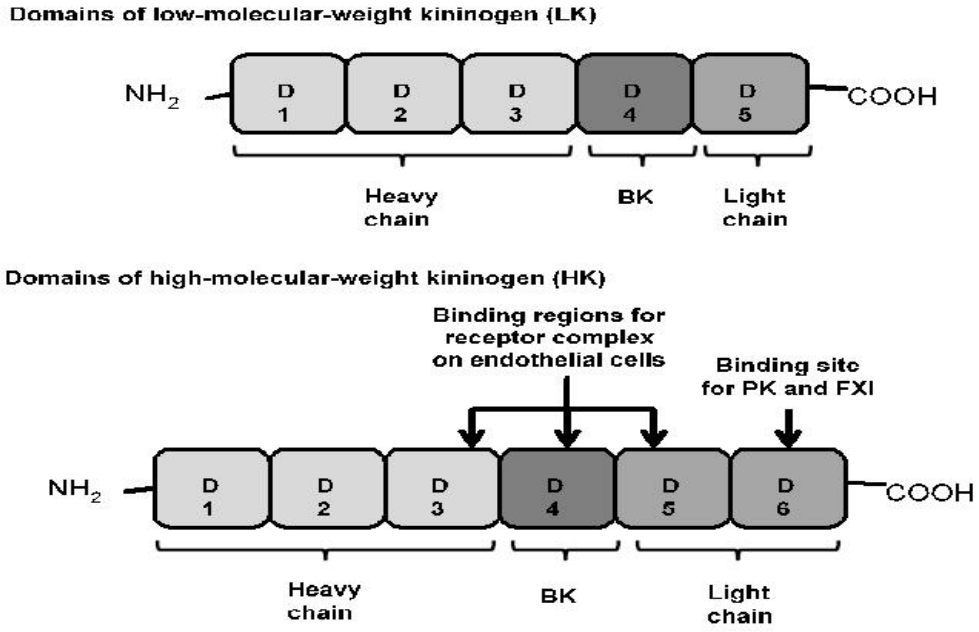


**Figure 1.1. High-molecular-weight kininogen and Low-molecular-weight kininogen structures.** D: domain; BK: bradykinin.

There are two general pathways by which BK is generated:

a) Intravascular formation: BK is formed from several complex interactions between the KKS and the endothelial cell surface. KKS is composed by one substrate, HK, and two zymogens, PK and FXII (Kaplan & Ghebrehiwet, 2010). Cleavage of HK will result in the release of BK (Muller-Esterl et al., 1982). Both zymogens require contact with negative charged surfaces for their enzymatic activity on HK (Colman & Schmaier, 1997). In plasma, HK is found forming a circulating complex together with 75-80% of circulating PK (Mandle et al., 1976; Scott & Colman, 1980), and with 95% of Factor XI of coagulation (FXI) (Thompson et al., 1979). Both, FXII and HK, bind to the endothelial surface and both compete for the zinc-dependent binding of a multiprotein-receptor-complex (Schmaier et al., 1988; Van Iwaarden et al., 1988; Reddigari et al. 1993a, 1993b). This multiprotein-receptor-complex is formed by the receptor for the globular heads of complement component C1q (gC1qR) (Joseph et al., 1996), urokinase plasminogen activator receptor (u-PAR) (Colman et al., 1997) and cytokeratin-1 receptor (CK-1) (Hasan et al., 1998). They can be found as bimolecular complex: gC1qR/CK-1 and u-PAR/CK-1, as well as without forming complex -gC1qR- (Joseph et al., 2004). Activated FXII (FXIIa) binds mainly to u-PAR/ CK-1 (Mahdi et al., 2002), while HK binds to gC1qR (Herwald et al. 1996) and gC1qR/CK-1 (Joseph et al., 1999; Shariat-Madar et al., 1999). In 2020, Kaira et al. have shown than gC1qR can simultaneously bind HK and FXII.

Contact activation can be FXII-dependent or FXII-independent:

- *FXII-dependent formation of BK* (figure 1.2): FXII is highly susceptible to auto-activation through a conformational change (Silverberg et al., 1980a; Tankersley and Finlayson, 1984; Citarella et al., 1997; Kaplan and Joseph, 2016). Several triggers on endothelial cell surface (damage, inflammation, negative surface charges, macromolecules, gC1qR, other proteins) produce this FXIIa. This auto-activation constitutes the key initiator of KKS cascade. The first step of the cascade is constituted by a slow auto-activation of FXII, until it is enough FXIIa to cleavage PK into PKa and FXI into activated FXI. New formed PKa in turn acts as a positive feedback that results in rapid transformation of FXII in FXIIa (Cochrane et al., 1973; Meier et al., 1997; Silverberg et al., 1980b). so most of the FXIIa

produced is due to activation by the PKa. The strong positive feedback between FXIIa and PKa allows full activation of KKS within minutes (de Maat et al., 2013). FXIIa presents two conformations (Kaplan and Austen, 1970, 1971; Revak et al., 1978): (1) αFXIIa, which has the ability to remain bound to the cellular surface and activate PK and FXI; and (2) βFXIIa –initially described as FXIIf- which is a fraction released into the plasma circulation, with the ability of activate PK in a fluid phase (it cannot bind to FXI or the vascular surface). BK production depends on local activation of KKS, systemic activation is not mandatory (Nussberger et al., 1999). However, βFXIIa allows BK production to continue in a fluid phase until the enzyme is inactivated, and therefore reactions could occur at distant sites on the initial vascular surface (Kaplan and Ghebrehiwet, 2010). Following interaction between PK-HK complex and endothelial cell surface binding sites, PKa is cleaved from the complex by the prolylcarboxypeptidase (PRCP) of the endothelial surface. PKa cleaves HK to release BK (Reddigari and Kaplan, 1989; Nishikawa et al., 1992).

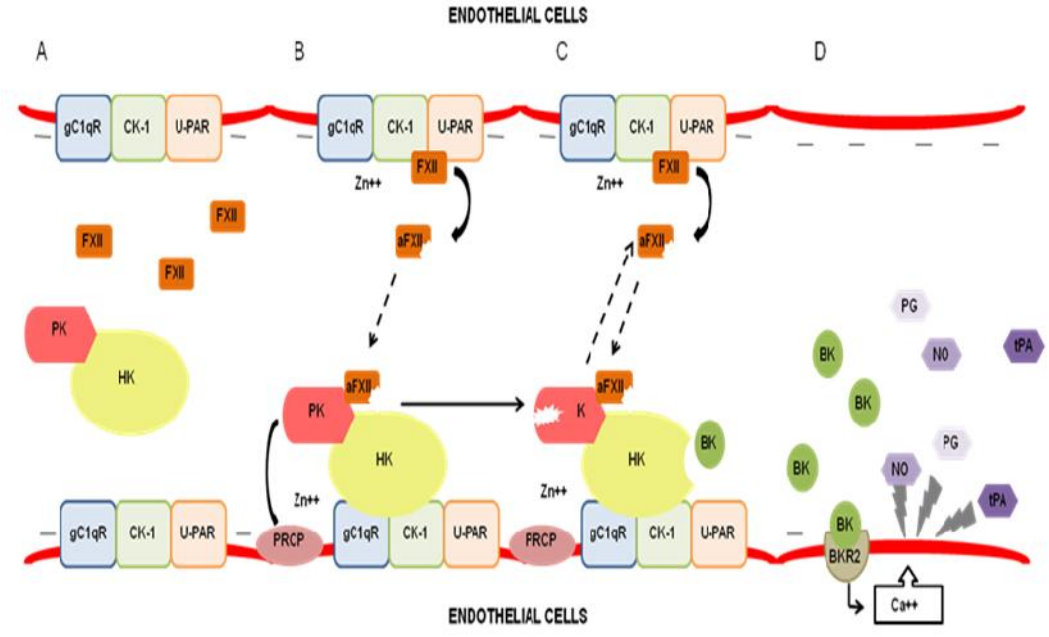


**Figure 1.2 FXII-dependent formation of bradykinin.** BK: bradykinin; BKR2: bradykinin-receptor 2; CK-1: cytokeratin-1 receptor; FXII: factor XII of coagulation; gC1qR: receptor for the globular heads of complement component C1q; HK: high-molecular-weight kininogen; K: kallikrein; NO: nitric oxide; PG: prostaglandins; PK: prekallikrein; PRCP: Prolylcarboxypeptidase**;** u-PAR: urokinase plasminogen activator receptor; tPA: tissue plasminogen activator.

There are several ways of feedback that allow an exponential activation of KKS (Figure 1.3).

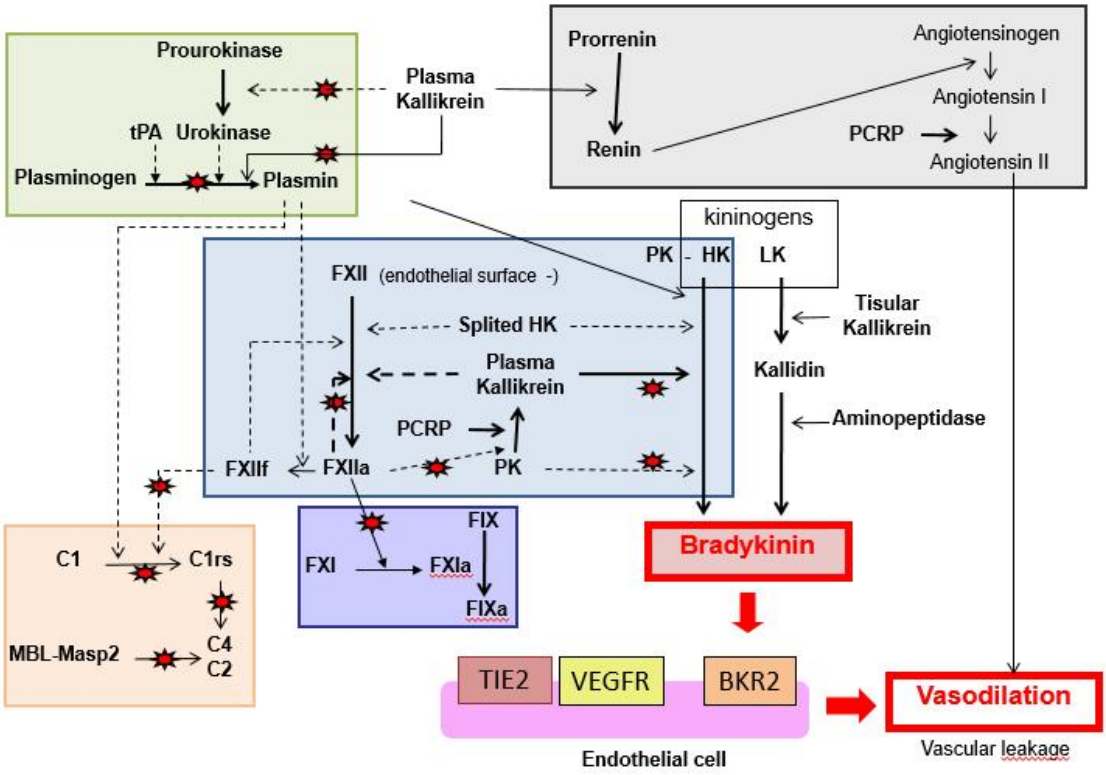


**Figure 1.3 Kallikrein-kinin system and its interactions with other systems.** Contact system (blue box), fibrinolytic system (green box), complement system (orange box), coagulation system (purple box) and renin-angiotensin-aldosterone system (grey box). tPA: tissue plasminogen activator; UK: urokinase; FXII(a): factor XII of coagulation (activated); FXI(a): factor XI of coagulation (activated); PK-HK: prekallikrein - high-molecular-weight kininogen complex; LK: low-molecular-weight kininogen; MBL: mannose-binding lectin. PCRP:prolylcarboxypeptidase; BKR2: bradykinin receptor 2; TIE2: tyrosine-protein kinasa; VEGFR: vascular endothelial growth factor. ✸ C1 inhibitor esterase inhibition. Discontinuous line: activator. Continuous lines: enzymatic activity.

- *FXII-independent forming of BK* (figure 1.4): formation is minimal and much slower. Binding of PK-HK complex to endothelial cells can activate the cascade in the absence of FXII (Rojkjaer et al., 1998; Joseph et al., 2001a, 2001b) On one side, PK exhibits a small enzymatic activity independent of PKa associated avtivity. When PK binds with HK, this zymogene acquires enzymatic capacity and, without the need to convert to PKa, is able to cleave HK releasing BK (Joshep et al., 2009). This reaction is FXII-independent and is strictly controlled by C1INH. On the other hand, two endothelial cell-derived factors with cascade-initiating properties have been described: heat shock protein 90 (HSP90) (Joseph et al., 2002)

and prolylcarboxypeptidase (Shariat-Madar et al., 2002). Both have the ability to activate PK when forming the PK-HK complex, but neither is a direct PK activator (Kaplan & Ghebrehiwet, 2010).

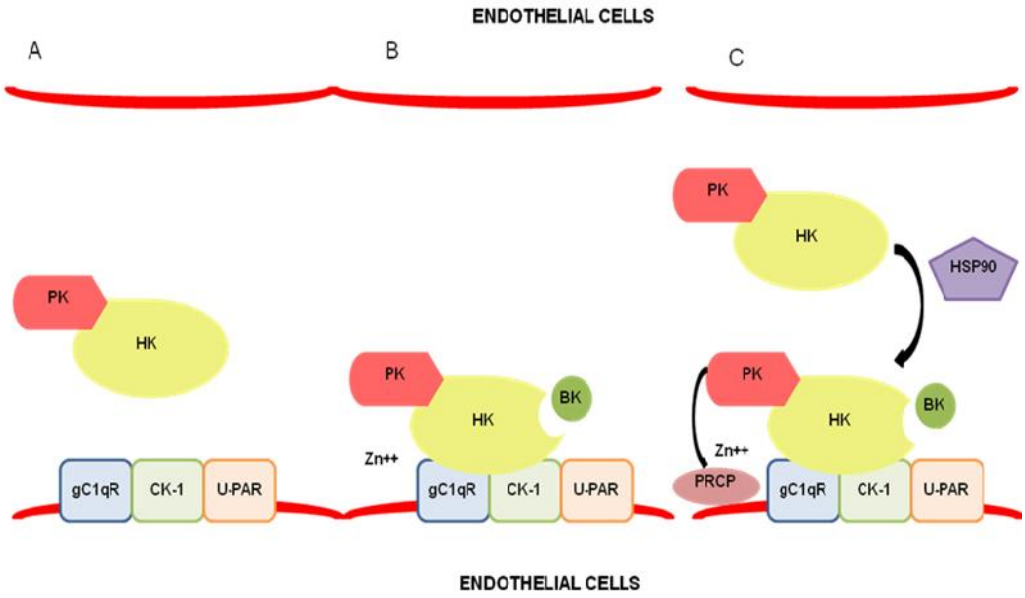


**Figure 1.4 FXII-independent forming of bradykinin.** BK: bradykinin; CK-1: cytokeratin-1 receptor; gC1qR: receptor for the globular heads of complement component C1q; HK: high-molecular-weight kininogen; PK: prekallikrein; PRCP: Prolylcarboxypeptidase; HSP90: heat shock protein 90.

b) Intracellular starting formation (figure 1.5): this pathway of formation is not yet well understood. Tissue kallikrein (TK) is formed from PK inside the cell and secreted into the intercellular space. There, LK is split by TK producing Kallidin or Lys-BK, which in turn is converted to BK by a zinc-dependent enzyme called aminopeptidase (Margolius, 1998). TK could probably produce a local increase in circulating kinins around it, but the principal kinin-formation activity comes from PKa (Ghannam et al., 2013).

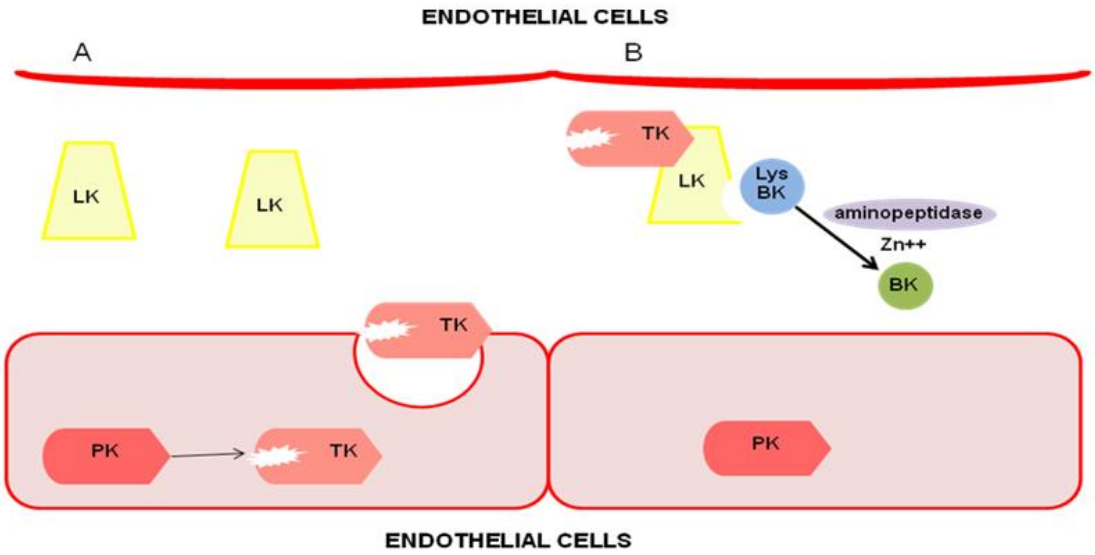


**Figure 1.5 Intracellular starting formation of bradykinin.** BK: bradykinin; LK: low-molecular-weight kininogen; Lys-BK: kallidin, PK: prekallikrein; TK: tissue kallikrein;

Although endothelial cells can be activated through binding to FXII and PK-HK complex, they are primarily activated by the binding of BK to their surface receptors (see 1.3.1.2 Bradykinin receptors).

In humans, the various regulatory activations cascades, including KKS, interact with each other and have a reciprocal influence. To summarise, figure 1.3 shows the relationship between KKS (blue box) and various activations pathways such fibrinolysis cascade (green box), complement system (orange box), coagulation system (purple box) and renin-angiotensine-aldosterone system (RAAS) (grey box).

### 1.3.1.2 *Bradykinin receptors*

The biological effects of BK on endothelium and smooth muscular cells are mediated by two 7-transmembrane domain-containing G-protein-coupled receptors called B1 receptor (BK1R) and B2 receptor (BK2R) (Leeb-Lundberg et al., 2005; Joseph & Kaplan, 2005; Bossi & Tedesco, 2013; de Maat et al., 2018):

- BK2R is ubiquitous and constitutively expressed on the endothelia and healthy tissues. It presents a high affinity for BK and Lys-BK. Upon stimulation of BK2R, the action of BK is amplified by the secondary release of vasodilators such as tissue plasminogen activator (tPA), nitric oxide, PG and TXA, which exacerbate the increase in permeability. The involvement of BK2R in the pathogenesis of HAE is supported by the study in *knockout* mice for C1INH and by the improvement of symptoms when patients are treated with a selective antagonist of BK2R, Icatibant (Cicardi et al., 2010b).

- BK1R is inducible and synthesized *de novo* in endothelial cells in response to inflammatory processes or tissues injury. BK1R shows affinity to des-Arg9-BK and Lys-des-Arg9-BK, the main metabolites of BK and Lys-BK respectively. Complete resolution of the AE episode with the use of Icatibant is not immediate, but requires several hours, suggesting that BK1R may be involved in the prolongation of AE attack. In 2009, Bossi et al demonstrated, *in vitro* and *in vivo* models, that use of BK1R or BK2R antagonist separately partially inhibit the increased permeabilization activity in serum from HAE patients during an acute attack, but is completely blocked when both antagonists are used. This blockade was also observed when gC1q receptor was inhibited. So it was demonstrated that both BK1R and gC1q receptor play a role in vascular leakage in HAE.

#### 1.3.1.3 *Bradykinin metabolism and metallopeptidases*

Under normal conditions, after formation, BK is rapidly metabolized by the action of several spontaneously active metallopeptidases (Cyr et al., 2001; Kaplan & Joseph, 2014) (figure 1.6): Angiotensina-converting enzyme (ACE), aminopeptidase P (APP), carboxipeptidase N (CPN), dipeptidyl peptidase IV (DPPIV), and neutral endopeptidase (NEP). ACE and APP represent the primary and secondary BK degradation pathways respectively, which much higher activity than NEP or DPPIV (Fryer et al., 2008). ACE, APP, DPPIV and NEP produce inactive metabolites, while CPN metabolize BK into des-Arginine9-Bradykinin (des-Arg9-BK) (Sheikh & Kaplan, 1986), an active BK1R agonist metabolite (Regoli & Barabe, 1980; Marceau & Bachvarov, 1998). Des-Arg9-BK is also

degraded by ACE and APP but, compared to BK, with a reversed degradation relevance.

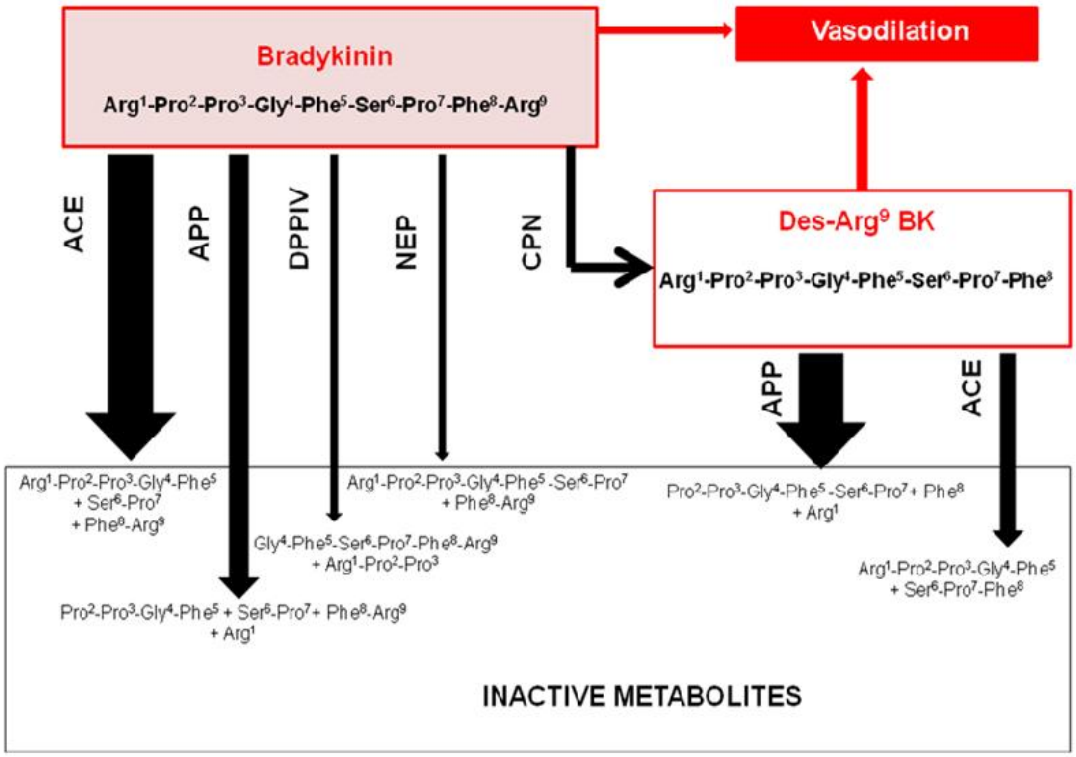


**Figure 1.6 Metabolism of bradykinin.** ACE: angiotensin converting enzyme; APP: aminopeptidase P; CPN: carboxypeptidase N; DPPIV: dipeptidyl peptidase IV; NEP: neutral endopeptidase.

### 1.3.1.4 *Inhibition of kallikrein - kinin system*

The main regulator of KKS is C1INH (Davis et al., 2004; Kaplan & Joseph, 2014). C1-INH belongs to serpin family (Serin Protease Inhibitor). It presents similar structure and functions to other members of this family (Bos et al., 2022). *In vivo*, its synthesis is basically produced in the hepatocytes (Johnson et al., 1971). The main biological activity of C1INH focus on the regulation of vascular permeability (through its role in maintaining vascular permeability) (Davis et al., 2008). C1INH also presents an anti-inflammatory effect, but appears to be related with other properties independent of its inhibitory power.

C1INH works as a suicide substrate (Patston et al., 1991; Huntington et al.,2000). Protease inactivation is based in a trapping mechanism where the protease recognizes the pseudosubstrate reactive center loop (RCL) of the serpin displayed above the surface of the molecule. Each molecule of C1INH forms binds irreversibly to the target proteases. This bind produces the cleavage of the peptide bound in RCL (P1-P1´) and triggers a serpin conformational change that moves the RCL to the opposite end of the serpin domain, taking the tethered protease with it. This structural disorder allows the proteolytic destruction of the complex. Mutations in C1INH can modify exposure and mobility of RCL which can produce a decrease or lost in C1INH inhibitor function (Zahedi et al., 1997; Janciauskiene, 2001).

C1INH blocks several steps in the KKS activation cascade (figure 1.3). It function covers the inhibition of (1) up to 93% of FXIIa in plasma (de Agostini et al., 1984), (2) 52% of PKa (Schapira et al., 1982) and (3) 47% of FXIa (Wuillemin et al., 1995). C1INH also slightly inhibits plasmin, but this action seems to present a limited physiological relevance (Harpel & Cooper, 1975).

In addition, the KKS cascade can be inactivated by other protease inhibitors, considered secondary, including α2-macroglobulin, α2-antiplasmin, antithrombin and α1-antitrypsin (de Agostini et al., 1984; Wuillemin et al., 1995.

### 1.3.2 Pathogenic mechanism of Hereditary Angioedema with C1 Inhibitor Deficiency

The main fact supporting the implication of C1INH as an obvious regulator of normal vascular permeability is the appearance of angioedema in C1INH deficiency. The first description of C1INH deficiency as responsible of HAE was made by Donaldson and Evans in 1963. Subsequently studies have demonstrated that C1INH is the primary regulator of KKS cascade and, therefore, of vascular permeability.

Under normal circumstances, the auto-activation capacity of KKS is controlled by C1INH, but in the absence of this serpin, this capacity produces an uncontrolled auto-activation and an excessive BK release. In 2013, a new plasma amidase assay to measure the spontaneous amidase activity of Serine proteases belonging to KKS and BK-related fibrinolysis system (PKa, FXII, plasmin and tissue-type plasminogen activator) was developed (Defendi et al., 2013). An elevation in spontaneous amidase activity was associated with increased HK cleavage, supporting that this amidase activity is comparable to kininogenic activity. Compared to healthy donors, spontaneous amidase activity was significantly elevated in plasma of HAE-C1INH at remission and symptomatic periods, independent of plasma C1INH levels. In addition, proenzyme activation was strongly decreased in HAE-C1INH. These results were interpreted by authors as zymogene consumption during contact phase activation in the context of an absence C1INH control capacity.

In 2014, Suffriti et al. evaluated the amidolytic activity of PKa in plasma from HAE-C1INH patients and the ability of this plasma to inhibit the amidolytic activity of exogenous PKa. Spontaneous plasma PKa activity was statistically significantly elevated during remission periods, and markedly decreased during acute attacks of HAE patients. Patients also had lower PKa inhibitory capacity during periods of remission, and these levels were further decreased during angioedema attacks. These results showed no differences based on expressed disease phenotype or in relation to the use of attenuated androgens (AAs) as long term prophylaxis (LTP). These results were supported by the presence, in periods of remission, of higher levels of HK cleavage in patients vs. controls, which in turn were higher during AE attacks. In relation to disease phenotype, levels of cleaved HK were higher in patients with moderate or severe phenotypes compared to patients with mild phenotype or controls. The use of long-term treatment did not change these levels. In plasma from HAE-C1INH patients vs. controls, C1INH activity correlated negatively with cleaved HK and spontaneous PKa activity, and positively with PKa inhibitory activity. Some years later, it was shown that increased amidase activity in serum from HAE-C1INH patients revealed higher diagnostic performance values than C4 levels, and it was suggested that this method could be used in conjunction

with C1INH levels for the diagnosis and monitoring of HAE (Charignon et al., 2017).

### 1.3.3 Pathogenic mechanism of Hereditary Angioedema with normal C1 Inhibitor

Since 2000, when this group was first described, some evidence strongly suggests the involvement of BK in the physiopathology of HAE-nC1INH: (1) identical clinical presentation compared with HAE-C1INH (Bork et al, 2007; Vitrat-Hincky et al., 2010); (2) response during an attack to treatment with a B2R antagonist (icatibant acetate) and plasma derived C1INH (Bouillet et al., 2017a, 2017b); (3) the absence of response with corticosteroids, antihistamines and adrenaline (Bork et al., 2009); (4) spontaneous amidase activity was found significantly increased in HAE-nC1INH (Defendi et al., 2013); (5) In 2020, for first time, BK elevation was confirmed in patients with HAE-FXII and HAE-PLG (Marceau et al., 2020).

Initially, HAE-nC1INH encompassed all hereditary forms of HAE without alterations in C1INH. However, in recent years, new discoveries in genetics (and thus physiopathology) have provided insight into this group. Future perspectives focus on the disaggregation of HAE-nC1INH according to the physiopathology involved. Some forms are clearly related to increased BK formation (HAE-FXII, HAE-PLG, HAE-KNG1, HAE-HS3ST6) and other forms are involved in intracellular transductions in nearby pathways (HAE-ANGPT1, HAE-MYOF, HAE-HS3ST6):

a) HAE-FXII: Mutations in *F12* gene described in HAE-FXII are located in the proline-rich region that binds to surfaces (Citarella et al., 1996). These mutations result in a conformational change that leads to an increase in FXII activation capacity, and thus gain-of-function variants (Cichon et al., 2006). This conformational change also extremely increases the susceptibility of FXII activation by plasmin in the fluid phase (de Maat et al., 2016; Ivanov et al., 2019).

b) HAE-PLG: Plasminogen is the inactivated precursor of plasmin. Plasmin is an activator of FXII, playing a role in BK formation. The described mutation (Bork et al., 2018) produces a conformational change of the wild-type protein, resulting in increased BK production. This year, Dickeson et al demonstrated, indirectly, that substitution of Lys311 for glutamic acid (Plm-Glu) in plasminogen induces bradykinin generation through HK cleavage once plasminogen is converted to plasmin. Plm-Glu also has the ability to cleave LH, so it may play a major role in the pathophysiology of these patients. Plm-Glu was originally theorized to have a high and rapid rate of activation and FXII cleavage, a primary FXII activator, but has been shown to have a direct ability to cleave HK and LK.

c) HAE-KNG: so far, a single mutation in *KNG* gene has been described. Two hypothesis have been proposed as pathophysiological mechanisms: (1) the mutation leads to the production of aberrant forms of released BK and Lys-BK that prevent a normal inactivation process by peptidases; (2) the mutation could enhance the release of BK by facilitating the accessibility of PKa to its cleavage sites in HK (Bork et al., 2019).

d) HAE-ANGPT: ANGPT is the ligand of the angiopoetin receptor (TIE2), expressed on vascular endothelial cells. ANGPT1-TIE2 contributes to the regulation of endothelial barrier function by inhibiting the effects of several permeability factors, such as vascular endothelial growth factor (VEGF) and BK. The pathogenic variant decreases the amount of AGPT1 in plasma, whereby ANGPT1-TIE2 is impaired by reduced binding to the TIE2 receptor (because of a mechanism of haploinsufficiency) (Baffuno et al., 2018; d'Apolito et al., 2019).

e) HAE-MYOF: *MYOF* gene encodes myoferlin 1, a membrane protein located in the plasma membrane of endothelial cells. This protein regulates VEGF signaling by preventing ubiquitination and degradation of its receptor. Functional studies performed in cell lines have shown that the described variant increases VEGF receptor (VEGFR) levels and improves its localization on the plasma membrane (Ariano et al., 2020).

f) HAE-HS3ST6: *heparan sulfate-glucosamine 3-sulfotransferase 6 (HS3ST6)* is a protein-coding gene. Heparan sulfate (HS) critically regulates

angiogenesis (Fuster & Wang, 2010). Functional studies of the variant have not been performed, but it is hypothesized that the mutation may affect PKa binding at the cell surface or its internalization via endocytosis, following increased cleavage, excessive production of BK and increased vascular permeability (Bork et al., 2021).

## 1.4 Clinical Characteristics of Bradykinin-Induced Angioedema

### 1.4.1 Hereditary forms of bradykinin - induced angioedema

1.4.1.1 *Hereditary Angioedema with C1 inhibitor deficiency*

HAE-C1INH is a rare disease considered to be a primary immunodeficiency (Notarangelo et al., 2004). It is the most common genetic disorder of the complement system (Carreer, 1992). The estimated frequency of the disease is 1:50.000 (Agostini & Cicardi, 1992). It has been documented for more than a century: the first description of the disease was made by Quincke in 1882, based on the observation of a family with HAE by his pupil Dinkelacher, and was initially called angioneurotic oedema (Strubing, 1885). In 1888 Osler first referred to the existence of a hereditary form of AE. It was not until 1963 that Donaldson & Evans first described C1INH deficiency in these patients.

There are two traditionally described types of HAE-C1INH, with identical clinical expression (Rosen et al., 1965): HAE-C1INH-1 which has decreased quantitative levels of C1INH (approximately 85% of patients); HAE-C1INH-2 which has normal levels of a dysfunctional protein (15% of patients).

AE can appear in any subcutaneous or submucosal location (face, larynx, genitalia, extremities or abdominal area). Cutaneous involvement, especially in the extremities, is the most frequent location of attacks (Agostoni et al., 2004; Bork et al., 2006; Zuraw et al., 2013). A high frequency of abdominal symptoms has been described as a hallmark of HAE-C1INH, as abdominal AE is less frequent in HAE of other origin (Agostoni et al., 2004, Bork et al., 2009). Acute episodes usually affect a single site, although it is not uncommon for them to occur simultaneously

or, more frequently, to progressively affecting several sites. Prodromes such as erythema marginatum, mood swings, anxiety and/or extreme tiredness several hours before the attack occur in 82-95% of patients (Prematta et al., 2009, Reshef et al., 2013). Although C1-INH deficiency is present from birth, most patients have a disease debut during school age. The rate of asymptomatic adults is estimated to be 5-13.7% (Agostoni, 1992; Roche et al., 2005). The frequency and duration of acute episodes vary markedly between different HAE patients, and even between patients with the same mutation or in the same patient at different times of life. Table 1.2 shows the clinical and laboratory criteria for the diagnosis of the disease.

**Table 1.2 Diagnostic criteria of hereditary angioedema with C1INH deficiency**

| |
|---|
| **Clinical criteria**<br><br>**Major:**<br><br>(1) Self-limiting, non-inflammatory subcutaneous angioedema without major urticarial rash, often recurrent and often lasting more than 12 hours.<br><br>(2) Self-remitting abdominal pain without clear organic etiology, often recurrent and often lasting more than 6 hours.<br><br>(3) Recurrent laryngeal edema.<br><br>**Minor:**<br><br>(4) Family history of recurrent angioedema and/or abdominal pain and/or laryngeal edema. |
| **Laboratory criteria**<br><br>(1) C1INH antigenic levels <50% of normal at 2 separate determinations (2-3 months interval normally) with patient in basal condition and after the first year of age.<br><br>(2) C1INH functional levels <50% of normal at 2 separate determinations with patient in basal condition and after the first year of age.<br><br>(3) Mutation in C1INH gene altering protein synthesis and/or function. |
| Diagnosis can be established in presence of 1 major (1-3) clinical criteria and 1 laboratory criteria |

C1INH: C1 esterase inhibitor. (Agostoni et al., 2004).

Attacks usually occur spontaneously. However, stress or anxiety, minor trauma (including surgery), infections and ACEi use are known triggers (Frank et al., 1976; Agostini et al., 2004; Bork et al., 2011a; Jurado-Palomo et al., 2013; Zuraw et al., 2013; Aygören-Pürsün et al., 2013). Endogenous (pregnancy, menstruation and/or ovulation) and exogenous (contraceptives and hormone replacement therapy) estrogens also frequently act as triggers (Bouillet et al, 2008; Martinez-Saguer et al., 2010; Caballero et al, 2012;).

#### 1.4.1.2 *Hereditary angioedema with normal C1 inhibitor*

Although the first description of this entity was made in 1986 by Warin et al., it was not until 2000 that two independent groups reported the first case series of HAE-nC1INH (Bork et al., 2000; Binkley & Davis, 2000). The clinical presentation of HAE-nC1INH is very similar to that of HAE-C1INH. However, HAE-nC1INH has normal quantitative and functional levels of C1INH and a different molecular basis. Despite their similarities, some clinical features may guide the diagnosis (table 1.3).

**Table 1.3. Features of Hereditary angioedema with normal C1INH that could distinguish it from Hereditary angioedema with C1INH deficiency**

| |
|---|
| - Normal C1INH quantitative and functional levels. |
| - Women are mainly affected. |
| - Symptoms starts in adulthood frequently, the incidence in childhood is being low. |
| - Disease-free interval is more frequent throughout the course of the disease. |
| - Facial swelling, mainly lips, as well as tongue are more frequents. |
| - Abdominal attacks are less frequents. |
| - No erythema marginatum has been observed in these patients. |
| - Hemorrhages or equimosis into skin swellings were observed. |

C1INH: C1 esterase inhibitor

HAE-nC1INH is characterized by a clear predominance of female involvement - male involvement is very rare - and by the occurrence of attacks especially in hyper-estrogenic states (both endogenous - pregnancy - and exogenous - contraceptives or hormone replacement therapy) (Martin et al., 2007; Baeza et al., 2011; Charingnon et al., 2014; Moreno et al., 2015; Deroux et al., 2016; Veronez et al., 2017; Veronez et al., 2018; Bork et al., 2018; Belbezier et al., 2018; Bork et al., 2015, 2019, 2020a).

This entity presents the same triggers as HAE-C1INH, but in this case, estrogens play a key role and there seems to be a greater sensitivity to female hormones than in HAE-C1INH. Depending on their relationship with estrogens, 3 profiles have been described that apply to both HAE-nC1INH and HAE-C1INH female patients (Bouillet & Gompel, 2013):

a) Estrogen-dependent: symptoms appear exclusively in hyper-estrogenic periods. This is the most frequent profile in HAE-nC1INH.

b) Estrogen-sensitive: symptoms are expressed, and clearly aggravated, in hyper-estrogenic states, but attacks also occur outside these specific contexts.

c) Estrogen-independent: symptoms are not expressed in relation to, or aggravated by, exogenous or endogenous estrogens.

There are currently no laboratory tests to confirm the diagnosis in cases where no genetic cause can be found, so the diagnosis is based on a concordant clinical history, exclusion of urticaria and urticaria-associated angioedema, and mainly: one or more family members must be affected (although they can often remain paucisymptomatic), and normal protein and functional C1-INH levels measured under baseline conditions (Cicardi et al., 2014).

### 1.4.2 Other forms of bradykinin - induced angioedema

#### 1.4.2.1 *Idiopathic non - histaminergic angioedema*

This group (AAE-InH) includes patients with recurrent episodes of angioedema with normal quantitative and qualitative C1INH values and negative family history. AE-MC, drug-induced AE, infections and autoimmune diseases should be excluded (Bork et al., 2013). The clinical presentation is comparable to HAE-nC1INH, except that they have no family history, and their diagnosis is based on the same clinical features. Although AAE-InH can be included among the acquired forms, it is mandatory to search for known mutations affecting HAE-nC1INH patients, as cases have been described of patients with HAE-FXII representing the only clinically affected family member, with other asymptomatic carriers in their family.

#### 1.4.2.2 *Acquired angioedema with C1 inhibitor deficiency*

AAE-C1INH represents the non-genetic form of C1INH deficiency, without mutations in *SERPING1* gene and without family history of angioedema. The frequency of AAE-C1INH is unknown, but it is estimated that there is one patient per 10-13 patients with HAE-C1INH (Cicardi & Zanichelli, 2010a; Bygum & Vestergaard, 2013; Baeza et al., 2022). It occurs as a consequence of hyperactivation of the classical human complement pathway, with accelerated consumption of C1INH. In the absence of the regulatory element, increased activation of the contact system leads to episodes of AE indistinguishable from hereditary forms, although the age of onset is usually from the age of 40 years. AAE-C1INH is associated with various diseases, mainly lymphoproliferative diseases, as well as cancer and infections. After confirmation of C1INH deficiency, the C1q level needs to be determined: the diagnosis is confirmed if C1q is reduced (70% of patients with AAE-C1INH). If C1q levels are normal, the presence of auto-antibodies against C1INH should be investigated. If antibodies are negative, the diagnosis is assumed after normal genetic screening (Agostini et al., 2004).

1.4.2.3 *Angioedema induced by use of angiotensin-converting enzyme inhibitor drugs*

As mentioned above, AAE-ACEI has a prevalence of 0.1-0.7%. Despite having a similar clinical presentation to the hereditary forms, there is a predominance of facial/laryngeal involvement (Beltrami et al., 2011; Zuraw et al., 2013). Diagnosis is based on resolution of symptoms after treatment discontinuation, although up to half of patients may present with an episode in the months following treatment withdrawal, due to alteration of the BK degradation pathway (Piñero-Saavedra et al., 2013; Byrd et al., 2006).

## 1.5 Genetics of Bradykinin - Induced Angioedema

### 1.5.1 Hereditary Angioedema with C1 Inhibitor Deficiency

1.5.1.1 *Mutations in SERPING1 gene*

C1-INH is encoded by the *SERPING1* gene (Gene Bank X54486; Swiss-Prot P05155; Online Mendelian Inheritance in Man #606860), located on chromosome 11 (locus 11q12 to q13.1) (Bock et al., 1986; Davis et al., 1986; Tosi et al., 1986; Theriault et al., 1990). It consists of 8 exons (of which 7 are protein coding) and 7 introns distributed along a DNA length of 17 Kb. Exon 2 contains the transcription start site and exons 3 to 8 encode the rest of the protein. Exon 8 also encodes the hinge region of the protein and the reactive centre of the C1INH molecule, essential for proper function (Verpy et al., 1995).

*SERPING1* gene is characterized by not being a highly polymorphic gene. The introns are particularly rich in *Alu* repeats, a source of genetic instability, which confers a high predisposition to uneven rearrangements between *Alu* sequences, including partial deletions and, less frequently, partial duplications (Ariga et al., 1990; Stoppa-Lyonnet et al., 1990; Carter et al., 1991; Stoppa-Lyonnet et al., 1991).

HAE-C1INH is inherited autosomal dominantly, mainly in heterozygosity (Späth & Wüthrich, 1998). The cases of homozygosity described are exceptional (Verpy et al., 1996; Blanch et al., 2006; Lopez-Lera et al., 2010; Rijavec et al.,

2013; Bafuno et al., 2013; Montinaro, 2013), as well as cases of mosaicism (Guarino et al., 2006; Yu et al., 2007; Ebo et al., 2017).

The occurrence of *de novo* mutations is estimated at around 25% of cases (Pappalardo et al., 2000). This high frequency seems to be related to the high frequency of *Alu* repeats (stoppa-Lyonnet, 1991), as well as to the existence of several mutational hotspots: the CpG dinucleotide at the end of exons 3 and 6, ATG codon duplications, and changes in the CpG dinucleotide of the reactive centre (Arg444Cys and Arg444Leu) by various mechanisms (Pappalardo et al., 2000). Heterozygous people have plasma C1INH levels around 5-30% of the normal value (Pappalardo et al., 2004), instead of the expected 50%. This is due not only to a defect in the production of the protein, but also to an increased metabolism of C1INH (Quastel et al., 1983), a decrease in mRNA expression of the normal allele and/or a translational trans-inhibition mechanism of normal C1INH by altered mRNA and/or protein (Kramer et al., 1993).

At least 748 mutations have now been described along the entire gene (Pappalardo et al., 2004; Roche et al., 2005; Kesim et al., 2011; Rijavec et al., 2013; Kalmar et al., 2003; Gösswein et al., 2008; Pappalardo et al., 2008; Bygum et al., 2011; Kesim et al., 2011; Yamamoto et al., 2012; Xu et al., 2012; Martinho et al., 2013; Bafunno et al, 2014; Johnsrud et al., 2015; Cagini et al., 2016; Steiner et al., 2017; Grivčeva-Panovska et al., 2018; Ponard et al., 2020), collected in international genetic databases (Human Genome Mutation Database, http://www.hgmd.cf.ac.uk/ac) and in a disease-specific database (HAEdb, http://www.hae.enzim.hu) (Kalmar et al., 2005). Mutations leading to HAE-C1INH-1 are very heterogeneous and are located throughout the gene. Point mutations, affecting one or several nucleotides, are the most frequent genetic alterations. Up to 20% of patients have large rearrangements (partial deletions, insertions or duplications), which are attributed to recombination of *Alu* sequences (Stoppa-Lyonnet et al., 1990; Carugati et al. 2001). On the other hand, HAE-C1INH-2 is caused by point mutations in exon 8 and nearby areas (Carugati et al. 2001; Blanch et al., 2002), which code for the hinge region of C1INH, resulting in a dysfunctional protein. Up to 70% of HAE-C1INH patients have a mutation in the P1 residue of the active centre, amino acid 444 (Arg) is replaced by another amino acid (His, Cys, Leu or Ser).

There are several studies that attempt to explain the large clinical variability in HAE. In 2013, the Hungarian HAE Study Group published a study revealing a lower disease severity score (based on lower symptom frequency and lower consumption of pdC1INH) in patients with missense mutations compared to other types of mutations (Bors et al., 2013). This result contrasts with several publications in which no relationship between phenotype and genotype was found (Pappalardo et al., 2000; Agostoni et al., 2004; Bygum et al., 2011; Xu et al., 2012). The single nucleotide polymorphism c.21T>C in exon 2, when transmitted with a pathogenic mutation, was associated with increased disease severity (Cumming et al., 2003; Duponchel et al., 2006). However, this could not be confirmed in subsequent studies (Bygum et al., 2011 Allergy, Bafunno et al., 2014). There is also no relationship between phenotype and C1INH levels (Cicardi et al., 1998; Cumming et al., 2003; Agostoni et al., 2004; Cugno et al., 2009; Bygum et al., 2011), which contrasts with data reported by Kelemen et al in 2010, who found a relationship between baseline C1INH activity levels and a higher disease severity score.

#### 1.5.1.2 *Mutations in other genes*

In accordance with the minimal or even non-existent relationship between *SERPING1* phenotype and genotype, other genetic factors (with involvement in HAE pathophysiology) have been evaluated, but no relationship with disease severity has been found: *ACE* I/D polymorphism (Freiberger et al., 2011), the most relevant polymorphism of this enzyme and responsible for up to 47% of plasma ACE levels (Rigat et al., 1990); polymorphisms in BK1R or BK2R - 58c/t and 181c/t polymorphism in BK1R, 699c/g and 1098g/c in BK2R-(Freiberger et al., 2011); mannose-binding lectin (MBL) levels (Cedzynski et al., 2008) or MBL polymorphisms-(Freiberger et al., 2011); peripheral blood mononuclear cell expression (Lopez-Lera 2013 Orphanet J Rare Dis).

Recently, a comparison was made between 14 patients with either *SERPING1* mutations or *PLG* mutations (Bork et al., 2020b). The presence of mutations in *SERPING1* was associated with the occurrence of abdominal or extra-limb AE, while patients with mutations in the *PLG* gene had AE of the lips and tongue, but

not of the limbs. In this group of patients, each mutation was associated with different symptom expression. In this group of patients, a relationship between clinical expression and genotype was established. This finding could explain not only the existence of divergent phenotypes in HAE patients, but also could support a genotype-phenotype correlation. However, more studies are needed.

### 1.5.2 Hereditary Angioedema with Normal C1 Inhibitor

The molecular basis of HAE-nC1INH is still poorly understood, and it has been suggested that this entity encompasses several forms with different pathophysiology. Therefore, at present, HAE-nC1INH remains a challenge in certain aspects. The only certainty is that patients with HAE-nC1INH do not have alterations in the *SERPING1* gene. So far, mutations in 6 different genes that are elements of the BK-forming cascade or vascular endothelial function have been described as pathological.

#### 1.5.2.1 *Mutations in F12 gene*

The *F12* gene (Online Mendelian Inheritance in Man #610619) codes for FXII, one of the core elements of the KKS. It is a 12 Kb gene (consisting of 14 exons), located on chromosome 5, subregion 5q33-5qter (Royle et al., 1988). The first description of a disease-related mutation was described by Dewald & Bork in 2006. Since then, a total of 4 mutations have been described:

a) Two different nonsense mutations at exactly the same position:

- c.983 C>A: this mutation results in a threonine to lysine substitution (p.Thr328Lys). It is by far the most frequent mutation reported in HAE-nC1INH, affecting numerous families - more than 400 patients (Cichon et al., 2006; Martin et al., 2007; Bouillet et al., 2007; Bell et al., 2008; Duan et al., 2009; Prieto et al., 2009; Hentges et al., 2009; Nagy et al., 2009; Picone et al., 2010; Baeza et al., 2011; Marcos et al., 2012; Gomez-Traseira et al., 2013; Charignon et al., 2014; Bork et al., 2015;

Mansi et al., 2015; Moreno et al., 2015; Stieber et al., 2015; Moreno et al., 2016; Deroux et al., 2016; Grumach et al., 2016; Bork et al., 2017; Veronez et al., 2018; Bova et al., 2020). This mutation has been mainly observed in Germany, France, Spain and Brazil. All patients were heterozygous for the respective mutations, but two patients from the same family were reported by Grumach et al. (2018).

- c.983 C>G: this mutation causes a threonine to arginine substitution (p.Thr328Arg). It has been described in 2 families (Bork et al., 2015).

b) A 72 base pair deletion (c.971_1018+24del72), starting at position c.971A of exon 9 (encoding p.Lys324) - loss of 48 bp from exon 9 - and ending in intron 9 - loss of 24 bp - affecting the donor splice site (Bork et al., 2011b). It has been described in 2 families (each of Turkish and Brazilian origin) (Bork et al., 2011b; Veronez et al. 2018).

c) A duplication of 18 base pairs (c.892_909dup), which produces a repetition of 6 amino acids (p.Pro298_Pro303dup). (Kiss et al., 2013).

The 4 *F12* gene alterations described are located in the same region, which encodes the proline-rich region of the FXII protein. De Maat et al. (2016) confirmed that these mutations developed a new cleavage site in this region, which accelerates liquid-phase activation by plasmin.

Mutations in the *F12* gene have been reported to be responsible for the disease in 15-30% of patients (Dewald & Bork 2006; Bork et al., 2007; Vitrat-Hincky et al., 2010). Testing of asymptomatic carriers of the *F12* mutation demonstrates autosomal dominant inheritance with a low penetrance. The overall male:female ratio was between 1:6 and 1:13, according to several reports (Bork et al., 2020a).

As in HAE-C1INH patients, there is no evident association between the clinical phenotype and the *F12* mutation presented, so a multigene origin for this disease has been postulated. In 2009, Duan et al studied the presence of 2 polymorphisms in ACE and APP enzymes in a family of patients with HAE-FXII: (1) the *ACE* gene I/D polymorphism (Rigat et al., 1992), whose I allele has been

associated with lower ACE gene mRNA expression (Suehiro et al., 2004) and reduced BK degradation (van Dijk et al., 2000); (2) the c.2399 A polymorphism (rs3788853) in the XPNPEP2 gene, which encodes APP, has been associated with lower enzyme activity and higher levels of BK and des-Arg9-BK (Duan et al., 2005; Molinaro et al., 2006; Cilia La Corte et al., 2011); all three HAE-FXII patients in this study had at least one copy of both the I allele of the ACE gene and the A allele of XPNPEP2, leading the authors to suggest that the combination of multiple loci variations may contribute to HAE based on their effects on BK levels.

#### 1.5.2.2 *Mutations in PLG gene*

In 2018, the c.988A>G (p.Lys330Glu) mutation in the *PLG* gene was linked to HAE-nC1INH (Bork et al., 2018). Since then, more than 140 patients, from 33 unrelated families, have been reported as HAE-PLG (Dewald, 2018; Germenis et al., 2018; Belbezier et al., 2018; Yakushiji et al., 2018; Recke et al., 2019; Bodian et al., 2019; Bork et al., 2020a; Bork et al., 2020b). In all cases this mutation has been described in heterozygosis, and they present an autosomal dominant transmission. The male:female ratio was 1:3 (Bork et al., 2020a).

The first finding of two HAE-specific mutations in a large family has been made, with the description of two patients with mutations in the *SERPING1* and *PLG* genes (Bork et al., 2020b).

#### 1.5.2.3 *Mutations in KNG gene*

In 2019, the c.1136 T>A (p.Met379Lys) mutation in the *KNG* gene was linked to HAE-nC1INH. A new variant, named HAE-KNG, was described in this family. The mutation is dominantly inherited. The mutation was located in the cleavage region of kinins, including BK (Bork et al., 2019).

#### 1.5.2.4 *Mutations in ANGPT gene*

The *ANGPT* gene encodes angiopoetin-1, a molecule with important functions in vascular development and angiogenesis. Also in 2018, the c.807G>T (p.Ala119Ser) mutation in the *ANGPT* gene was described as responsible for HAE-ANGPT (Bafunno et al., 2018). This mutation has been described in several members of the same family, with an autosomal dominant character for inheritance.

Considering that ANGPT1 is not directly related to KKS, this discovery opens new frontiers in the understanding of HAE-nC1INH. Physiologically, AGNPT1 acts through the TIE 2 receptor by reinforcing the cell cytoskeleton arrangement and decreasing vascular permeability. AGNPT2 acts by antagonizing ANGPT1, leading to increased permeability. The p.Ala119Ser mutation reduces ANGPT1 levels, and the ANGPT1/ANGPT2 ratio is decreased (D'apolito et al., 2019). Therefore, it appears that this mutation leads to impaired interactions with its membrane receptor, which could lead to increased plasma leakage of BK.

#### 1.5.2.5 *Mutations in MYOF gene*

The *MYOF* gene encodes for myoferlin 1, a membrane protein of endothelial cells that regulates VEGF signalling. Recently, a new mutation in this gene has been described in an Italian family. This new subtype of HAE-nC1-INH has been named HAE-MYOF. The c.651G > T (p.Arg217Ser) mutation increases VEGFR-2 levels in response to VEGF stimuli by modifying the subcellular distribution of the protein and enhancing its localisation at the plasma membrane (Ariano et al., 2020).

#### 1.5.2.5 *Mutations in HS6ST2 gene*

One year ago, the mutation c.430A>T (p.Thr144Ser) in *HS3ST6* gene was described in a 3-member family diagnosed with HAE-nC1INH (Bork et al., 2021). The current hypothesis is that this mutation may produce changes in the active center of the protein, altering its action.

### 1.5.3 Metallopeptidases

The clinical importance of uncovering the genetic mechanisms of AE-BK stems from the urgent need to identify predictive markers for the development of this potentially fatal disease.

Due to the relevance of metallopeptidases in BK metabolism and the previous suggestion of their possible involvement in disease severity, in this study we have selected to study *ACE* and *APP* gene polymorphisms that have been previously linked to enzyme activity.

#### 1.5.3.1 *ACE gene*

ACE is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells (Campbell, 2003). In addition to its involvement in the renin-angiotensin system, ACE also plays an important role in vasodilation and blood pressure regulation via the kallikrein-kinin cascade (Atlas, 2007). Plasma ACE levels are stable when measured repeatedly in the same individual, whereas large interindividual differences are observed. A possible genetic origin is suggested (Sayed-Tabatabaei et al., 2006).

In humans, the *ACE* gene is located on the long arm of chromosome 17 (17q23). There are more than 160 polymorphisms in the *ACE* gene, most of which are SNPs. Studies in Caucasian populations indicated that most polymorphisms are closely related to a functionally relevant polymorphism described in intron 16 (Sayed-Tabatabaei et al., 2006): a 287 bp *Alu* repeat is present (allele I: insertion) or absent (allele D: deletion). The I/D allele of ACE is common in the general population, with reported genotypic frequencies of 25.6% (II), 48.3% (ID) and 26.1% (DD) (Scheer et al., 2005). In Caucasians, genotypic frequencies were 22.7% (II), 42.5% (ID) and 34.8% (DD).

The I/D polymorphism explained approximately 47% of the observed variance in ACE levels (Rigat et al., 1990): mean ACE activity levels in DD carriers were approximately twice those found in genotype II individuals, and subjects with ID genotype had intermediate levels indicating codominance. The I allele has been

found to be associated with lower serum enzyme activity, but also with lower ACE mRNA expression (Suehiro et al., 2004) and lower bradykinin degradation compared to the D allele (van Dijk et al., 2000).

The II allele is associated with low BK degradation and would therefore be expected to correlate with the occurrence of AE. This possibility has been studied in two groups of AE-BK: HAE-C1INH and AAE-ACEI. Freiberger et al (2011) studied the possible association of the HAE-C1INH phenotype and the ACE I/D polymorphism in a Czech population: no association was found between this allele and disease severity, location or frequency of attacks or age of disease onset. Several studies, including a recent genome-wide association study, have been conducted on the possible link between AAE-ACEI and the *ACE* I/D polymorphism (Gulec et al., 2008; Bas et al., 2010; Pare et al., 2013; Moholisa et al., 2013): all of them conclude that the *ACE* I/D polymorphism is not involved in the development of AAE-ACEI. However, some data lead us to follow the line that there must be some kind of association with AE: it has been shown that ACE activity was significantly decreased in patients with AAE-ACEI (Moholisa et al., 2013). Furthermore, Akcali et al. found a statistically significantly higher frequency of allele I in patients with ordinary chronic urticaria with associated AD compared to AD-negative patients and the control. Although the *ACE* I/D phenotype does not seem to be related to AE-BK when considered individually, several groups have postulated a possible link between disease expression and a conjunction of several polymorphisms of the enzyme.

#### 1.5.3.2 *XPNPEP2 gene*

APP is an important kinase *in vivo* (Kitamura et al., 1995). It is responsible for cleaving any terminal amino acid of an Xaa-Pro sequence that allows the subsequent action of prolyl aminopeptidase (Harbeck & Mentlein, 1991). Studies on APP activity began in the 1990s in animal models (Ahmad et al., 1992; Kitamura et al., 1995; Orawski & Simmons, 1995; Ersahin & Simmons, 1997) and its involvement in BK metabolism has been demonstrated. Human APP exists in both

cytosolic and membrane-bound forms, the latter being most likely responsible for plasma activity (Molinaro et al., 2005).

Previous studies have shown low levels of circulating APP in HAE-ACEI (Adam et al., 2002; Molinaro et al., 2002) and in HAE-C1INH (Drouet et al., 2008), which has been suggested as a possible association with increased susceptibility to AE attacks. Furthermore, Drouet et al demonstrated that APP activity showed a significant inverse relationship with disease severity.

The gene encoding membrane-bound APP (*XPNPEP2*) is located on the X chromosome (Xq26.1). In 2005, Duan et al demonstrated that genetic variants at the *XPNPEP2* locus are partially responsible for the variability in plasma APP activity. In particular, the single nucleotide polymorphism (SNP) C-2399A (rs3788853) has been associated with low plasma APP activity and thus with increased levels of BK and des-Arg9-BK (Duan et al., 2005; Molinaro et al., 2006; Cilia La Corte et al., 2011). A significant association of this SNP with AAE-ACEI has been found in several studies (Duan et al., 2005; Cilia La Corte et al., 2011; Woodard-Grice et al., 2010), only in one of them with a dependence on sex –male- and race –black- (Woodard-Grice et al., 2010). Excluding AAE-ACEI, it has not yet been studied whether this polymorphism could be related to disease severity or to an increased risk of AE in other forms of AE-BK. Only one report of three patients with HAE-FXII carrying both polymorphisms (ACE I/D and XPNPEP2 c2339A SNP) has been published, suggesting a polygenic origin of the disease.

## 1.6 Aims and organization of the thesis

As with other rare diseases, the low incidence difficult the study of HAE. In the absence of international studies and registries that could shed light on this matter, patient series are currently our major source of information on the disease. In this regard, our main objective is to contribute to the clinical description of two specific HAE subpopulations. On the one hand, Chapter 2 contains a detailed study of the clinical and genetic characteristics of a HAE-FXII population. For the first time, this study investigates in a HAE-FXII population the previously proposed

hypothesis of the involvement of polymorphisms in the ACE and XPNPEP2 genes in disease expression (Duan et al., 2009). On the other hand, Chapter 3 details the clinical characteristics of pregnancy and childbirth in patients with HAE-C1INH, which has been scarcely studied to date. Finally, Chapter 4 contains the general considerations of this study.

# Chapter 2

# Hereditary angioedema with F12 mutation: Clinical features and enzyme polymorphisms in 9 Southwestern Spanish families

# Abstract

Background: Information on *F12* mutation HAE is still limited, but Spain is now recognized as having one of the highest concentrations of cases in Western Europe.

Objective: To describe unique features of HAE in Spanish carriers of the *F12* mutation and investigate a potential role for ACE and APP polymorphisms in disease expression.

Methods: This was a prospective observational cohort study of 35 individuals (80% females) from 9 unrelated families carrying the p.Thr309Lys mutation. We analyzed detailed medical records and complement activity (C4, C1q, C1 inhibitor) and screened for mutations in exon 9 of the *F12* gene and 2 polymorphisms: *XPNPEP2* c-2399A and the *ACE* insertion/deletion polymorphism.

Results: The p.Thr309Lys mutation was found in all individuals. Three of the 9 index patients had a clinically negative family history, and 72% of males and 29% of females were asymptomatic. Sixteen females (44% estrogen dependent, 56% estrogen sensitive) were clearly symptomatic. The most common locations of attacks were the abdomen (63%), face (25%), and peripheral structures (6%). Triggers other than hyperestrogenic states included stress and minor trauma or pressure. Short-term treatment with C1-inhibitor concentrate and icatibant and LTP with tranexamic acid were useful. The combination of the I allele and A allele was detected in 17% of patients.

Conclusion: The polymorphisms analyzed were not a major determinant of disease expression in our population. We recommend searching for *F12* mutations in women with edema attacks without associated wheals and with normal C1-inhibitor levels, particularly when they develop symptoms during hyperestrogenic states or are of Western European or African origin.

## 2.1 Introduction

The 3 types of HAE types I, II, and III have an autosomal dominant mode of inheritance. They can all cause subcutaneous or submucosal edema in any part of the body and are clinically indistinguishable (Caballero et al., 2011).

Estrogen-induced familial angioedema, without C1INH deficiency, was first described by Warin et al in 1986. However, it was not until 2000 that a new type of HAE type III, or HAE-nC1INH was suggested (Binkley & Davis, 2000; Bork et al., 2000). In 2006, 2 different missense mutations located in the same position in exon 9 of the *F12* gene (encoding HAE-FXII) were described in approximately 25% of a cohort of patients with HAE-nC1INH (Dewald & Bork, 2006). The most common of the 2 mutations predicts a threonine-to-lysine substitution in the secreted zymogen protein (c.983C>A, p.Thr309Lys, also referred to as p.Thr328Lys with the addition of the leader protein), whereas the second mutation predicts a threonine-to-arginine substitution (c.983C>G, p.Thr309Arg). A 72-base pair (bp) deletion (Bork et al., 2011b) and an 18-bp duplication (Kiss et al., 2013) were also described in the same proline-rich region of the *F12* gene.

In the case of HAE-FXII, an autosomal dominant mode of inheritance with a very low penetrance is now recognized, particularly in males, because more than 90% of male carriers are asymptomatic compared with just 40% of females (Caballero et al., 2014). The cosegregation of *F12* mutations in a substantial proportion of individuals who experience angioedema attacks provides strong evidence that these gene mutations are responsible for disease susceptibility. Nevertheless, the underlying pathophysiologic mechanism remains unknown in most of families with nC1-INHHAE - (Bork et al., 2009). Nonetheless, the importance of contact pathway dysregulation in HAE-nC1INH is increasingly recognized.

The description of HAE-UNK and HAE-FXII has stimulated new research on contact system activation and interrelations with other homeostatic systems. Furthermore, the new millennium spawned investigations into rare diseases, such as HAE, and the identification of new forms is helping to understand certain biological and clinical implications in common conditions, such as cardiovascular

disorders (Moreau et al., 2010). The HAE-FXII subgroup provides an objective basis to analyze patients with similar pathologic forms of angioedema within the heterogeneous area of familial angioedema without wheals and without C1INH deficiency. *F12* mutations therefore constitute an essential genetic marker for advancing knowledge in this new, specific field of study.

The existence of factors other than described mutations (genetic or otherwise) that could explain the low expression of HAE is at the center of research in this field (Ghannam et al., 2013). Genetic variants that affect the activity of the ACE and APP enzymes that degrade bradykinin have been postulated as possible modifiers of phenotypic expression in HAE-FXII (Duan et al., 2009). The aims of this study were to describe and characterize the phenotypic features of southern Spanish individuals who share the same mutation, p.Thr309Lys, and to investigate the potential role of ACE and APP polymorphisms in phenotypic expression.

## 2.2 Methods

### 2.2.1 Design and Participants

This was a descriptive, prospective, observational study performed at the Angioedema Reference Unit of Hospital Universitario Virgen del Rocío in Seville, Southwest Spain. We studied 9 young index females with HAE-FXII from different parts of Andalusia (Seville, Granada, Malaga, Jaen, and Huelva) and from nearby Badajoz in Extremadura. Screening for *F12* mutations was extended to close relatives who agreed to participate. The 9 families were unrelated. This study complies with the guidelines for good clinical practice and was performed in concordance with the Declaration of Helsinki. All participants gave their written informed consent, and the local ethics committee approved the study.

Screening for *F12* mutations in 57 individuals from the 9 unrelated families identified the FXII p.Thr309Lys mutation in 35 individuals, who were recruited for the study between January 2009 and April 2015. After an initial clinical evaluation, the patients were assigned to 3 phenotype groups: asymptomatic carriers, paucisymptomatic patients (those who had experienced a single episode or sporadic

episodes of HAE), and symptomatic patients. We then explored the association between these clinical phenotypes and genetic characterization based on the *ACE* insertion/deletion polymorphism and the *XPNPEP2* c-2399A polymorphism.

### 2.2.2 Clinical Data Collection

We first obtained a detailed medical history from standardized written questionnaires (answered multiple times), electronic medical records, and the hospital's HAE patient registry. After an initial evaluation, we prospectively collected data on clinical characteristics and specific treatment up until April 2016; data were obtained from regular follow-up interviews, patient symptom diaries, and direct telephone contact between patients who experienced attacks and physicians from the HAE Reference Unit. All patients had at least 12 months of follow-up.

### 2.2.3 Laboratory Methods

#### *2.2.3.1 Complement investigations*

Complement component 4 (C4), complement component C1q (C1q), and C1INH levels were measured by radial immunodiffusion at least twice. C1INH activity was determined using chromogenic assays (Berichrom; Siemens, Marburg, Germany) and assessed at the reference laboratory in Barcelona, Spain. Biologic samples were determined during periods of remission in all patients. Two of the patients were additionally studied while symptomatic.

#### *2.2.3.2 Genetic Study*

*Samples obtaining*

The genetic study was carried out from peripheral blood collection through venipuncture into EDTA containing tubes to prevent coagulation. Samples were stored at -80 °C until use.

*DNA isolation*

Genomic DNA isolation was performed starting from 400 µl of blood in the automatic equipment "MagNa Pure Compact" (Roche laboratory) with the commercial kit "MagNa Pure Compact Nucleic Acid Isolation Kit I", following manufacturer's instructions. Isolated DNA was stored at 4ºC until use.

*Screening for mutation in exon 9 of Coagulation Factor XII gene*

a) Amplification of exon 9 by polymerase chain reaction (PCR): exon 9 of *F12* gene was amplified by using the primers previously described by Dewald and Bork (2006) (table 2.1).

**Table 2.1. Primers of exon 9, *F12* gene**

| | |
|---|---|
| Primer Forward (F) | 5'-GAACGTGACTGCCGAGCAAG-3' |
| Primer Reverse (R) | 5'-AGGAGCAGGGGCTGAGGAC-3' |

Reactants volumes and concentrations used during PCR amplification are described in table 2.2.

**Table 2.2. Reactants volumes and concentrations**

| Reactants | Volume | Final concentration |
|---|---|---|
| ADN | 3 µl | 10 ng/µl |
| Tampon PCR 10X | 3 µl | 1X |
| Primer F | 0,1 µl | 0,3 µM |
| Primer R | 0,1 µl | 0,3 µM |
| dNTPs (25 µM) | 0,4 µl | 300 µM |
| $Cl_2Mg$ (25 µM) | 0,5 µl | 0,42 µM |
| Taq Polimerase (Amersham Biosciences) | 0,2 µl | 0,03 U/µl |
| $ddH_2O$ | 22,7 µl | -- |
| Total | 30 µl | -- |

Amplification was performed in a ThermoCycler using the following parameters (figure 2.1).

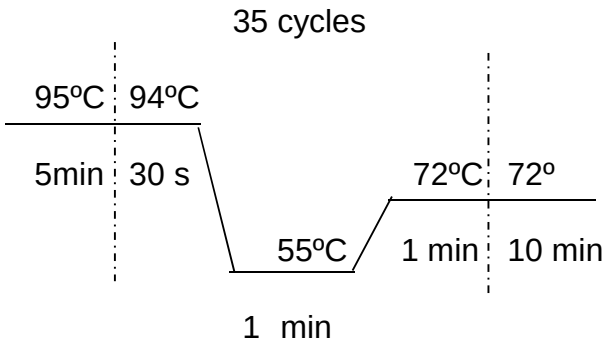


**Figure 2.1. Parameters of amplification.**

b) Bidirectional sequencing and alignment of exo 9: product of amplification was sequencing using the "CEQ Dye Terminator Cycle Sequencing with Quick Start Kit". Sequencing reagents and cycling conditions are described in table 2.3 and figure 2.2 (previously described by Dewald and Bork, 2006; Cichon et al., 2006). Sequencing was carried out in a Beckman CEQ 8000 sequencer following manufacturer's instructions.

**Table 2.3. Sequencing reagent volumes**

| Reagent | Volume |
|---|---|
| DNA | 1 µl of amplification product |
| "DTCS Quick Start Master Mix" | 4 µl |
| Primers (3µM) | 1 µl |
| dd$H_2O$ | 4 µl |

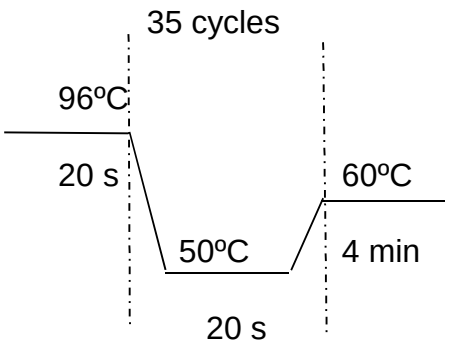


**Figure 2.2. Cycling conditions of sequencing.**

The sequences obtained were alignment with previously genomic sequences published to FXII (GenBank Accession no. NM_000505.2).

*Genotyped for SNP -2399A in XPNPEP2*

The XPNPEP2 c-2399A polymorphism (chromosome X) was genotyped using a real-time PCR TaqMan assay (Life Technologies, Carlsbad, California) following the manufacturer's indications.

*Genotyped for ACE I/D polymorphism*

The *ACE* insertion/deletion polymorphism (chromosome 17) was detected by PCR using previously reported primers and PCR conditions (Rigat et al., 1992) – table 2.4, figure 2.3.

**Table 2.4. Primers of I/D polymorphism in *ACE***

| | |
|---|---|
| Primer F | 5'-CTGGAGACCACTCCCATCCTTTCT-3' |
| Primer R | 5'-GATGTGGCCATCACATTCGTCAGAT-3' |

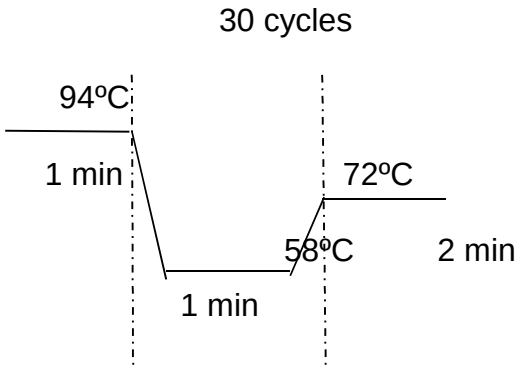


**Figure 2.3. Conditions of PCR**

The amplified products (10 µl) were analyzed by electrophoresis on 1.5% agarose gel. Amplification resulted in 490 and 287 bp bands corresponding to the I allele and D allele, respectively. An additional 335 bp product is present for heterozygotes (heteroduplex DNA fragment).

### 2.2.4 Statistical analysis

Descriptive statistics were used to summarize and analyse the collected data. Quantitative variables were described as means and ranges; qualitative variables were recorded as absolute values and percentages. Associations between polymorphisms and clinical conditions were evaluated using the Fisher exact test.

Data were analyzed using the Open Epi software, version 3.03, for Windows (www.OpenEpi.com). $P < .05$ was considered statistically significant.

## 2.3 Results

### 2.3.1 Clinical Data

Thirty-five individuals from 9 unrelated families (see figure 2.4 for pedigrees) had the FXII p.Thr309Lys mutation. Twenty-eight individuals (80%), including the 9 index patients, were female, and 7 (20%), from 5 different families, were male.

#### 2.3.1.1 *Clinical Characteristics*

The clinical phenotypes are shown in figure 2.5. Three of the 9 index patients (B:II:1-D:II:1-J:II:1) reported a negative family history at the first visit, but symptomatic ancestors were discovered on requestioning the patients after the laboratory tests. Information on time to diagnosis, age at onset, and characteristics of attacks (duration, frequency, affected locations, and most common locations) is given in table 2.5. No wheals were observed, but 1 patient had hemorrhagic lesions (figure 2.6), 5 had ecchymosis in the edematous area, and 2 experienced large, recurrent blisters on their lips. Oral intubation was required once in 2 patients because of a swollen uvula. Another 2 had experienced recurrent transient dysphonia and a foreign-body sensation in the throat. In 7 patients, medical imaging revealed free peritoneal fluid during at least 1 acute episode or in several attacks at different moments; 2 of these patients also had gastrointestinal wall thickening. Four patients had their appendices removed after a diagnosis of acute surgical abdomen; at least 2 of the appendices were normal.

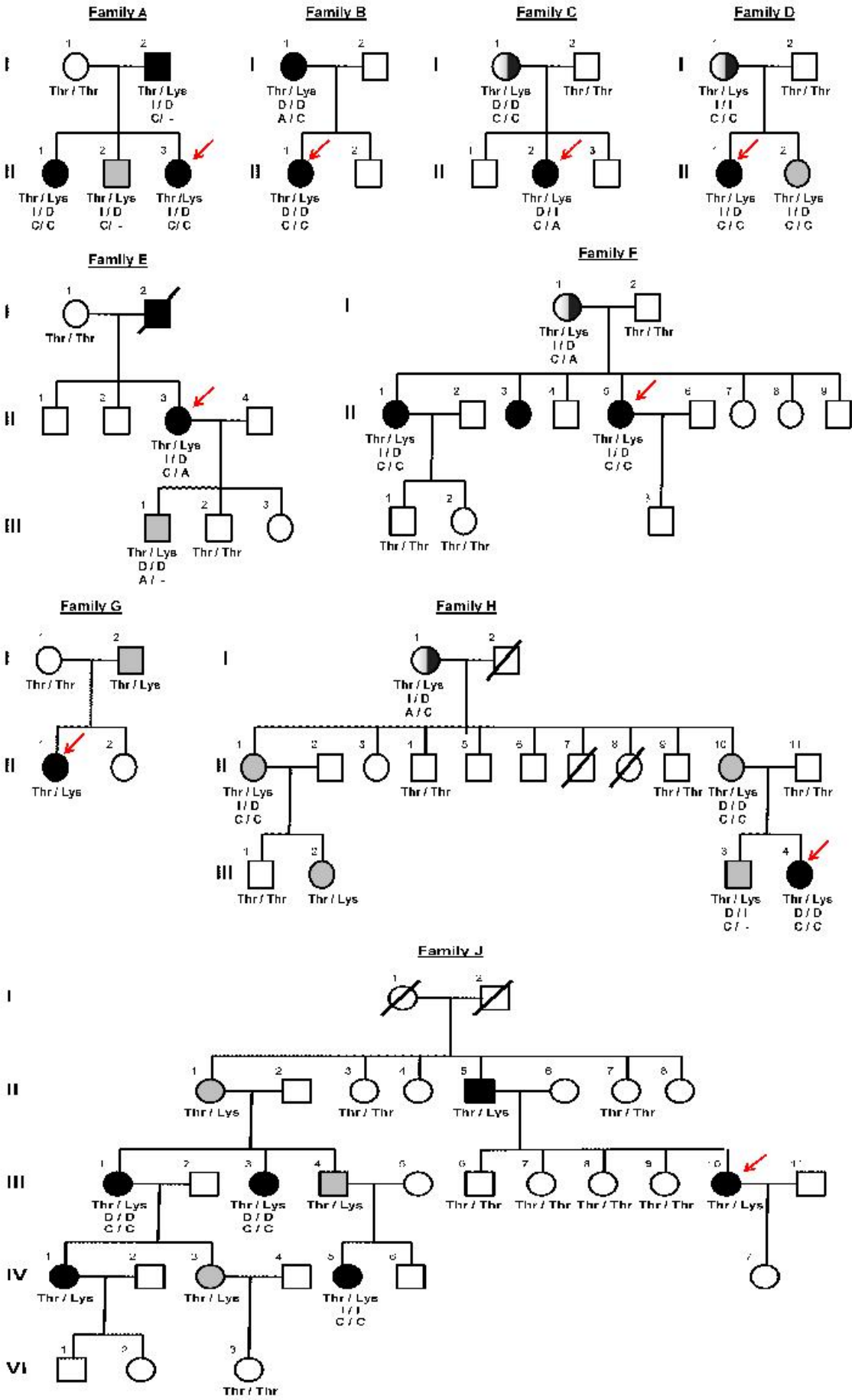
Family A
Family B
Family C
Family D
Family E
Family F
Family G
Family H
Family J

**Figure 2.4**. **Pedigrees of all 9 families carrying the p.Thr309Lys mutation in the *F12* gene.** Black filled symbols represent individuals with recurrent angioedema symptoms, half-filled symbols represent paucisymptomatic patients, and gray filled symbols represent asymptomatic carriers. Arrows indicate the index patients. Carriers (Thr/Lys) and noncarriers (Thr/Thr) of the p.Thr309Lys mutation are shown. D/D, I/D, II indicates *ACE* insertion/deletion polymorphism alleles; C/C (C/- males), C/A, A/- (males), XPNPEP2 c-2399A polymorphism alleles.

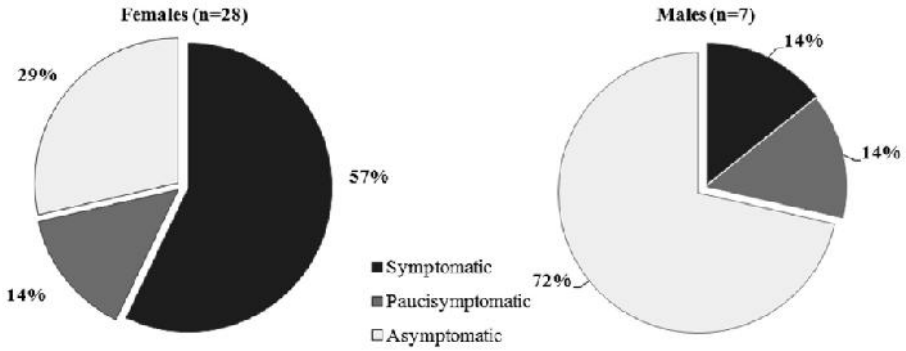


**Figure 2.5. Distribution of clinical phenotypes.**

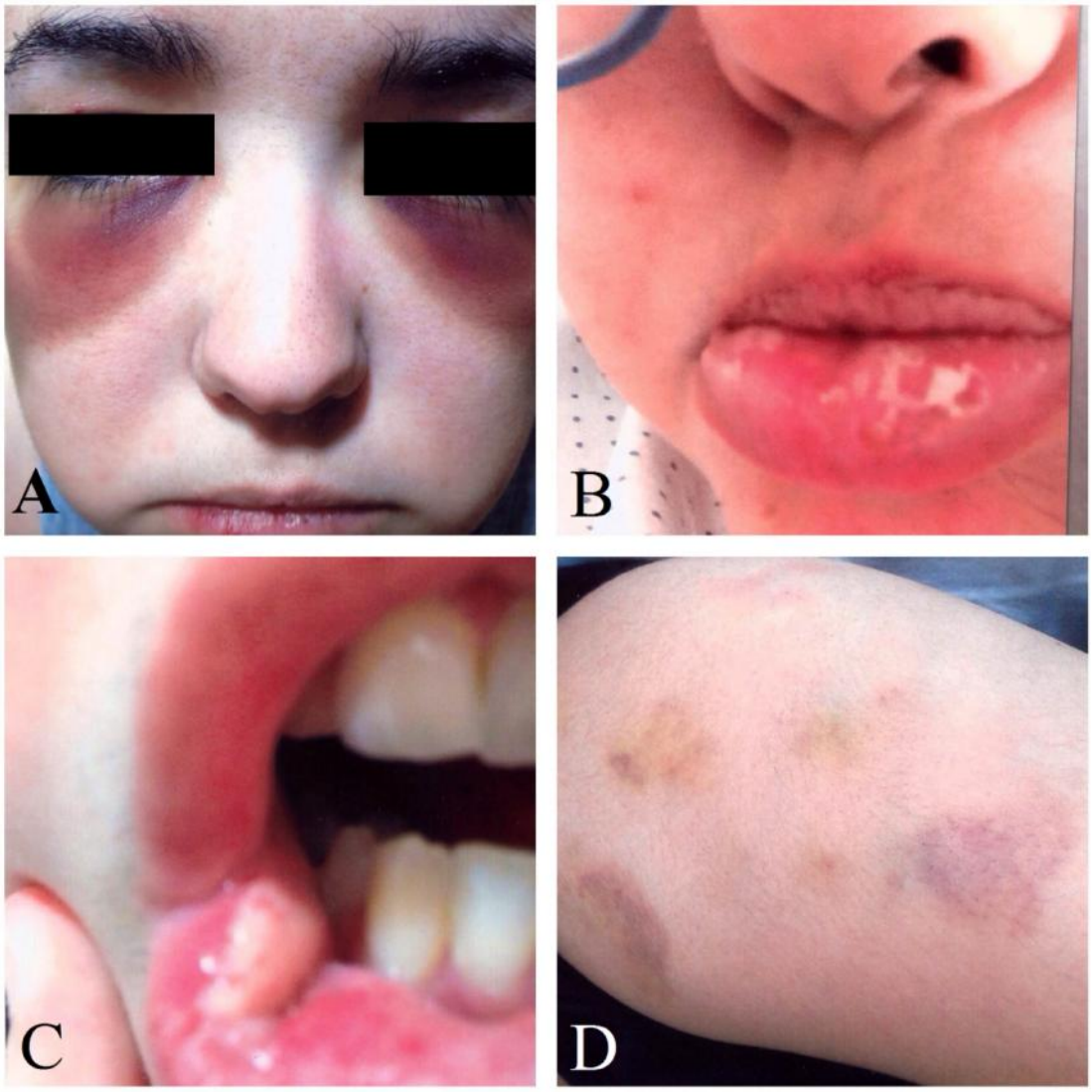


**Figure 2.6**: Hemorrhagic extensive lesions in the angioedema area (A: face; D: thigh). Lip angioedema (B) and detail of a large blister (C).

**Table 2.5. Clinical Characteristics of 22 Symptomatic and Paucisympomatic Patients Carrying the p.thr309lys Mutation in the *F12* Gen[a]**

| | Females | | Males (n=2) |
|---|---|---|---|
| | Symptomatic (n=16) | Paucisymp (n=4) | |
| ·Mean diagnosis delay (range) | 16.5 years (1-47) | Σ | 8.5 (3-14) |
| ·Mean age at onset (range) | 19.9 years (14-28) | Σ | 61.5 (55-68) |
| ·Detailed affected location of attacks at least once | | | |
| Face | 12 (75%) | - | - |
| Eyelid | 10 (63%) | 1 | - |
| Lip | 14 (88%) | 2 | - |
| Tongue | 7 (44%) | 1 | 1 |
| Upper-respiratory tract | 14 (88%) | - | 1 |
| Abdomen | 14 (88%) | - | - |
| Limb | 10 (63%) | - | - |
| Genital | 5 (31%) | - | - |
| Migratory attacks | 6 (34%) | - | - |
| ·More frequent location of attacks | | | |
| Facial area | 4 (25%) | 3 | - |
| Tongue | - | 1 | 1 |
| Upper-respiratory tract | - | - | 1 |
| Abdominal | 10 (63%) | - | - |
| Peripheral | 1 (6%) | - | - |
| Not known[Σ] | 1 (6%) | - | - |
| ·Duration of attacks | | | |
| <48h | 1 (6%) | Σ | 1 |
| 48-96h | 11 (69%) | Σ | Σ |
| >96h | 4 (25%) | 1 | Σ |
| ·Frequency (attacks/year) | | | |
| Hyperoestrognic states | | | |
| Ocassional | - | 3 | NA |
| <3 attacks/year | - | - | NA |
| 4-12 attacks | 7 (44%) | - | NA |
| >12 attacks/year | 7 (44%) | - | NA |
| Not known[Σ] | 2 (12%) | 1 | NA |
| Non hyperestrogenic states | | | |
| Asymptomatic | 8 (50%) | 3 | - |
| Occasional | - | - | 1 |
| <3 attacks/year | 4 (25%) | - | - |
| 4-12 attacks | - | - | 1 |
| >12 attacks/year | 2 (13%) | - | - |
| Not known[Σ] | 2 (12%) | 1 | - |

Paucisymp: paucisymptomatic patients; NA: not applicable; [Σ]: missing data

2.3.1.2 *Estrogen sensitivity, triggers and prodromal symptoms*

Estrogen sensitivity, triggers, and prodromal symptoms are listed for the female patients in table 2.6. Five full-term pregnancies and 1 early pregnancy interruption were registered in 4 patients during the prospective study period (T.G.-Q. and M.P.-S., unpublished data, 2016). No miscarriages were reported. All the women experienced symptoms during pregnancy. Five patients reported undergoing dental procedures without prophylactic treatment, and 3 had noticed local edema (the other 2 had not). One patient had been taking sitagliptin (DPPIV) for several years and was asymptomatic. One male patient experienced angioedema symptoms on using an ACEI. No other triggers or prodromal symptoms were found in male patients.

**Table 2.6. Oestrogen sensitivity, triggers and prodromal symptoms**

| | Females | | Males (n=2) |
|---|---|---|---|
| | Symptomatic (n=16) | Paucisymp (n=4) | |
| ·Estrogen sensitivity | | | |
| Estrogen-dependent | 7 (44%) | 3 | NA |
| Estrogen-sensible | 9 (56%) | - | NA |
| Not known$^{\Sigma}$ | - | 1 | NA |
| ·HES debut | 13 (81%) | 3 | NA |
| ·Triggers | | | |
| OCC | 12/13 | - | NA |
| Pregnancy | (93%) | 4/4 | NA |
| Menstruation | 8/8 | - | NA |
| Ovulation | (100%) | - | NA |
| Stress | 12 (75%) | - | - |
| Pressure/Trauma | 8 (50%) | - | - |
| Infections | 9 (56%) | - | - |
| Cold | 9 (56%) | - | - |
| ACEi | 3 (19%) | 1*/1 | 1/1 |
| ·Prodromal symptoms | 1 (6%) | | |
| Erythema marginatum | - | - | - |
| Unusual fatigue | 1 (6%) | - | - |
| Chest discomfort/ | 3 (19%) | - | - |
| palpitations | 3 (19%) | | |

Paucisymp: paucisymptomatic patients; HES: hyperestrogenic states; OCC: oestrogen-containing contraceptives; ACEi: angiotensin-converting enzyme inhibitors; NA: not applicable; *Not angioedema symptoms but a very disturbing cough.

2.3.1.3 *Long - term prophylactic treatment*

Oral tranexamic acid (1,500 mg/d) was temporarily used in 3 female patients and led to a reduction in the frequency and severity of symptoms. A fourth patient, who was simultaneously taking estrogen-containing contraceptive, did not have any improvement with this treatment. The same patient took danazol (600 mg/d) for 1 year but experienced no improvement in symptoms until she stopped taking estrogen-containing contraceptives. One patient with a more severe form of disease (no clear periods of complete remission between attacks and continuous abdominal pain and discomfort not explained by other causes) required the temporary addition of plasma-derived human C1 inhibitor (pdhC1INH) to tranexamic acid 1,500 mg (1,000 U per week for the first month followed by 500 U every 2weeks for 2.5 months) to treat subintrant abdominal attacks. This treatment allowed her to stay out of the emergency department for more than 6 consecutive weeks. Another patient remained asymptomatic for at least 2.5 years while taking a daily desogestrel pill. The rest of the patients did not require LTP treatment after discontinuation of estrogen-containing contraceptives.

2.3.1.4. *Short-term prophylaxis*

Treatment with intravenous pdhC1INH administered 1 to 2 hours before 4 deliveries and 1 shoulder operation was successful.

2.3.1.5 *Treatment of attacks*

Five of 6 patients who had an attack noticed an improvement with pdhC1INH, and 2 patients (including the nonresponder to pdhC1INH) noticed an improvement with a single injection of icatibant (Table 7). In 1 case, icatibant was administered late (>6 hours after the onset of the attack) and was not effective, but the patient responded well to fresh frozen plasma (2 U). One patient described faster resolution of symptoms (with subsidence of swelling in <24 hours) with a supply of on-hand oral tranexamic acid (500 mg 3 times daily) compared with no treatment. High corticosteroid doses, antihistamines, and/or adrenaline were received several times

by all patients but did not result in disease improvement. Adverse effects were observed with corticosteroid therapy and were particularly severe in 1 case.

**Table 2.7. Treatment of acute attacks**

| | pdhC1INH | Icatibant | Tranexamic acid |
|---|---|---|---|
| Nº (%) of acute attacks (patients) | 11 (5) | 8 (2) | 2 (2) |
| Onset of improvement, min | 20-30 | 15-30 | |
| Objective symptoms relief, h | 1 | 1-2 | 2 |
| Complete resolution, h | 3-24 | 24 | 24-48 |

pdhC1INH: Plasma-derived human C1 esterase inhibitor concentrate.

## 2.3.2 Laboratory Data

### 2.3.2.1 *Complement analysis*

All patients had normal levels of C4, quantitative C1INH, and C1q measured during periods of remission. One female patient had reduced C1INH activity (58%) during a symptomatic period (reference range, 70%-130%). This activity had been measured at the hospital, where she had been misdiagnosed with HAE-C1INH-2 during 8 years of follow-up by the internal medicine department. Another patient had levels in the lower to normal range (70%) while symptomatic and levels of more than 87% when retested during remission. With the exception of 1 patient with C1INH activity levels of 75%, all other patients had activity levels of more than 90% during remission.

### 2.3.2.2 *Genetic study*

The c.983C>A mutation (p.Thr309Lys), heterozygous in all cases, was found in the *F12* gene of 35 patients (Figures 2.3 & 2.7).

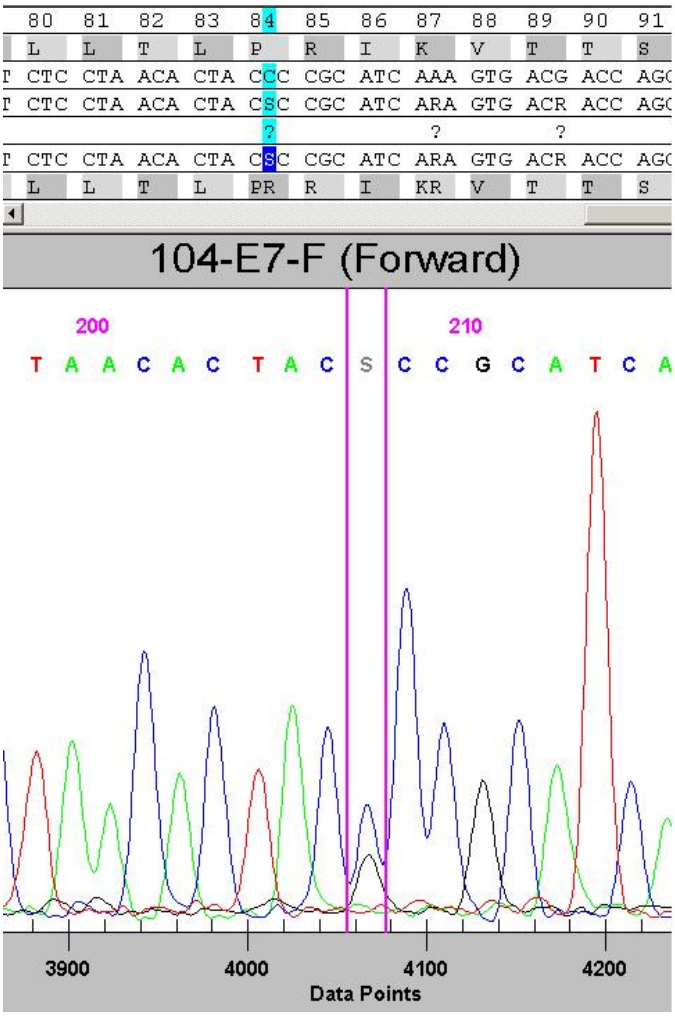


**Figure 2.7. Mutation c.983 C>A in *F12* gene**.

The results for the *XPNPEP2* c-2399A and *ACE* insertion/deletion polymorphisms are detailed in table 2.8 and figure 2.3.

**Table 2.8. Polymorphisms in *ACE* gene and X*PNPEP2* gene.**

| | Total (n=24) | Symptomatic (n=14) | Paucisymptomatic (n=4) | Asymptomatic (n=6) |
|---|---|---|---|---|
| Insertion/deletion (rs1799752) *ACE gene* | | | | |
| D/D | 8 (33%) | 5 | 1 | 2 |
| I/D | 14 (58%) | 8 | 2 | 4 |
| I/I | 2 (8%) | 1 | 1 | 0 |
| SNP 2399-A (rs3788853) *XPNPEP2 gene* | | | | |
| C/C | 15 (63%) | 10 | 2 | 3 |
| C/- | 3 (13%) | 1 | 0 | 2 |
| A/C | 5 (21%) | 3 | 2 | 0 |
| A/A | 0 | 0 | 0 | 0 |
| A/- | 1 (4%) | 0 | 0 | 1 |
| Allele I + allele A | 4 (17%) | 2 | 2 | 0 |

No significant differences were found between the expression of symptoms and the presence of [1] combination of (allele I +allele A) vs no combination – p=0.5759 ; [2] D/D vs (I/D; I/I) – p=0.999; [3] C/C or C/- vs A/C or A/- – p=0.999. Fisher's exact test.

## 2.4 Discussion

We have reported clinical and genetic results for 35 individuals with the *F12* gene p.Thr309Lys mutation from 9 families with HAE-FXII in southwest Spain. The p.Thr309Lys mutation is the most common *F12* mutation found thus far: approximately 179 families worldwide have been reported to carry it vs 2 families with p.Thr309Arg, 1 family with a *F12* deletion and 1 family with a duplication mutation (Bork et al., 2020a). There is a clear predominance of HAE-FXII involvement in populations of Western European, and the results of a haplotype analysis performed in 2006 pointed to a common ancestor from the 11th century. (Cichon et al., 2006). The same haplotype was found in 2 subsequent reports (Nagy

et al., 2009; Firinu et al., 2015). Our study provides unique insights into features of this variant of HAE.

We detected a significant delay in HAE-FXII diagnosis (mean delay, 16.5 years; range, 1-47 years), supporting findings by Firinu et al. This long time to diagnosis is similar to that seen in classic forms of HAE-C1INH (Zanichelli et al., 2013) and has obvious implications in terms of delayed initiation of necessary treatment.

According to reports to date, skin involvement, particularly of the perioral area, is the most common feature of symptomatic HAE (Bork et al., 2009; Zuraw et al., 2012; Cicardi et al., 2014; Craig et al., 2014; Marcos et al., 2014; Mansi et al., 2014; Firinu et al., 2015). Ours is the first large case series in which the gastrointestinal area was the most common site of attacks, although other sites were also involved. Specifically, abdominal involvement was observed in 62.5% of the 16 symptomatic patients (table 2.1). The abdomen is also the most common site for attacks in classic forms of HAE-C1INH (Agostoni et al., 2004), but the likelihood of attributing attacks to other causes of abdominal pain is probably greater in patients with HAE-FXII. Recurrent abdominal or gastrointestinal attacks in female patients were also more common in our series (14 of 16 [87.5%]) than in other large series described to date. Like Bork et al. (2009), we detected a wide diversity of symptoms in the upper respiratory tract mucosa (tongue, uvula, palatal-mouth swelling, bolus foreign-body sensation). Although these symptoms were experienced by 14 of the 16 patients (87.5%), they were fortunately infrequent. Nonetheless, this high rate of laryngeal swelling suggests, contrary to what is commonly thought (Bork et al., 2007), that nearly all patients with HAE-FXII could be at risk of a life-threatening attack.

In 2007, Bork et al. drew attention to the presence of hemorrhagic lesions and ecchymosis in edematous areas in patients with HAE-nC1INH. In our series, these clinical features were present in a third of patients. The case of 1 female patient with severe disease is particularly noteworthy. This patient experienced multiple, extensive hemorrhagic lesions under the eyes together with bruising in several locations (figure 2.3) during different angioedema episodes that led emergency department staff to suspect mental insanity and acts of self-injury. Such situations

may be amplified by feelings of guilt, which were expressed by at least 2 of our young patients. The image of our patient strongly resembles that of a patient with an *F12* mutation described by Bork et al.

Multi-location attacks have been described as rare in HAE-nC1INH and HAE-FXII (Craig et al., 2014), but 37.5% of patients in our series had migratory attacks, supporting the possible implication of an FXII fragment, as reported by Kaplan & Joseph (2014).

In our study, 28 of 35 carriers (80%) of the p.Thr309Lys mutation were women, and 12 of 28 (42.9%) were asymptomatic or paucisymptomatic; this rate is slightly higher than that of 34% reported by Charignon et al (2014). Notably, 7 of these 12 women were older than 50 years (data not shown), and it can therefore be assumed that there is little likelihood that they will have any clear signs of involvement in the future. It also indicates that up to 25% of women might not be easily recognized as transmitters of this life threatening disease.

As expected, estrogen-containing contraceptive use played a relevant role in the expression of disease in our patients. Pregnancy was also a trigger, but it seemed to have a lesser impact in terms of severity of symptoms (T.G.-Q. and M.P.-S., unpublished data, 2016). Other endogenous sex hormones movements also acted as triggers in our female population. More than 50% of symptomatic women reported additional, non-sex hormonal triggers (particularly stress and pressure or minor trauma) in both hyperestrogenic and non-hyperestrogenic states.

Prodromal symptoms have been poorly described in HAE-FXII. In our series, 18.8% of symptomatic females reported unusual fatigue, whereas another 18.8% reported chest discomfort or palpitations.

Normal C1INH function has been proposed as a necessary condition for the diagnosis of HAE-nC1INH and hence HAE-FXII (Zuraw et al., 2012; Cicardi et al., 2014; Craig et al., 2014). In our case series, only 3 of the 35 individuals (9.1%) had transiently decreased functional C1INH values (below or around the lower normal range), and one of these had been misdiagnosed with HAE type II for several years. We would like to highlight the importance of confirming decreases in functional values to avoid misdiagnoses with other forms of HAE. Slightly

decreased C1INH activity has been previously reported, but in most cases, levels were measured during pregnancy (Bouillet et al., 2007; Martin et al., 2007; Marco et al., 2012), or estrogen-containing contraceptive use (Bouillet et al., 2007; Marcos et al., 2014) hence, the hypothesis that decreases may be the result of high proteolytic activity during symptomatic periods (Charignon et al., 2014).

Similar to previous reports (Bork et al., 2009; Marcos et al., 2012; Charignon et al., 2014) our study found high interpatient variability in the frequency and severity of symptoms, even among members of the same family. Until recently, there was no explanation for this extreme variability, which is also seen in individuals over time. In addition to the genetic basis of HAE, exaggerated kinin formation, combined with some form of impaired kinin catabolism, seems to have a central, additive role in HAE-nC1INH as a whole (Ghannam et al., 2013; Cichon et al., 2006; Charignon et al., 2014; Kaplan & Joseph, 2014). Kinin overproduction was recently confirmed in both HAE-FXII (Charignon et al., 2014) and HAE-C1INH (Suffritti et al., 2014). However, levels do not seem to be sufficient to distinguish among different phenotypes, lending strength to the idea that individual variations in kinin degradation enzyme activity influence disease expression. Further supporting this idea, Charignon et al found a correlation between a severe phenotype of HAE-FXII and low kinin catabolism and decreased carboxypeptidase N and ACE activity (a condition favorable to bradykinin accumulation).

Regarding a possible genetic influence on kinin catabolism, Duan et al (2009) suggested that the *ACE* insertion/deletion polymorphism - the major *ACE* gene locus that influences serum ACE concentration (Rigat et al., 1992)- and the APP-regulatory polymorphism -*XPNPEP2* c-2399A - might be involved in reduced APP activity. They found copies of an I allele (associated with low ACE serum levels) and an A allele (associated with low APP serum activity) in 3 symptomatic female patients from the same family and hypothesized that this genetic combination might contribute to disease expression in FXII mutation carriers.

Our study incorporated additional analyses of bradykinin degradation enzyme polymorphisms. Our results, however, do not support the hypothesis proposed by Duan et al, because we found no statistically significant differences in the distribution of the *ACE* insertion/deletion polymorphism or the APP regulatory

polymorphism, evaluated together and separately, among the clinical phenotypes established in FXII mutation carriers. It must, however, be remembered that the statistical power of our study was limited by the small sample size.

There are no approved treatments for HAE-FXII so all our patients were treated off-label with their explicit informed consent. Tranexamic acid, progesterone, and danazol have been reported to be effective as LTP (Bork et al., 2009; Bouillet et al., 2007; Marcos et al., 2012). Disease control improved in all 5 patients (31.3%) in our series who received LTP: 4 received tranexamic acid, temporarily associated with pdhC1INH in 1 case, and the fifth received progesterone.

The use of pdhC1INH as short-term prophylaxis (STP) during deliveries was effective, as previously reported (Bouillet et al., 2007; Picone et al., 2010). The likelihood of symptoms during dental procedures (Bork et al., 2009; Bell et al., 2008; Prieto et al., 2009; Marcos et al., 2012) and invasive diagnostic medical techniques, such as endoscopies (Marcos et al., 2012), should be considered to justify the use of STP.

A positive response to pdhC1INH (despite absence of C1INH deficiency) and icatibant (a bradykinin receptor 2 antagonist) was observed during 20 attacks in 7 patients, supporting previous reports of the general effectiveness of these 2 drugs for attacks (Bouillet et al., 2007; Bork et al., 2009; Marcos et al., 2012; Mansi et al., 2014; Craig et al., 2014; Firinu et al., 2015).

The small number of symptomatic patients and inclusion of some retrospective data could be considered a limitation of our study. However, HAE is a rare disease, and we consider that our data add to the limited body of knowledge in this area. Analysis of electronic medical records also constitutes an essential and objective tool for the identification of symptoms that frequently go unnoticed.

In summary, our findings indicate that certain HAE-FXII populations may not necessarily have different symptom patterns to patients with HAE-C1INH, as initially described. Involvement of locations other than the skin is common and could even be the main expression of disease. The significant delay in HAE-FXII diagnosis (mean of 16.5 years) suggests a possible lack of awareness in the medical

community about this life-threatening disease. The absence of a family history in women with recurrent angioedema without wheals and without C1INH deficiency is clearly not reason enough to exclude genetic testing. The enzyme polymorphisms studied were not a major determinant of disease expression in our population.

## 2.5 Acknowledgments

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# Chapter 3

# Management of Pregnancy and Delivery in Patients with Hereditary Angioedema with C1 Inhibitor Deficiency

# Abstract

Background and Objective: There is little information on pregnancy and delivery in patients with HAE-C1INH. The aim of this study was to describe the effect of pregnancy and deliveries on symptoms of HAE-C1INH and review the need for and safety of treatments available during the study period.

Methods: Retrospective review using a purpose-designed questionnaire of 61 HAE-C1INH patients from 5 hospitals specialized in the management of HAE in Spain. The outcomes measured were number of pregnancies, changes in symptoms during pregnancy and delivery, mode of delivery, type of anesthesia during delivery, treatments received, and tolerance of treatments.

Results: We reviewed 125 full-term pregnancies (89 without a prior diagnosis of HAE-C1INH), 14 miscarriages, and 4 induced abortions. Patients reported an increased frequency of HAE-C1INH symptoms in 59.2% of pregnancies (74/125) and the presence of symptoms throughout pregnancy in 40% (50/125). Prophylactic HAE-C1INH therapy was used during 9 (7.2%) of the 125 pregnancies. Nine patients—in 11 pregnancies (8.8 %)—received treatment for acute attacks. Most deliveries (n=110, 88%) were vaginal. A caesarean section was necessary in 15 cases (12%). STP with pdhC1INH was administered before 14 deliveries (11.2 %); 111 deliveries (88.8 %) were performed without premedication and were well tolerated. Anesthesia was used in 51 deliveries (40.8%).

Conclusions: Pregnancy has a variable influence on the clinical expression of HAE-C1INH. Attacks tend to occur more frequently but not to increase in severity. Vaginal delivery was mostly well tolerated. pdhC1INH prophylaxis should be administered prior to caesarean delivery and is also recommended before vaginal delivery if there are additional risk factors. pdhC1INH should always be available in the delivery room.

## 3.1 Introduction

HAE-C1INH is a rare disease (Mansi et al., 2015). In Spain, a minimal prevalence of 1.09 cases per 100 000 inhabitants has been reported (Roche et al., 2005). Two subtypes of HAE-C1INH have been described: HAE-C1INH-1, with reduced functional C1 inhibitor levels and HAE-C1INH-2, with normal or high C1INH protein levels but reduced C1INH function (Rosen et al., 1965). Another type of HAE with normal C1INH levels has also been described (Bork et al., 2000; Binkley & Davis, 2000), but in this work we focus just on types I and II. HAE-C1INH is characterized by nonpruritic, nonpitting edema that typically affects different locations. Abdominal pain, distension, nausea, or vomiting may also be present secondary to edema of submucosal tissues of the gastrointestinal tract (Agostoni et al., 2004). Upper airway involvement can be fatal, and mortality due to suffocation can be as high as 33% in inappropriately treated patients (Bork et al., 2012). Estrogens, trauma, and stressful situations can worsen the course of disease and consequently, pregnancy and delivery may be special periods for female patients (Longhurst, 2005; Bouillet et al., 2008; Browen et al., 2010). Three treatment options are available for HAE-C1INH: LTP, STP, and acute treatment. LTP consists mainly of AAs, but these can cross the placental barrier, possibly producing virilization, and should therefore be strictly avoided during pregnancy and lactation; antifibrinolytic agents are also used for LTP, though they are less effective (Caballero et al., 2012, 2014). pdhC1INH can be used for LTP when other treatments are contraindicated, ineffective, or poorly tolerated. STP with pdhC1INH is the most effective preventive therapy for patients undergoing surgery. pdhC1INH has traditionally been the treatment of choice for acute attacks during pregnancies; an alternative, though less safe, option is fresh frozen plasma (Craig et al., 2012; de Serres et al., 2003). Icatibant acetate, a BK2R blocker, as well as a recombinant version of the human C1 inhibitor protein, have been approved by the European Medicines Agency and the US Food and Drug Administration, and together with ecallantide, are licensed for the treatment of acute edema attacks in adult patients; they have not yet, however, been approved for use in pregnancy (Gras, 2009; Zuraw et al., 2010; Levy et al., 2010). In this study, we describe the experience of 5 major HAE centers in Spain in managing HAE-C1INH during

pregnancy and delivery with the aim of adding to the body of knowledge regarding the management of obstetric events in this setting.

## 3.2 Patients and Methods

### 3.2.1 Study population

Patients from 5 HAE reference hospitals in Spain were recruited for the study. The participating centers were Hospital Universitario Virgen del Rocío in Sevilla, Andalusia, Hospital Universitario La Paz in Madrid, Complejo Hospitalario Universitario de Vigo in Vigo, Galicia, Hospital Universitario Vall d'Hebron in Barcelona, Catalonia, and Hospital General Universitario Gregorio Marañón in Madrid.

### 3.2.2 Methods

We performed a retrospective study of HAE-C1INH patients who had become pregnant before or after HAE-C1INH diagnosis. A specific questionnaire was designed and filled out with information from clinical charts or from telephone interviews when data were missing. Information about full-term pregnancies, miscarriages, and induced abortions was also included. The research ethics committees of Hospital Universitario Virgen del Rocío and Hospital Universitario La Paz approved the study.

The criteria to define worsening of symptoms compared with the baseline condition included an increase in attack frequency as well as the duration of single attacks. Increased severity was also considered a criterion of worsening but there were no cases (ie, there were no changes in the distribution of sites of involvement). The criteria to define an improvement in HAE symptoms included no symptoms, fewer symptoms, or milder attacks.

### 3.2.3 Statistical analysis

Descriptive statistics were used to summarize and analyse the collected data. Analysis was performed using the IBM SPSS v19.0 statistical package. Quantitative variables were described as means and ranges; qualitative variables were recorded as absolute values and percentages. Categorical variables were compared using the $\chi 2$ Pearson test. P values of less than .05 was considered to be statistically significant.

## 3.3 Results

We reviewed 125 pregnancies in 61 patients as well as 14 miscarriages and 4 induced abortions. The percent distribution of patients and full-term pregnancies among the 5 study hospitals are shown in the Figure 1.

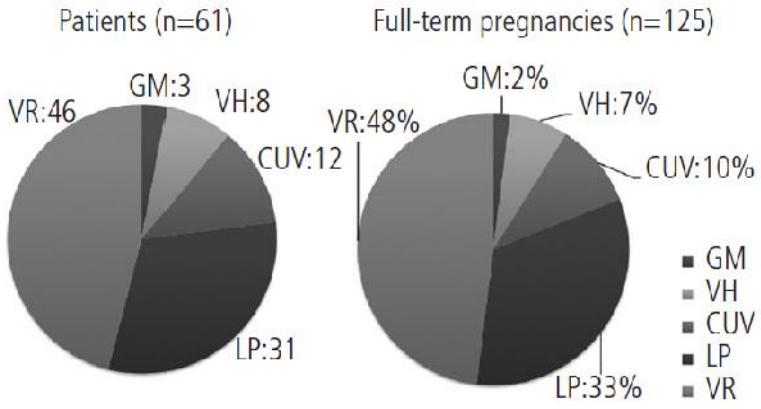


**Figure 3.1. Distribution of patients and full-term pregnancies among the 5 reference hospitals for hereditary angioedema**. CUV indicates Complejo Hospitalario Universitario de Vigo; GM, Hospital General Universitario Gregorio Marañón; LP, Hospital Universitario La Paz; VH, Hospital Universitario Vall d'Hebron; VR, Hospital Universitario Virgen del Rocío.

### 3.3.1 Clinical characteristics of the pregnancies

The mean age of patients at the time of HAE-C1INH diagnosis was 30.6 years (range, 6-60 years), and the mean age at the start of pregnancy was 27.1 years (range, 16-42 years). There was a prior diagnosis of HAE-C1INH in just 44 (30.7 %) of the 143 initial gestations and in 36 (28.8 %) of the 125 full-term pregnancies. Thirty-five women (57.4%) were not aware that they had HAE during 89 pregnancies (71.2%). Twenty of the 61 patients had only 1 pregnancy. Data were collected between the years 2006 and 2010 and corresponded to pregnancies that had occurred more than 20 years previously in nearly half of the patients (49.2%), 6 of whom had only been pregnant once.

A greater frequency or duration of acute attacks was reported for 59.2% of the pregnancies, no changes with respect to baseline symptoms were reported in 26.4% of cases, and symptoms improved in 14% of cases. There were no differences in attack severity from one trimester to the next, and in 40.0 % (50/125) of pregnancies HAE symptoms were present throughout the pregnancy. Patients with more than 1 gestation (67.2%) generally described a similar course for each of their pregnancies (similar C1-INH-HAE symptoms in 85.4% of cases). There were no changes in sites of involvement but there was an increase in the frequency of mild abdominal crises. Non-life-threatening symptoms were reported in the 125 full-term pregnancies or abortions.

We found no statistically significant differences for disease course on comparing percentages between the group of women with a known HAE-C1INH diagnosis before pregnancy (n=36) and the group of women with an unknown HAE-C1INH diagnosis at the time of pregnancy (n=89) (Table 1).

**Table 3.1. Changes in HAE-C1INH symptoms during pregnancy in patients with and without a previous diagnosis of HAE-C1INH[a]**

| | | Previous diagnosis of C1-INH-HAE | |
|---|---|---|---|
| | | Yes n=36 (28'8%) | No N=89 (71'2%) |
| Worsening of C1-INH-HAE symptoms during pregnancy | No n=51 (40'8%) | 12 (33'3%) | 39 (43'8%) |
| | Yes n=74 (59'2%) | 24 (66'7%) | 50 (56'2% |

HAE-C1INH, hereditary angioedema with C1 inhibitor deficiency. [a] No statistically significant differences were noted between groups.

### 3.3.2 Clinical characteristics of the deliveries

The vast majority of deliveries (n=110, 88%) were vaginal; forceps and vacuum extraction were used in 9 and 5 deliveries, respectively (table 3.2).

**Table 3.2. Mode of Delivery and Type of Anesthesia**

| Mode of delivery | Type of Anesthesia | | | |
|---|---|---|---|---|
| | Epidural | General | Pudendal block | No anesthesia |
| Caesarean section (n=15) | 6 | 9 | 0 | 0 |
| Vaginal delivery | | | | |
| Non-instrumental (n= 96) | 21 | 4 | 0 | 71 |
| Vacuum (n= 5) | 4 | 2 | 3 | 0 |
| Forceps (n= 9) | 0 | 2 | 0 | 3 |
| Total (n=125) | 31 | 17 | 3 | 74 |

Caesarean sections were necessary in 15 deliveries (the reasons are summarized in table 3.3). Only 5 of the 15 women who underwent a caesarean section knew that they had HAE-C1INH and had been treated with pdhC1INH prior to delivery; tolerance was good in all cases and the 10 women who did not receive premedication for the caesarean section did not recall any adverse perioperative events. Anesthesia was used in 51 deliveries (40.8%) and in the 4 induced abortions. No specific complications associated with the anesthesia were reported.

**Table 3.3. Reasons for Cesarean Delivery (Rate 12%)**

| Patient | Year | Pregnancy number | Age, y | Type | Reason |
|---|---|---|---|---|---|
| 1 | 1975 | Second | 28 | A | Dystocia |
| 2 | 1983 | First | 19 | E | Narrow pelvic opening |
| 3 | 1983 | First | 23 | A | Anomalies of umbilical cord |
| 4 | 1984 | Third | 39 | E | Other complications |
| 5 | 1985 | Third | 25 | A | Dystocia |
| 6 | 1993 | Second | 40 | E | Other complications |
| 7 | 1994 | First | 29 | A | Dystocia |
| 8 | 1994 | Second | 27 | E | HAE diagnosed during pregnancy |
| 9 | 1996 | First | 27 | E | Narrow pelvic opening |
| 10 | 1998 | First | 28 | E | Unknown. Known diagnosis of HAE |
| 11 | 2001 | Second | 37 | E | Narrow pelvic opening |
| 12 | 2002 | First | 27 | A | Fetal bradycardia. Known diagnosis of HAE |
| 13 | 2005 | First | 36 | E | Unknown. Known diagnosis of HAE |
| 14 | 2006 | Second | 35 | E | Fetus in breech position. Known diagnosis of HAE |
| 15 | 2007 | Third | 28 | A | Dystocia |

### 3.3.3 Long-term prophylaxis, Short-term prophylaxis and acute treatment

Only 13 patients (21.3 %) were receiving LTP before they became pregnant. Nine patients had been receiving AAs. Seven of these stopped AA therapy before they conceived and the other 2 stopped 1 month after conception, on confirmation of their pregnancy.

LTP was only used during 9 of the 125 pregnancies: epsilon-amino-caproic acid was used in 1 case, tranexamic acid in 2 cases, AAs in 2 cases, and pdhC1INH

(Berinert, CSL-Behring) in 4 cases. In the 2 cases in which AAs were temporarily used (for 8 and 12 weeks), the drug was administered after confirmation that the fetus was male. (As mentioned previously, 2 other patients interrupted LTP with AAs when they became aware they were pregnant).

STP with pdhC1INH was only administered prior to 14 deliveries; 5 patients received 1000 U and 9 patients received 500 U. All the patients demonstrated good tolerance of the infusion and no adverse events were reported. Of the 14 patients who received STP before delivery, 8 had experienced a worsening of C1INH-HAE symptoms during pregnancy and 4 reported no changes with respect to before the pregnancy. None of these 14 patients had HAE symptoms during delivery or in the next 48 hours. No premedication was used in 111 deliveries and mild local C1INH-HAE symptoms were observed in just 6 vaginal deliveries (5.4%). These local symptoms ceased spontaneously.

Nine patients received treatment for an acute attack during 11 pregnancies. pdhC1INH (Berinert) was administered in all 9 cases and 1 of the patients additionally received tranexamic acid and other corticosteroids. This last patient was not diagnosed with C1-INH-HAE until 5 years later. Seven patients were administered a total of 618 vials of pdhC1INH 500 U (Berinert) to treat acute attacks and as LTP (4 cases) in 9 pregnancies (average of 4.16 vials/mo); 1 patient received, as LTP, a total of 356 vials in 2 consecutive pregnancies (average 4.94 vials/wk); no adverse effects were reported. None of the newborns developed health problems or experienced adverse effects attributable to any of the drugs used.

### 3.3.4 Miscarriages and abortions

Our patients reported 14 miscarriages, most of which occurred during the first trimester. Additionally, there were 4 registered abortions. There were also 3 fetal deaths and 1 premature delivery with complications (deafness and visual problems). None of the miscarriages or cases of fetal damage occurred in patients temporarily exposed to AAs.

## 3.4 Discussion

We have reported on the course and management of 125 full-term pregnancies and deliveries, 14 miscarriages, and 4 induced abortions in 61 HAE-C1INH patients through a nationwide cooperation study. A clear set of international HAE-C1INH guidelines containing recommendations for delivery and pregnancy follow-up and information on the benefits and risks associated with each of the available treatments was published over the last decade (Caballero et al., 2012; Craig et al., 2012). Three case series have been published (Chinniah & Katelaris, 2009; Czaller et al., 2010; Martinez-Saguer et al., 2010), but most publications on HAE and pregnancy report on few pregnancies and deliveries (between 1 and 6). Our study is the largest to date and can be considered representative of the approach to and management of pregnancies and deliveries in HAE-C1INH patients in Spain in recent decades.

Mild aggravation of HAE-C1INH symptoms was experienced in 59.2% of the pregnancies in our series; this figure is similar to previous reports (Czaller et al., 2010; Martinez-Saguer et al., 2010). The mild worsening detected is more likely to be attributable to changes associated with pregnancy rather than to discontinuation of HAE treatment, as most patients were not receiving regular treatment before they became pregnant.

No statistically significant differences were observed for course of disease on comparing patients with a previous diagnosis of HAE-C1INH and those without one (table 3.1). Of the 36 pregnancies in which there was a confirmed diagnosis of HAE before pregnancy, there was an improvement in clinical signs in 6 cases (16.7%) and no changes in another 6.

The majority of the 41 patients with more than 1 pregnancy (85.4%) noted that the disease manifested itself in a similar way during each pregnancy and only 4 patients described a change in symptoms from one pregnancy to the next.

The course of symptoms has been reported to vary greatly between pregnancy trimesters (Czaller et al., 2010; Martinez-Saguer et al., 2010), but in more than half

of the pregnancies in our series, there were reports of worsening of symptoms throughout the pregnancies, with no clear differences observed between trimesters.

Chinniah & Katelaris (2009) described a group of 7 patients with 16 pregnancies who experienced fewer attacks as the pregnancy progressed and more attacks during the first trimester. In all cases, the patients had edema attacks, but the authors did not report whether symptoms worsened during pregnancy in comparison with previous or posterior periods.

In our case series, none of the 14 miscarriages were directly related to angioedema attacks and this rate of 10.1% can be considered low compared with average rates reported for the general European population (10%-20%) (Bouillet et al. 2008). The decision to terminate the pregnancy was at least partially related to the diagnosis of HAE in 2 of the 4 voluntary interruptions. One of the 3 fetal deaths was due to a congenital cardiopathy and 2 (in the same woman) were due to Rh incompatibility. There was just 1 new-born who developed complications because of premature delivery (at 6 months). None of these cases were related to the temporary use of AAs.

Almost all studies of patients with HAE-C1INH have reported good progression of HAE symptoms during pregnancy and delivery. Although HAE symptoms may be present, they are usually mild and rarely life-threatening. McGlinchey et al. (2000) reported a life-threatening laryngeal angioedema attack in the 25th week of gestation that was treated with fresh frozen plasma and 2000 U of pdhC1INH and resolved completely. Postnikoff & Pritzker (1979) wrote about the only death reported of a pregnant HAE woman 110 hours after delivery. This patient developed perineal swelling and a purulent discharge from the episiotomy 48 hours postpartum. Autopsy revealed edema of the subcutaneous tissues, which was most prominent in the perineal region, with severe effusions present within all body cavities and septic shock.

LTP during pregnancy was seldom used in the pregnancies reported in this case series; in some cases the patients had not yet been diagnosed with HAE-C1INH, while in others presumably little was known at the time about the safety of

HAE-C1INH treatments. We administered C1INH concentrate as LTP in 4 pregnancies in 2009 and 2010; the drug was administered off-label, with the patients' informed consent, due to a clear exacerbation of symptoms following discontinuation of effective AA therapy.

In our series vaginal delivery without prophylactic treatment was well tolerated, and although trauma and stress are known to trigger attacks, very few patients developed mild local edema. There have been many isolated reports of pregnant HAE-C1INH patients who experienced no edema attacks after a vaginal or cesarean delivery with prophylactic pdhC1INH treatment (Cox & Holdcroft, 1995; Hawthorne & Gooi, 1996; Marescal et al., 1999; Nathani et al., 2006; Caliskaner et al., 2007; Montinaro & Castellano, 2010). There have also been reports of 2 cesarean deliveries (Chappatte & de Swiet, 1988) and 2 vaginal deliveries (Stiller et al., 1984) that were well tolerated without prophylactic therapy. Anecdotal reports of STP with fresh frozen plasma and danazol prior to delivery, with good outcomes, have also been published (Galan et al., 1996; Boulos et al., 1994). In the series reported by Martinez-Saguer et al (2010), all the patients received prophylactic pdhC1INH treatment prior to delivery. Chinniah & Katelaris (2009) did not report any symptomatic deliveries in their case series, although prophylactic pdhC1INH was used in only 5 of the 16 deliveries. In the series by Czaller et al (2010), prophylactic treatment prior to delivery was used in 9 of 82 deliveries, with good tolerance in all cases; the same authors described asymptomatic deliveries in the 73 deliveries performed without prophylaxis. The proportion of deliveries without pretreatment and the good outcomes in cases in which prophylaxis was not used match the findings of our case series.

Only 5 of 15 women who underwent a cesarean section in our series knew about their condition and were treated with pdhC1INH prior to delivery. Of the 10 cesarean deliveries that did not include prophylactic treatment, a single patient experienced mild HAE symptoms during delivery and 48 hours postpartum and subsequently required treatment with pdhC1INH. Czaller et al (2010) described the use of prophylactic treatment prior to cesarean delivery in 1 of 8 cases and reported no symptoms in any of these deliveries. Martinez-Saguer et al (2010), in turn, reported 8 cesarean sections out of 35 deliveries; in all cases, the patients underwent

prophylactic treatment with pdhC1INH immediately before delivery and no attacks were reported.

Our study has certain limitations. Information was obtained retrospectively through a questionnaire completed using clinical records and telephone interviews in the case of missing data. Due to the retrospective nature of the study, the information corresponded to pregnancies from several years earlier (up to 20 years in nearly half of the patients). It is also important to note that HAE-C1INH had not been previously diagnosed in 89 pregnancies, and in the majority of cases, a diagnosis was not made until several years later. Therefore, the patients may have underestimated the severity of symptoms or failed to associate them with HAE-C1INH. Nevertheless, the fact that most patients had not been diagnosed with HAE-C1INH before conception and did not receive any treatment during pregnancy provides interesting insights into the natural course of HAE-C1INH during pregnancy and labor.

In conclusion, after reviewing data from our series we can conclude that pregnancy appears to have a variable influence on the clinical expression of HAE-C1INH, and may differ from one patient to another and to a lesser extent, from one pregnancy to the next. Attacks tend to occur more frequently but they do not appear to increase in severity. pdhC1INH prophylaxis should be administered prior to cesarean delivery and is also highly recommended for vaginal delivery in patients with additional risks factors or severe HAE-C1INH symptoms during pregnancy or previous deliveries. pdhC1INH (2000 U) should always be available in the delivery room and during hospitalization. To improve outcomes in pregnant women with HAE-C1INH, it would be wise to maintain observation for 48 hours postpartum.

## 3.5Acknowledgments

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# Chapter 4

# General Conclusions

HAE is a rare underdiagnosed disease. It is characterised by episodes of HAE of the skin and mucous membranes, abdomen, as well as pharyngeal and laryngeal episodes that can lead to death by asphyxia (Cicardi et al., 2014). Two main groups have been described:

(1) HAE-C1INH is the most frequent and best characterised form, presenting a well-known pathophysiological mechanism of C1INH deficiency. Despite this, its epidemiological aspects are not yet fully understood, as no aggregated data exist. Clinical phenotypes of the disease are described (Cicardi & Zuraw, 2018), however, patients cannot be stratified because we lack prospective data in large populations, collected with the same criteria, on the frequency, duration and severity of attacks. There was an attempt at a European Registry 20 years ago, that could not be sustained (Zingale et al, 2007). Recently a new Global Registry has been launched (Zanichelli et al., 2021). At the moment it only collects basic data, implementations are a future project. However, they have reported that there is a lack of participation, with more than 90% of the data collected being from a single country, which obviously limits its main objective. Therefore, to date, the main data we have on the disease are based on published case series and systematic reviews.

(2) HAE-nC1INH includes those patients who do not have a C1INH deficiency. Advances in the description of the pathophysiological mechanisms of this group seem to suggest that it is a heterogeneous group encompassing several pathophysiological alterations affecting both KKS and intrinsic endothelial dysfunction. To date, 9 mutations have been identified: 4 located in the *F12* gene, and the others in the *PLG, ANGPT, KNG, MYOF* and *HS3ST6* genes. In many families, the genetic background is still unknown (Bork et al., 2015). Despite recent discoveries, there are still many gaps in terms of pathophysiology, clinical aspects, treatment and epidemiology.

In our first part of the project, we conducted a clinical study, as detailed as possible, of a HAE-FXII population. All our patients had the p.Thr309Lys mutation, to date described as the most frequent mutation found in HAE-nC1INH. Although no global data are available, a prevalence of 1:400,000 has been estimated

in Germany (Bork et al., 2015). From a clinical point of view, our work has allowed several relevant contributions: it is the first series study where a significant occurrence of abdominal symptoms, which may often be underestimated or misdiagnosed; we also found a higher rate of pharyngeal/laryngeal symptoms than in previous reports (Bork et al., 2007). This demonstrate that virtually all patients with HAE-FXII may be at risk of having a life-threatening attack; we describe for the first time in the literature the existence of previously unknown prodromal symptoms. Recently, the first systematic review of the clinical features of genetically characterized HAE-nC1INH was performed (Bork et al., 2020a). This review highlighted the unique treatment contributions (especially for STP and LTP) covered in this work.

No obvious cause has yet been found to explain the wide variety in clinical expression among individuals diagnosed with HAE, both in the classical form, HAE-C1INH, and HAE-nC1INH. Conjectures focus on an exaggerated production of bradykinin or a decrease in its metabolization. Therefore, the hypothesis that there may be other genetic alterations that may influence this clinical expression has been put on the table several times. Following the finding in 3 patients diagnosed with HAE-FXII (Duan et al., 2009), it was postulated that the co-presentation of polymorphisms associated with a decrease in the enzymatic activity of ACE and APP, the main bradykinin degrading enzymes, could influence the severity of this disease. In our first part of the project, we also studied the presence of the main polymorphisms that have been shown to downregulate ACE and APP enzyme activity and its relation with disease expression. However, no differences were observed based on these polymorphisms, so the previously launched hypothesis could not be confirmed.

The second part of the project focused on the study of the obstetric history (course and management of pregnancies, deliveries, miscarriages and abortions) in a group of patients with HAE-C1INH. Previously published data on this topic were limited to 3 case series, although most publications consisted of reports. To this end, a nationwide cooperative study was carried out in Spain, involving 5 HAE reference centres. To date, our series is the most extensive published. As findings

to be highlighted, no differences were observed between those patients who had a previous diagnosis and those who did not. In the majority of patients with more than one pregnancy, there were no major variations between pregnancies. Contrary to previous publications (Czaller et al., 2010; Martinez-Saguer et al., 2010), no differences between trimesters were observed in our series. Although there is an increase in the number of attacks during pregnancy, it does not appear that there is usually an increase in severity, as they are mild and rarely life-threatening. Having HAE does not contraindicate vaginal delivery. Most data to date indicate that it is well tolerated, as are caesarean sections even without prophylaxis, and mild local AE is rare. However, the use of PTS with pdhC1INH is currently recommended. No disease-related miscarriages were observed. In the absence of clinical trials, the use of pdhC1INH as LTP in pregnant patients has been shown to be safe.

The inclusion of retrospective data, as well as the inclusion of a relatively small number of patients, are considered the main limitations of our study. However, as this is a rare disease, we consider our contribution in both respects to be quite important. Future directions are oriented towards international collaborations and the development of prospective studies, which will allow us to learn more reliably how the disease works and to advance our knowledge of it globally.

# Chapter 5

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# Appendix 1

# Manuscripts

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# Hereditary angioedema with *F12* mutation

## Clinical features and enzyme polymorphisms in 9 Southwestern Spanish families

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ABSTRACT

**Background:** Information on *F12* mutation hereditary angioedema (HAE) is still limited, but Spain is now recognized as having one of the highest concentrations of cases in Western Europe.
**Objective:** To describe unique features of HAE in Spanish carriers of the *F12* mutation and investigate a potential role for angiotensin-converting enzyme (ACE) and aminopeptidase-P polymorphisms in disease expression.
**Methods:** This was a prospective observational cohort study of 35 individuals (80% females) from 9 unrelated families carrying the p.Thr309Lys mutation. We analyzed detailed medical records and complement activity (C4, C1q, C1 inhibitor) and screened for mutations in exon 9 of the *F12* gene and 2 polymorphisms: *XPNPEP2* c-2399A and the *ACE* insertion/deletion polymorphism.
**Results:** The p.Thr309Lys mutation was found in all individuals. Three of the 9 index patients had a clinically negative family history, and 72% of males and 29% of females were asymptomatic. Sixteen females (44% estrogen dependent, 56% estrogen sensitive) were clearly symptomatic. The most common locations of attacks were the abdomen (63%), face (25%), and peripheral structures (6%). Triggers other than hyperestrogenic states included stress and minor trauma or pressure. Short-term treatment with C1-inhibitor concentrate and icatibant and long-term prophylaxis with tranexamic acid were useful. The combination of the I allele and A allele was detected in 17% of patients.
**Conclusion:** The polymorphisms analyzed were not a major determinant of disease expression in our population. We recommend searching for *F12* mutations in women with edema attacks without associated wheals and with normal C1-inhibitor levels, particularly when they develop symptoms during hyperestrogenic states or are of Western European or African origin.


## Introduction

The 3 types of hereditary angioedema (HAE)—types I, II, and III—have an autosomal dominant mode of inheritance. They can all cause subcutaneous or submucosal edema in any part of the body and are clinically indistinguishable.[1]

Estrogen-induced familial angioedema, without C1 inhibitor (C1-INH) deficiency, was first described by Warin et al in 1986.[2] However, it was not until 2000 that a new type of HAE—type III, or HAE with normal C1-INH (nC1-INH-HAE)—was suggested.[3,4] In 2006, 2 different missense mutations located in the same position in exon 9 of the *F12* gene (encoding factor XII [FXII] HAE) were described in approximately 25% of a cohort of patients with nC1-INH-HAE.[5] The most common of the 2 mutations predicts a threonine-to-lysine substitution in the secreted zymogen protein (c.983C>A, p.Thr309Lys, also referred to as p.Thr328Lys with the addition of the leader protein), whereas the second mutation predicts a threonine-to-arginine substitution (c.983C>G, p.Thr309Arg). A 72–base pair (bp) deletion[6] and an 18-bp

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duplication[7] were also recently described in the same proline-rich region of the *F12* gene.

In the case of FXII-HAE, an autosomal dominant mode of inheritance with a very low penetrance is now recognized, particularly in males, because more than 90% of male carriers are asymptomatic compared with just 40% of females.[8] The cosegregation of *F12* mutations in a substantial proportion of individuals who experience angioedema attacks provides strong evidence that these gene mutations are responsible for disease susceptibility. Nevertheless, the underlying pathophysiologic mechanism remains unknown in families with nC1-INH-HAE, 75% of whom do not have *F12* mutations (unknown HAE).[9] Nonetheless, the importance of contact pathway dysregulation in both groups of individuals with nC1-INH-HAE (unknown HAE and FXII-HAE) is increasingly recognized.

The description of unknown HAE and FXII-HAE has stimulated new research on contact system activation and interrelations with other homeostatic systems. Furthermore, the new millennium spawned investigations into rare diseases, such as HAE, and the identification of new forms is helping to understand certain biological and clinical implications in common conditions, such as cardiovascular disorders.[10] The FXII-HAE subgroup provides an objective basis to analyze patients with similar pathologic forms of angioedema within the heterogeneous area of familial angioedema without wheals and without C1-INH deficiency. *F12* mutations therefore constitute an essential genetic marker for advancing knowledge in this new, specific field of study.

The existence of factors other than *F12* mutations (genetic or otherwise) that could explain the low expression of HAE is at the center of research in this field.[11] Genetic variants that affect the activity of the angiotensin-converting enzyme (ACE) and aminopeptidase P (APP)—2 enzymes that degrade bradykinin—have been postulated as possible modifiers of phenotypic expression in FXII-HAE.[12] The aims of this study were to describe and characterize the phenotypic features of southern Spanish individuals who share the same mutation, p.Thr309Lys, and to investigate the potential role of ACE and APP polymorphisms in phenotypic expression.

## Methods

### *Design and Participants*

This was a descriptive, prospective, observational study performed at the Angioedema Reference Unit of Hospital Universitario Virgen del Rocío in Seville, Southwest Spain. We studied 9 young index females with FXII-HAE from different parts of Andalusia (Seville, Granada, Malaga, Jaen, and Huelva) and from nearby Badajoz in Extremadura. Screening for *F12* mutations was extended to close relatives who agreed to participate. The 9 families were unrelated. All participants gave their written informed consent, and the local ethics committee approved the study.

Screening for *F12* mutations in 57 individuals from the 9 unrelated families identified the FXII p.Thr309Lys mutation in 35 individuals, who were recruited for the study between January 2009 and April 2015. After an initial clinical evaluation, the patients were assigned to 3 phenotype groups: asymptomatic carriers, paucisymptomatic patients (those who had experienced a single episode or sporadic episodes of HAE), and symptomatic patients. We then explored the association between these clinical phenotypes and genetic characterization based on the *ACE* insertion/deletion polymorphism and the *XPNPEP2* c-2399A polymorphism.

### *Clinical Data Collection*

We first obtained a detailed medical history from standardized written questionnaires (answered multiple times), electronic medical records, and the hospital's HAE patient registry. After an initial evaluation, we prospectively collected data on clinical characteristics and specific treatment up until April 2016; data were obtained from regular follow-up interviews, patient symptom diaries, and direct telephone contact between patients who experienced attacks and physicians from the HAE Reference Unit. All patients had at least 12 months of follow-up.

### *Laboratory Methods*

#### *Complement investigations*

C4, C1q, and C1-INH levels were measured by radial immunodiffusion at least twice. C1-INH activity was determined using chromogenic assays (Berichrom; Siemens, Marburg, Germany) and assessed at the reference laboratory in Barcelona, Spain. Biologic samples were determined during periods of remission in all patients. Two of the patients were additionally studied while symptomatic.

#### *Genetic study*

Genomic DNA was isolated from total blood samples using the MagNa Pure automated system (Roche, Barcelona, Spain). Screening of exon 9 of the *F12* gene (chromosome 5) was performed by polymerase chain reaction (PCR) and bidirectional sequencing of PCR products, using primers and cycling conditions as previously described.[5,13] The resulting sequences were compared with the reference sequence ENSG00000131187 in the Ensembl database.

The *ACE* insertion/deletion polymorphism (chromosome 17) was detected by PCR using previously reported primers and PCR conditions.[14] The amplified products were analyzed by electrophoresis on 1.5% agarose gel. The *XPNPEP2* c-2399A polymorphism (chromosome X) was genotyped using a real-time PCR TaqMan assay (Life Technologies, Carlsbad, California) following the manufacturer's indications.

### *Statistical Analysis*

Associations between polymorphisms and clinical conditions were evaluated using the Fisher exact test. Data were analyzed using the Open Epi software, version 3.03, for Windows (www.OpenEpi.com). $P < .05$ was considered statistically significant.

## Results

### *Clinical Data*

Thirty-five individuals from 9 unrelated families (see Fig 1 for pedigrees) had the FXII p.Thr309Lys mutation. Twenty-eight individuals (80%), including the 9 index patients, were female, and 7 (20%), from 5 different families, were male.

The clinical phenotypes are shown in Figure 2. Three of the 9 index patients (B:II:1-D:II:1-J:II:1) reported a negative family history at the first visit, but symptomatic ancestors were discovered on requestioning the patients after the laboratory tests. Information on time to diagnosis, age at onset, and characteristics of attacks (duration, frequency, affected locations, and most common locations) is given in Table 1. No wheals were observed, but 1 patient had hemorrhagic lesions (Fig 3), 5 had ecchymosis in the edematous area, and 2 experienced large, recurrent blisters on their lips. Oral intubation was required once in 2 patients because of a swollen uvula. Another 2 had experienced recurrent transient dysphonia and a foreign-body sensation in the throat. In 7 patients, medical imaging revealed free peritoneal fluid during at least 1 acute episode or in several attacks at different moments; 2 of these patients also had gastrointestinal wall thickening. Four patients had their appendices removed after a diagnosis of acute surgical abdomen; at least 2 of the appendices were normal.

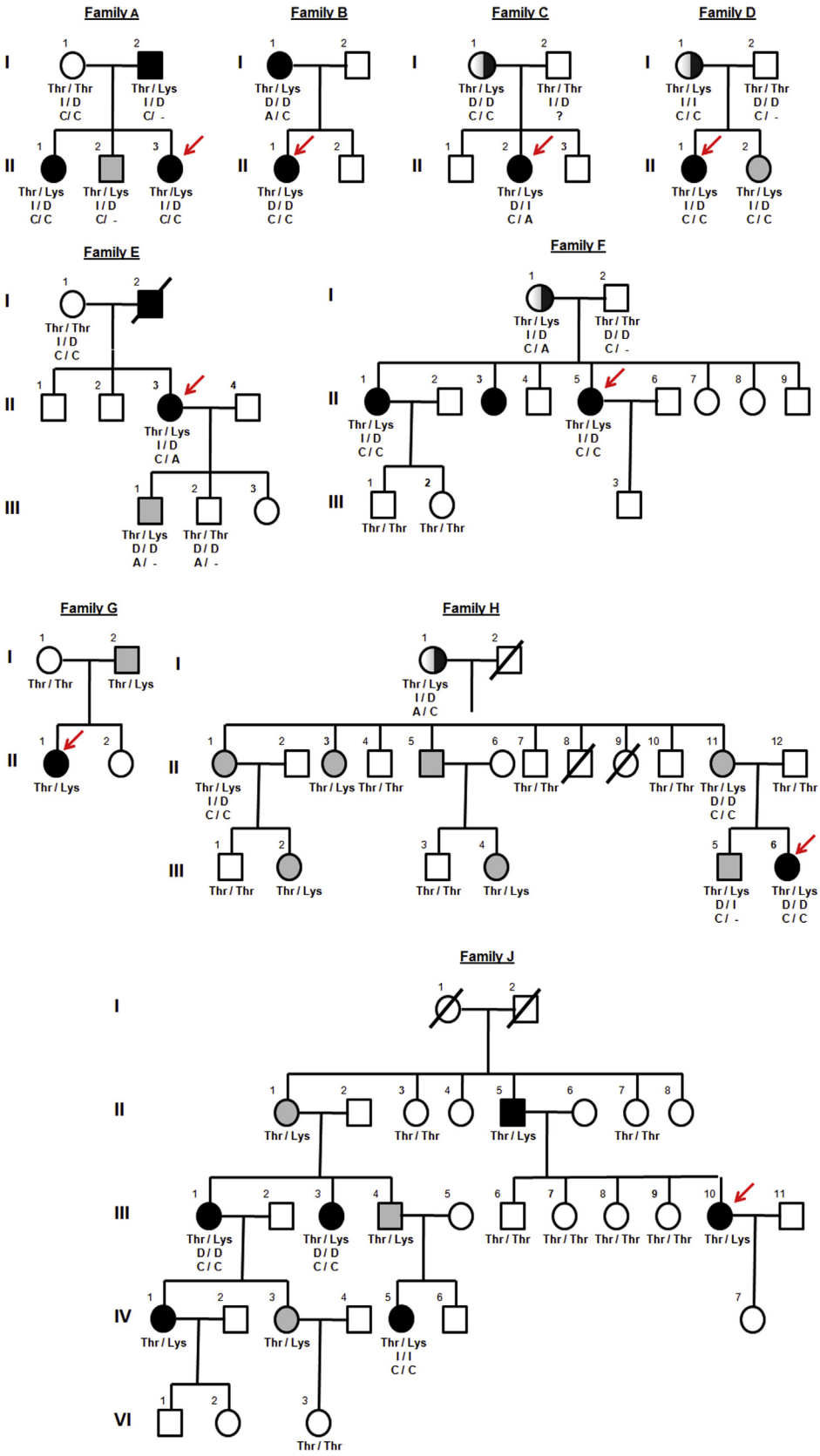


**Figure 1.** Pedigrees of all 9 families carrying the p.Thr309Lys mutation in the *F12* gene. Black filled symbols represent individuals with recurrent angioedema symptoms, half-filled symbols represent paucisymptomatic patients, and gray filled symbols represent asymptomatic carriers. Arrows indicate the index patients. Carriers (Thr/Lys) and noncarriers (Thr/Thr) of the p.Thr309Lys mutation are shown. D/D, I/D, II indicates *ACE* insertion/deletion polymorphism alleles; C/C (C/- males), C/A, A/- (males), *XPNPEP2* c-2399A polymorphism alleles.

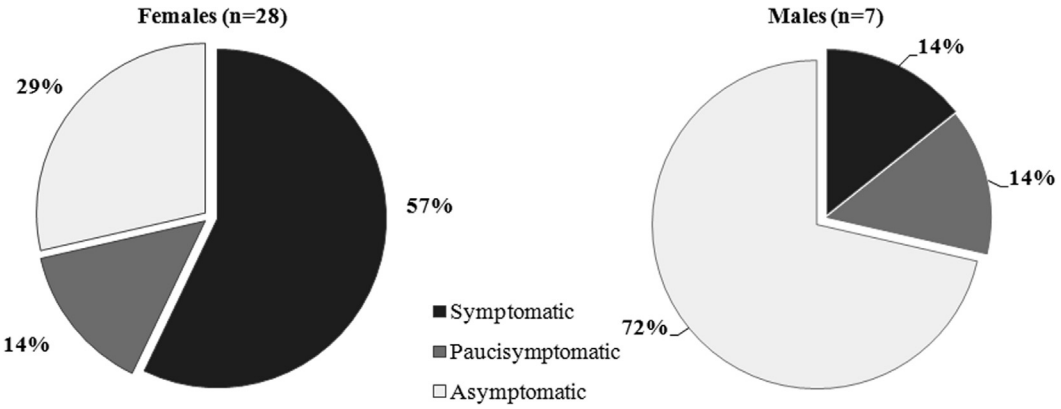


**Figure 2.** Distribution of clinical phenotypes.

Estrogen sensitivity, triggers, and prodromal symptoms are listed for the female patients in Table 2. Five full-term pregnancies and 1 early pregnancy interruption were registered in 4 patients during the prospective study period (T.G.-Q. and M.P.-S., unpublished data, 2016). No miscarriages were reported. All the women experienced symptoms during pregnancy. Five patients reported undergoing dental procedures without prophylactic treatment, and 3 had noticed local edema (the other 2 had not). One patient had been taking sitagliptin (anti–dipeptidyl-peptidase IV) for several years and was asymptomatic. One male patient experienced angioedema symptoms on using an ACE inhibitor. No other triggers or prodromal symptoms were found in male patients.

**Table 1**
Clinical Characteristics of 22 Symptomatic and Paucisympomatic Patients Carrying the p.thr309lys Mutation in the *F12* Gene[a]

| Characteristic | Females | | Males (n = 2) |
|---|---|---|---|
| | Symptomatic (n = 16) | Paucisymptomatic (n = 4) | |
| Diagnostic delay, mean (range), y | 16.5 (1–47) | MD | 8.5 (3–14) |
| Age at onset, mean (range), y | 19.9 (14–28) | MD | 61.5 (55–68) |
| Location of ≥1 attacks | | | |
| Face | 12 (75.0) | | |
| Eyelid | 10 (62.5) | 1 | |
| Lip | 14 (87.5) | 2 | |
| Tongue | 7 (43.8) | 1 | 1 |
| Upper respiratory tract | 14 (87.5) | | 1 |
| Abdomen | 14 (87.5) | | - |
| Limb | 10 (62.5) | | - |
| Genitals | 5 (31.2) | | - |
| Migratory attacks | 6 (37.5) | | - |
| Most common location of attacks | | | |
| Facial area | 4 (25.0) | 3 | - |
| Tongue | | 1 | 1 |
| Upper respiratory tract | | | 1 |
| Abdomen | 10 (62.5) | | - |
| Peripheral structures | 1 (6.3) | | - |
| Not known | 1 (6.3) | | - |
| Duration of attacks, h | | | |
| <48 | 1 (6.3) | MD | 1 |
| 48–96 | 11 (68.8) | MD | MD |
| >96 | 4 (25.0) | 1 | MD |
| Frequency (attacks per year) | | | |
| Hyperestrogenic states | | | |
| Occasional | | 3 | NA |
| <3 attacks per year | | - | NA |
| 4–12 attacks per year | 7 (43.8) | - | NA |
| >12 attacks per year | 7 (43.8) | - | NA |
| Not known | 2 (12.5) | 1 | NA |
| Nonhyperestrogenic states | | | |
| Asymptomatic | 8 (50.0) | 3 | |
| Occasional | | | 1 |
| <3 attacks per year | 4 (25.0) | | |
| 4–12 attacks per year | | | 1 |
| >12 attacks per year | 2 (12.5) | | |
| Not known | 2 (12.5) | 1 | |

Abbreviations: MD, missing data; NA, not applicable.
[a]Data are presented as number (percentage) of patients unless otherwise indicated.

*Long-term prophylactic treatment*

Oral tranexamic acid (1,500 mg/d) was temporarily used in 3 female patients and led to a reduction in the frequency and severity of symptoms. A fourth patient, who was simultaneously taking estrogen-containing contraceptive, did not have any improvement with this treatment. The same patient took danazol (600 mg/d) for 1 year but experienced no improvement in symptoms until she stopped taking estrogen-containing contraceptives. One patient with a more severe form of disease (no clear periods of complete remission between attacks and continuous abdominal pain and discomfort not explained by other causes) required the temporary addition of plasma-derived human C1 inhibitor (pdhC1INH) to tranexamic acid 1,500 mg (1,000 U per week for the first month followed by 500 U every 2 weeks for 2.5 months) to treat subintrant abdominal attacks. This treatment allowed her to stay out of the emergency department for more than 6 consecutive weeks. Another patient remained asymptomatic for at least 2.5 years while taking a daily desogestrel pill. The rest of the patients did not require long-term prophylactic treatment after discontinuation of estrogen-containing contraceptives.

*Short-term prophylaxis*

Treatment with intravenous pdhC1INH administered 1 to 2 hours before 4 deliveries and 1 shoulder operation was successful.

*Treatment of attacks*

Five of 6 patients who had an attack noticed an improvement with pdhC1INH, and 2 patients (including the nonresponder to pdhC1INH) noticed an improvement with a single injection of icatibant (Table 3). In 1 case, icatibant was administered late (>6 hours after the onset of the attack) and was not effective, but the patient responded well to fresh frozen plasma (2 U). One patient described faster resolution of symptoms (with subsidence of swelling in <24 hours) with a supply of on-hand oral tranexamic acid (500 mg 3 times daily) compared with no treatment.

High corticosteroid doses, antihistamines, and/or adrenaline were received several times by all patients but did not result in disease improvement. Adverse effects were observed with corticosteroid therapy and were particularly severe in 1 case.

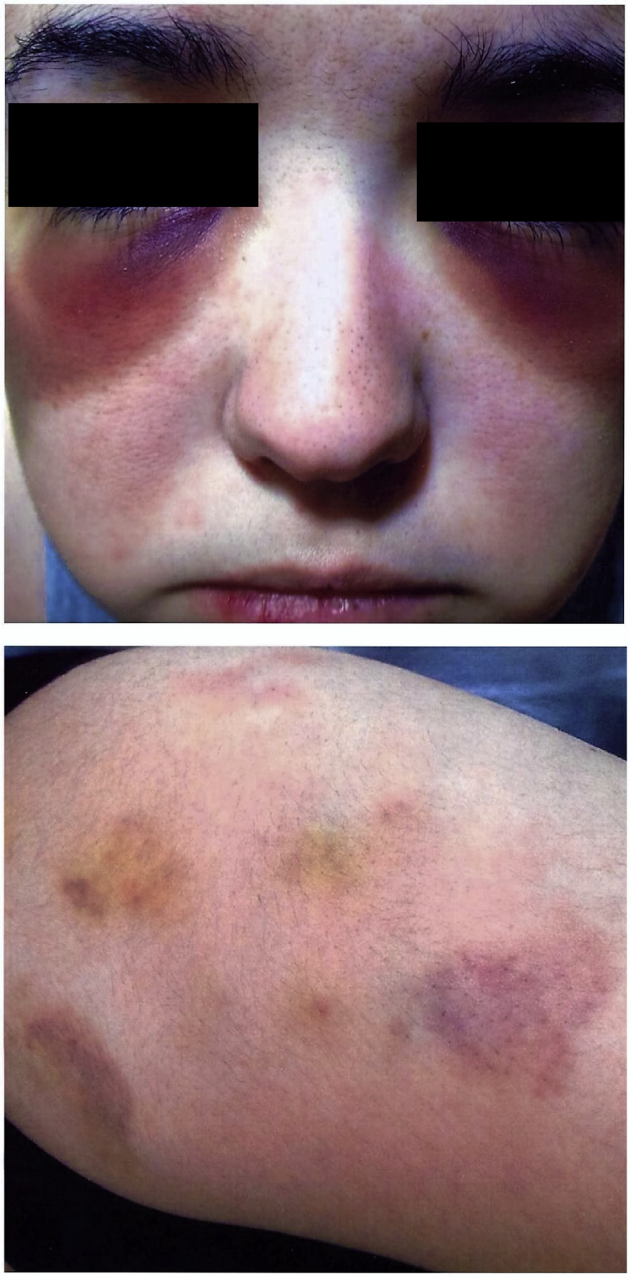

**Figure 3.** Extensive hemorrhagic lesions in areas of the face and thigh affected by angioedema.

*Laboratory Data*

*Complement analysis*

All patients had normal levels of C4, quantitative C1-INH, and C1q measured during periods of remission. One female patient had reduced C1-INH activity (58%) during a symptomatic period (reference range, 70%–130%). This activity had been measured at the hospital, where she had been misdiagnosed with HAE type II during 8 years of follow-up by the internal medicine department. Another patient had levels in the lower to normal range (70%) while symptomatic and levels of more than 87% when retested during remission. With the exception of 1 patient with C1-INH activity levels of 75%, all other patients had activity levels of more than 90% during remission.

**Table 2**
Estrogen Sensitivity, Triggers, and Prodromal Symptoms in Female

| Variable | No. (%) of Females | |
|---|---|---|
| | Symptomatic (n = 16) | Paucisymptomatic (n = 4) |
| Estrogen sensitivity | | |
| Estrogen dependent | 7 (43.8) | 3 |
| Estrogen sensitive | 9 (56.3) | |
| Not known | | 1 |
| HES | 13 (81.3) | 3 |
| Triggers | | |
| ECC use | 12/13 (92.3) | |
| Pregnancy | 8/8 (100) | 4/4 |
| Menstruation | 12 (75.0) | |
| Ovulation | 8 (50.0) | |
| Stress | 9 (56.3) | |
| Pressure/trauma | 9 (56.3) | |
| Infections | 3 (18.9) | |
| Cold | 1 (6.3) | |
| ACEi use | | 1[a]/1 |
| Prodromal symptoms | | |
| Unusual fatigue | 3 (18.8) | |
| Chest discomfort or palpitations | 3 (18.8) | |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitors; ECC, estrogen-containing contraceptive; HES, hyperestrogenic state; NA: not applicable.
[a]No angioedema symptoms but a very disturbing cough.

*Genetic study*

The c.983C>A mutation (p.Thr309Lys), heterozygous in all cases, was found in the *F12* gene of 35 patients (Fig 1). The results for the *XPNPEP2* c-2399A and *ACE* insertion/deletion polymorphisms are detailed in Table 4 and Figure 1.

## Discussion

We have reported clinical and genetic results for 35 individuals with the *F12* gene p.Thr309Lys mutation from 9 families with FXII-HAE in southwest Spain. On the basis of several case reports[5,12,13,15–26] and 3 large case series,[9,27,28] the p.Thr309Lys mutation is the most common *F12* mutation found thus far: approximately 66 families worldwide have been reported to carry it vs 2 families with p.Thr309Arg and 1 family each with the duplication and deletion. There is a clear predominance of FXII-HAE involvement in populations of Western European or Maghreb origin, and the results of a haplotype analysis performed in 2006 pointed to a common ancestor from the 11th century.[13] The same haplotype was found in 2 subsequent reports.[19,26] Our study provides unique insights into features of this variant of HAE.

We detected a significant delay in FXII-HAE diagnosis (mean delay, 16.5 years; range, 1–47 years), supporting recent findings by Firinu et al.[26] This long time to diagnosis is similar to that seen in classic forms of C1-INH-HAE[29] and has obvious implications in terms of delayed initiation of necessary treatment.

According to reports to date, skin involvement, particularly of the perioral area, is the most common feature of symptomatic FXII-HAE.[9,25–27,30–32] Ours is the first large case series in which the gastrointestinal area was the most common site of attacks, although other sites were also involved. Specifically, abdominal

**Table 3**
Treatment of Acute Attacks

| Variable | pdhC1INH | Icatibant | Tranexamic acid |
|---|---|---|---|
| No. (%) of acute attacks (patients) | 11 (5) | 9 (2) | 2 (2) |
| Onset of improvement, min | 20–30 | 15–30 | |
| Objective symptom relief, h | 1 | 1–2 | 2 |
| Complete resolution, h | 3–24 | 24 | 24–48 |

Abbreviation: pdhC1INH: Plasma-derived human C1 esterase inhibitor concentrate.

**Table 4**
Polymorphisms in the *ACE* and *XPNPEP2* Genes[a]

| Gene | Total (n = 24) | Symptomatic (n = 14) | Paucisymptomatic (n = 4) | Asymptomatic (n = 6) |
|---|---|---|---|---|
| Insertion/deletion (rs1799752) | | | | |
| *ACE* gene | | | | |
| D/D | 8 (33) | 5 | 1 | 2 |
| I/D | 14 (58) | 8 | 2 | 4 |
| I/I | 2 (8) | 1 | 1 | 0 |
| SNP 2399-A (rs3788853) | | | | |
| *XPNPEP2* gene | | | | |
| C/C | 15 (63) | 10 | 2 | 3 |
| C/- | 3 (13) | 1 | 0 | 2 |
| A/C | 5 (21) | 3 | 2 | 0 |
| A/A | 0 | 0 | 0 | 0 |
| A/- | 1 (4) | 0 | 0 | 1 |
| Allele I + allele A | 4 (17) | 2 | 2 | 0 |

[a]Data are presented as number (percentage). No significant differences were found between the expression of symptoms and the presence of combination of (allele I + allele A) vs no combination ($P = .58$), D/D vs (I/D; I/I) ($P = .10$), or C/C or C/- vs A/C or A/- ($P = .10$) (Fisher exact test).

involvement was observed in 62.5% of the 16 symptomatic patients (Table 1). The abdomen is also the most common site for attacks in classic forms of C1-INH-HAE,[33] but the likelihood of attributing attacks to other causes of abdominal pain is probably greater in patients with FXII-HAE. Recurrent abdominal or gastrointestinal attacks in female patients were also more common in our series (14 of 16 [87.5%]) than in other large series described to date.[9,27,28] Like Bork et al,[9] we detected a wide diversity of symptoms in the upper respiratory tract mucosa (tongue, uvula, palatal-mouth swelling, bolus foreign-body sensation). Although these symptoms were experienced by 14 of the 16 patients (87.5%), they were fortunately infrequent. Nonetheless, this high rate of laryngeal swelling suggests, contrary to what is commonly thought,[34] that nearly all patients with FXII-HAE could be at risk of a life-threatening attack.

In 2007, Bork et al[35] drew attention to the presence of hemorrhagic lesions and ecchymosis in edematous areas in patients with nC1-INH-HAE. In our series, these clinical features were present in a third of patients. The case of 1 female patient with severe disease is particularly noteworthy. This patient experienced multiple, extensive hemorrhagic lesions under the eyes together with bruising in several locations (Fig 3) during different angioedema episodes that led emergency department staff to suspect mental insanity and acts of self-injury. Such situations may be amplified by feelings of guilt, which were expressed by at least 2 of our young patients. The image of our patient strongly resembles that of a patient with an *F12* mutation described by Bork et al.[35]

Multilocation attacks have been described as rare in nC1-INH-HAE and FXII-HAE,[32] but 37.5% of patients in our series had migratory attacks, supporting the possible implication of an FXII fragment, as reported by Kaplan and Joseph.[36]

In our study, 28 of 35 carriers (80%) of the p.Thr309Lys mutation were women, and 12 of 28 (42.9%) were asymptomatic or paucisymptomatic; this rate is slightly higher than that of 34% reported by Charignon et al.[28] Notably, 7 of these 12 women were older than 50 years (data not shown), and it can therefore be assumed that there is little likelihood that they will have any clear signs of involvement in the future. It also indicates that up to 25% of women might not be easily recognized as transmitters of this life-threatening disease.

As expected, estrogen-containing contraceptive use played a relevant role in the expression of disease in our patients. Pregnancy was also a trigger, but it seemed to have a lesser impact in terms of severity of symptoms (T.G.-Q. and M.P.-S., unpublished data, 2016). Other endogenous sex hormones movements also acted as triggers in our female population. More than 50% of symptomatic women reported additional, non–sex hormonal triggers (particularly stress and pressure or minor trauma) in both hyperestrogenic and non-hyperestrogenic states.

Prodromal symptoms have been poorly described in FXII-HAE. In our series, 18.8% of symptomatic females reported unusual fatigue, whereas another 18.8% reported chest discomfort or palpitations.

Normal C1-INH function has been proposed as a necessary condition for the diagnosis of nC1-INH-HAE and hence FXII-HAE.[30–32] In our case series, only 3 of the 35 individuals (9.1%) had transiently decreased functional C1-INH values (below or around the lower-normal range), and one of these had been misdiagnosed with HAE type II for several years. We would like to highlight the importance of confirming decreases in functional values to avoid misdiagnoses with other forms of HAE. Slightly decreased C1-INH activity has been previously reported, but in most cases, levels were measured during pregnancy[15,16,27] or estrogen-containing contraceptive use[15,27]; hence, the hypothesis that decreases may be the result of high proteolytic activity during symptomatic periods.[28]

Similar to previous reports,[9,27,28] our study found high interpatient variability in the frequency and severity of symptoms, even among members of the same family. Until recently, there was no explanation for this extreme variability, which is also seen in individuals over time. In addition to the genetic basis of HAE, exaggerated kinin formation, combined with some form of impaired kinin catabolism, seems to have a central, additive role in nC1-INH-HAE as a whole.[11,13,28,36] Kinin overproduction was recently confirmed in both FXII-HAE[28] and C1-INH-HAE.[37] However, levels do not seem to be sufficient to distinguish among different phenotypes, lending strength to the idea that individual variations in kinin degradation enzyme activity influence disease expression. Further supporting this idea, Charignon et al[28] found a correlation between a severe phenotype of FXII-HAE and low kinin catabolism and decreased carboxypeptidase N and ACE activity (a condition favorable to bradykinin accumulation).

Regarding a possible genetic influence on kinin catabolism, Duan and Binkley[38] suggested that the *ACE* insertion/deletion polymorphism—the major *ACE* gene locus that influences serum ACE concentration[14]—and the APP-regulatory polymorphism—*XPNPEP2* c-2399A—might be involved in reduced APP activity. They found copies of an I allele (associated with low ACE serum levels) and an A allele (associated with low APP serum activity) in 3 symptomatic female patients from the same family and hypothesized that this genetic combination might contribute to disease expression in FXII mutation carriers.

Our study incorporated additional analyses of bradykinin degradation enzyme polymorphisms. Our results, however, do not support the hypothesis proposed by Duan and Binkley[38] because we found no statistically significant differences in the distribution of the *ACE* insertion/deletion polymorphism or the APP-regulatory polymorphism, evaluated together and separately, among the clinical phenotypes established in FXII mutation carriers. It must, however, be remembered that the statistical power of our study was limited by the small sample size.

There are no approved treatments for FXII-HAE, so all our patients were treated off-label with their explicit informed consent. Tranexamic acid, progesterone, and danazol have been reported to be effective as long-term prophylactic treatment.[9,15,27] Disease control improved in all 5 patients (31.3%) in our series who received long-term prophylactic treatment: 4 received tranexamic acid, temporarily associated with pdhC1INH in 1 case, and the fifth received progesterone.

The use of pdhC1INH as short-term prophylaxis during deliveries was effective, as previously reported.[15,21] The likelihood of

symptoms during dental procedures[9,17,18,27] and invasive diagnostic medical techniques, such as endoscopies,[27] should be considered to justify the use of short-term prophylaxis.

A positive response to pdhC1INH (despite absence of C1-INH deficiency) and icatibant (a bradykinin receptor 2 antagonist) was observed during 20 attacks in 7 patients, supporting previous reports of the general effectiveness of these 2 drugs for attacks.[9,15,25–27,32]

The small number of symptomatic patients and inclusion of some retrospective data could be considered a limitation of our study. However, HAE is a rare disease, and we consider that our data add to the limited body of knowledge in this area. Analysis of electronic medical records also constitutes an essential—and objective—tool for the identification of symptoms that frequently go unnoticed.

In summary, our findings indicate that certain FXII-HAE populations may not necessarily have different symptom patterns to patients with C1-INH-HAE, as initially described. Involvement of locations other than the skin is common and could even be the main expression of disease. The significant delay in FXII-HAE diagnosis (mean of 16.5 years) suggests a possible lack of awareness in the medical community about this life-threatening disease. The absence of a family history in women with recurrent angioedema without wheals and without C1-INH deficiency is clearly not reason enough to exclude genetic testing. The enzyme polymorphisms studied were not a major determinant of disease expression in our population.

## Acknowledgments

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ORIGINAL ARTICLE

# Management of Pregnancy and Delivery in Patients With Hereditary Angioedema Due to C1 Inhibitor Deficiency

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## Abstract

*Background and Objective:* There is little information on pregnancy and delivery in patients with hereditary angioedema due to C1 inhibitor deficiency (C1INH-HAE). The aim of this study was to describe the effect of pregnancy and deliveries on symptoms of C1INH-HAE and review the need for and safety of treatments available during the study period.
*Methods:* Retrospective review using a purpose-designed questionnaire of 61 C1INH-HAE patients from 5 hospitals specialized in the management of HAE in Spain. The outcomes measured were number of pregnancies, changes in symptoms during pregnancy and delivery, mode of delivery, type of anesthesia during delivery, treatments received, and tolerance of treatments.
*Results:* We reviewed 125 full-term pregnancies (89 without a prior diagnosis of C1INH-HAE), 14 miscarriages, and 4 induced abortions. Patients reported an increased frequency of C1INH-HAE symptoms in 59.2% of pregnancies (74/125) and the presence of symptoms throughout pregnancy in 40% (50/125). Prophylactic C1INH-HAE therapy was used during 9 (7.2%) of the 125 pregnancies. Nine patients—in 11 pregnancies (8.8 %)—received treatment for acute attacks. Most deliveries (n=110, 88%) were vaginal. A cesarean section was necessary in 15 cases (12%). Short-term prophylaxis with pdhC1INH was administered before 14 deliveries (11.2 %); 111 deliveries (88.8 %) were performed without premedication and were well tolerated. Anesthesia was used in 51 deliveries (40.8%).
*Conclusions:* Pregnancy has a variable influence on the clinical expression of C1INH-HAE. Attacks tend to occur more frequently but not to increase in severity. Vaginal delivery was mostly well tolerated. pdhC1INH prophylaxis should be administered prior to cesarean delivery and is also recommended before vaginal delivery if there are additional risk factors. pdhC1INH should always be available in the delivery room.

Key words: Hereditary angioedema. Pregnancy. Delivery. C1 inhibitor. Anesthesia. Treatment.

## Resumen

*Antecedentes y Objetivo:* Existe escasa información sobre la evolución del embarazo y el parto en pacientes con angioedema hereditario con déficit de C1 Inhibidor (AEH-C1INH). El objetivo del estudio fue describir el efecto de embarazo y parto en los síntomas de AEH-C1INH y la necesidad y seguridad de las terapias disponibles durante dicho período.

*Diseño:* Revisión retrospectiva de datos registrados en 5 centros hospitalarios españoles expertos en AEH.
*Pacientes y Métodos:* 61 mujeres con diagnóstico de AEH-C1INH antes o después de su(s) embarazo(s). Se rellenó un cuestionario específico. Fue evaluado: número de embarazos, evolución de síntomas de AEH durante embarazo(s) y parto(s), tipo de parto, tipo de anestesia durante el parto, tratamientos recibidos y su tolerancia.
*Resultados:* Se revisaron 125 embarazos a término (en 89 embarazos las pacientes estaban sin diagnosticar de AEH) y 18 abortos. Hubo aumento en la frecuencia de síntomas de AEH en 59,2% de embarazos (74/125) y los síntomas estuvieron presentes a lo largo de todos los trimestres en el 40% (50/125). Se usó tratamiento preventivo en 9 de los 125 embarazos (7,2%). Nueve pacientes -en 11 embarazos- (8,8%) recibieron tratamiento para crisis agudas. 110 partos (88%) fueron vaginales, mientras que 15 (12%) fueron cesáreas. Se usó tratamiento profiláctico con concentrado de C1-Inhibidor (pdhC1INH) antes de 14 partos (11,2%). Se completaron 111 partos (88,8%) sin ningún tipo de premedicación y resultaron bien tolerados. Se usó anestesia en 51 partos (41,6%).
*Conclusiones:* La influencia del embarazo en la expresión clínica de la enfermedad es variable, no obstante las crisis tienden a aumentar en frecuencia pero no en gravedad. El parto vaginal fue habitualmente bien tolerado. El pdhC1INH debe administrarse antes de un parto mediante cesárea y también se recomendaría en caso de parto vaginal si existiera algún factor de riesgo adicional. El pdhC1INH debe estar siempre disponible en la sala de partos.

Palabras clave: Angioedema hereditario. Embarazo. Parto. C1-Inhibidor. Anestesia. Tratamiento.

## Introduction

Hereditary angioedema (HAE) due to C1 inhibitor deficiency (C1INH-HAE) is a rare disease [1]. In Spain, a minimal prevalence of 1.09 cases per 100 000 inhabitants has been reported [2]. Two subtypes of C1INH-HAE have been described: type I C1INH-HAE with reduced yet functional C1 inhibitor levels and type II C1INH-HAE, with normal or high C1INH protein levels but reduced C1INH function [3]. Another type of HAE with normal C1INH levels has also been described [4,5], but in this paper we focus just on types I and II.

C1INH-HAE is characterized by nonpruritic, nonpitting edema that typically affects different locations. Abdominal pain, distension, nausea, or vomiting may also be present secondary to edema of submucosal tissues of the gastrointestinal tract [6]. Upper airway involvement can be fatal, and mortality due to suffocation can be as high as 33% in inappropriately treated patients [7]. Estrogens, trauma, and stressful situations can worsen the course of disease and consequently, pregnancy and delivery may be special periods for female patients [8-10].

Three treatment options are available for C1INH-HAE: long-term prophylaxis (LTP), short-term prophylaxis (STP), and acute treatment. LTP consists mainly of attenuated androgens (AAs), but these can cross the placental barrier, possibly producing virilization, and should therefore be strictly avoided during pregnancy and lactation; antifibrinolytic agents are also used for LTP, though they are less effective [11,12]. Plasma-derived human C1 inhibitor (pdhC1INH) can be used for LTP when other treatments are contraindicated, ineffective, or poorly tolerated. STP with pdhC1INH is the most effective preventive therapy for patients undergoing surgery. pdhC1INH has traditionally been the treatment of choice for acute attacks during pregnancies; an alternative, though less safe, option is fresh frozen plasma [13,14]. Icatibant acetate, a B2 receptor blocker, as well as a recombinant version of the human C1 inhibitor protein, have been approved by the European Medicines Agency and the US Food and Drug Administration, and together with ecallantide, are licensed for the treatment of acute edema attacks in adult patients; they have not yet, however, been approved for use in pregnancy [15-17]. In this study, we describe the experience of 5 major HAE centers in Spain in managing C1INH-HAE during pregnancy and delivery with the aim of adding to the body of knowledge regarding the management of obstetric events in this setting.

## Patients and Methods

Patients from 5 HAE reference hospitals in Spain were recruited for the study. The participating centers were Hospital Universitario Virgen del Rocío in Sevilla, Andalusia, Hospital Universitario La Paz in Madrid, Complejo Hospitalario Universitario de Vigo in Vigo, Galicia, Hospital Universitario Vall d'Hebron in Barcelona, Catalonia, and Hospital General Universitario Gregorio Marañón in Madrid. We performed a retrospective study of C1INH-HAE patients who had become pregnant before or after C1INH-HAE diagnosis. A specific questionnaire was designed and filled out with information from clinical charts or from telephone interviews when data were missing. Information about full-term pregnancies, miscarriages, and induced abortions was also included. The research ethics committees of Hospital Universitario Virgen del Rocío and Hospital Universitario La Paz approved the study.

The criteria to define worsening of symptoms compared with the baseline condition included an increase in attack frequency as well as the duration of single attacks. Increased severity was also considered a criterion of worsening but there were no cases (ie, there were no changes in the distribution of sites of involvement). The criteria to define an improvement in HAE symptoms included no symptoms, fewer symptoms, or milder attacks.

Descriptive statistics were used to summarize and analyze the collected data. Analysis was performed using the IBM SPSS v19.0 statistical package. Quantitative variables were described as means and ranges; qualitative variables were recorded as absolute values and percentages. Categorical variables were compared using the $\chi^2$ Pearson test. *P* values of less than .05 was considered to be statistically significant.

## Results

We reviewed 125 pregnancies in 61 patients as well as 14 miscarriages and 4 induced abortions. The percent distribution of patients and full-term pregnancies among the 5 study hospitals are shown in the Figure.

The mean age of patients at the time of C1INH-HAE diagnosis was 30.6 years (range, 6-60 years), and the mean age at the start of pregnancy was 27.1 years (range, 16-42 years). There was a prior diagnosis of C1INH-HAE in just 44 (30.7 %) of the 143 initial gestations and in 36 (28.8 %) of the 125 full-term pregnancies. Thirty-five women (57.4%) were not aware that they had HAE during 89 pregnancies (71.2%). Twenty of the 61 patients had only 1 pregnancy. Data were collected between the years 2006 and 2010 and corresponded to pregnancies that had occurred more than 20 years previously in nearly half of the patients (49.2%), 6 of whom had only been pregnant once.

Only 13 patients (21.3 %) were receiving LTP before they became pregnant. Nine patients had been receiving AAs. Seven of these stopped AA therapy before they conceived and the other 2 stopped 1 month after conception, on confirmation of their pregnancy.

A greater frequency or duration of acute attacks was reported for 59.2% of the pregnancies, no changes with respect to baseline symptoms were reported in 26.4% of cases, and symptoms improved in 14% of cases. There were no differences in attack severity from one trimester to the next, and in 40.0 % (50/125) of pregnancies HAE symptoms were present throughout the pregnancy. Patients with more than 1 gestation (67.2%) generally described a similar course for each of their pregnancies (similar C1INH-HAE symptoms in 85.4% of cases). There were no changes in sites of involvement but there was an increase in the frequency of mild abdominal crises. Non-life-threatening symptoms were reported in the 125 full-term pregnancies or abortions.

We found no statistically significant differences for disease course on comparing percentages between the group of women with a known C1INH-HAE diagnosis before pregnancy (n=36) and the group of women with an unknown C1INH-HAE diagnosis at the time of pregnancy (n=89) (Table 1).

LTP was only used during 9 of the 125 pregnancies: epsilon-amino-caproic acid was used in 1 case, tranexamic acid in 2 cases, AAs in 2 cases, and pdhC1INH (Berinert, CSL-Behring) in 4 cases. In the 2 cases in which AAs were temporarily used (for 8 and 12 weeks), the drug was administered after confirmation that the fetus was male. (As mentioned previously, 2 other patients interrupted LTP with AAs when they became aware they were pregnant.)

Our patients reported 14 miscarriages, most of which occurred during the first trimester. Additionally, there were 4 registered abortions. There were also 3 fetal deaths and 1 premature delivery with complications (deafness and visual problems). None of the miscarriages or cases of fetal damage occurred in patients temporarily exposed to AAs.

Nine patients received treatment for an acute attack during 11 pregnancies. pdhC1INH (Berinert) was administered in all 9 cases and 1 of the patients additionally received tranexamic acid and other corticosteroids. This last patient was not diagnosed with C1INH-HAE until 5 years later.

Seven patients were administered a total of 618 vials of pdhC1INH 500 U (Berinert) to treat acute attacks and as LTP (4 cases) in 9 pregnancies (average of 4.16 vials/mo); 1 patient received, as LTP, a total of 356 vials in 2 consecutive pregnancies (average 4.94 vials/wk); no adverse effects were reported.

None of the newborns developed health problems or experienced adverse effects attributable to any of the drugs used.

The vast majority of deliveries (n=110, 88%) were vaginal; forceps and vacuum extraction were used in 9 and 5 deliveries, respectively (Table 2). Cesarean sections were necessary in 15

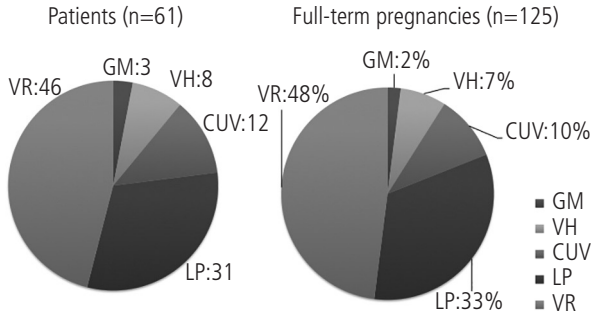


Figure 1. Distribution of patients and full-term pregnancies among the 5 reference hospitals for hereditary angioedema. CUV indicates Complejo Hospitalario Universitario de Vigo; GM, Hospital General Universitario Gregorio Marañón; LP, Hospital Universitario La Paz; VH, Hospital Universitario Vall d'Hebron; VR, Hospital Universitario Virgen del Rocío.

Table 1. Changes in C1INH-HAE Symptoms During Pregnancy in Patients With and Without a Previous Diagnosis of C1INH-HAE[a]

| | | Previous diagnosis of C1INH-HAE | |
|---|---|---|---|
| | | Yes n=36 (28.8%) | No n=89 (71.2%) |
| Worsening of C1INH-HAE symptoms during pregnancy (%) | No 51 (40.8) | 12 (33.3) | 39 (43.8) |
| | Yes 74 (59.2) | 24 (66.7) | 50 (56.2) |

Abbreviation: C1INH-HAE, hereditary angioedema due to C1 inhibitor deficiency.
[a]No statistically significant differences were noted between groups.

Table 2. Mode of Delivery and Type of Anesthesia

| Mode of Delivery | Type of Anesthesia | | | |
|---|---|---|---|---|
| | Epidural | General | Pudendal Block | No Anesthesia |
| Cesarean section (n=15) | 6 | 9 | 0 | 0 |
| Vaginal delivery | | | | |
| Noninstrumental (n=96) | 21 | 4 | 0 | 71 |
| Vacuum (n= 5) | 4 | 2 | 3 | 0 |
| Forceps (n= 9) | 0 | 2 | 0 | 3 |
| Total (n=125) | 31 | 17 | 3 | 74 |

Table 3. Reasons for Cesarean Delivery (Rate 12%)

| Patient | Year | Pregnancy Number | Age, y | Type | Reasons |
|---|---|---|---|---|---|
| 1 | 1975 | Second | 28 | Acute | Dystocia |
| 2 | 1983 | First | 19 | Elective | Narrow pelvic opening |
| 3 | 1983 | First | 23 | Acute | Anomalies of umbilical cord |
| 4 | 1984 | Third | 39 | Elective | Other complications |
| 5 | 1985 | Third | 25 | Acute | Dystocia |
| 6 | 1993 | Second | 40 | Elective | Other complications |
| 7 | 1994 | First | 29 | Acute | Dystocia |
| 8 | 1994 | Second | 27 | Elective | HAE diagnosed during pregnancy |
| 9 | 1996 | First | 27 | Elective | Narrow pelvic opening |
| 10 | 1998 | First | 28 | Elective | Unknown. Known diagnosis of HAE |
| 11 | 2001 | Second | 37 | Elective | Narrow pelvic opening |
| 12 | 2002 | First | 27 | Acute | Fetal bradycardia. Known diagnosis of HAE |
| 13 | 2005 | First | 36 | Elective | Unknown. Known diagnosis of HAE |
| 14 | 2006 | Second | 35 | Elective | Fetus in breech position. Known diagnosis of HAE |
| 15 | 2007 | Third | 28 | Acute | Dystocia |

Abbreviation: HAE, hereditary angioedema.

deliveries (the reasons are summarized in Table 3). Only 5 of the 15 women who underwent a cesarean section knew that they had C1INH-HAE and had been treated with pdhC1INH prior to delivery; tolerance was good in all cases and the 10 women who did not receive premedication for the cesarean section did not recall any adverse perioperative events.

Anesthesia was used in 51 deliveries (40.8%) and in the 4 induced abortions. No specific complications associated with the anesthesia were reported.

STP with pdhC1INH was only administered prior to 14 deliveries; 5 patients received 1000 U and 9 patients received 500 U. All the patients demonstrated good tolerance of the infusion and no adverse events were reported. Of the 14 patients who received STP before delivery, 8 had experienced a worsening of C1INH-HAE symptoms during pregnancy and 4 reported no changes with respect to before the pregnancy. None of these 14 patients had HAE symptoms during delivery or in the next 48 hours. No premedication was used in 111 deliveries and mild local C1INH-HAE symptoms were observed in just 6 vaginal deliveries (5.4%). These local symptoms ceased spontaneously.

## Discussion

We have reported on the course and management of 125 full-term pregnancies and deliveries, 14 miscarriages, and 4 induced abortions in 61 C1INH-HAE patients through a nationwide cooperation study. A clear set of international C1INH-HAE guidelines containing recommendations for delivery and pregnancy follow-up and information on the benefits and risks associated with each of the available treatments was published only recently [12,13]. Three case series have been published [18-20], but most publications on HAE and pregnancy report on few pregnancies and deliveries (between 1 and 3). Our study is the largest to date and can be considered representative of the approach to and management of pregnancies and deliveries in C1INH-HAE patients in Spain in recent decades.

Mild aggravation of C1INH-HAE symptoms was experienced in 59.2% of the pregnancies in our series; this figure is similar to previous reports [18,19]. The mild worsening detected is more likely to be attributable to changes associated with pregnancy rather than to discontinuation of HAE treatment, as most patients were not receiving regular treatment before they became pregnant.

No statistically significant differences were observed for course of disease on comparing patients with a previous diagnosis of C1INH-HAE and those without one (Table 1). Of the 36 pregnancies in which there was a confirmed diagnosis of HAE before pregnancy, there was an improvement in clinical signs in 6 cases (16.7%) and no changes in another 6.

The majority of the 41 patients with more than 1 pregnancy (85.4%) noted that the disease manifested itself in a similar way during each pregnancy and only 4 patients described a change in symptoms from one pregnancy to the next.

The course of symptoms has been reported to vary greatly between pregnancy trimesters [18,19], but in more than half of the pregnancies in our series, there were reports of worsening of symptoms throughout the pregnancies, with no clear differences observed between trimesters.

Chinniah and Katelaris [20] described a group of 7 patients with 16 pregnancies who experienced fewer attacks as the pregnancy progressed and more attacks during the first trimester. In all cases, the patients had edema attacks, but the authors did not report whether symptoms worsened during pregnancy in comparison with previous or posterior periods.

In our case series, none of the 14 miscarriages were directly related to angioedema attacks and this rate of 10.1% can be considered low compared with average rates reported for the general European population (10%-20%) [10]. The decision to terminate the pregnancy was at least partially related to the diagnosis of HAE in 2 of the 4 voluntary interruptions. One of the 3 fetal deaths was due to a congenital cardiopathy and 2 (in the same woman) were due to Rh incompatibility. There was just 1 newborn who developed complications because of premature delivery (at 6 months). None of these cases were related to the temporary use of androgens.

Almost all studies of patients with C1INH-HAE have reported good progression of HAE symptoms during pregnancy and delivery. Although HAE symptoms may be present, they are usually mild and rarely life-threatening. Mc Glinchey et al [21] reported a life-threatening laryngeal angioedema attack in the 25th week of gestation that was treated with fresh frozen plasma and 2000 U of pdhC1INH and resolved completely. Postnikoff and Pritzker [22] wrote about the only death reported of a pregnant HAE woman 110 hours after delivery. This patient developed perineal swelling and a purulent discharge from the episiotomy 48 hours postpartum. Autopsy revealed edema of the subcutaneous tissues, which was most prominent in the perineal region, with severe effusions present within all body cavities and septic shock.

LTP during pregnancy was seldom used in the pregnancies reported in this case series; in some cases the patients had not yet been diagnosed with C1INH-HAE, while in others presumably little was known at the time about the safety of C1INH-HAE treatments. We administered C1INH concentrate as LTP in 4 pregnancies in 2009 and 2010; the drug was administered off-label, with the patients' informed consent, due to a clear exacerbation of symptoms following discontinuation of effective AA therapy.

In our series vaginal delivery without prophylactic treatment was well tolerated, and although trauma and stress are known to trigger attacks, very few patients developed mild local edema. There have been many isolated reports of pregnant C1INH-HAE patients who experienced no edema attacks after a vaginal or cesarean delivery with prophylactic pdhC1INH treatment [23-28]. There have also been reports of 2 cesarean deliveries [29] and 2 vaginal deliveries [30] that were well tolerated without prophylactic therapy. Anecdotal reports of STP with fresh frozen plasma and danazol prior to delivery, with good outcomes, have also been published [31,32]. In the series reported by Martinez-Saguer et al [19], all the patients received prophylactic pdhC1INH treatment prior to delivery. Chinniah and Katelaris [20] did not report any symptomatic deliveries in their case series, although prophylactic pdhC1INH was used in only 5 of the 16 deliveries. In the series by Czaller et al [18], prophylactic treatment prior to delivery was used in 9 of 82 deliveries, with good tolerance in all cases; the same authors described asymptomatic deliveries in the 73 deliveries performed without prophylaxis. The proportion of deliveries without pretreatment and the good outcomes in cases in which prophylaxis was not used match the findings of our case series.

Only 5 of 15 women who underwent a cesarean section in our series knew about their condition and were treated with pdhC1INH prior to delivery. Of the 10 cesarean deliveries that did not include prophylactic treatment, a single patient experienced mild HAE symptoms during delivery and 48 hours postpartum and subsequently required treatment with pdhC1INH. Czaller et al [18] described the use of prophylactic treatment prior to cesarean delivery in 1 of 8 cases and reported no symptoms in any of these deliveries. Martinez-Saguer et al [19], in turn, reported 8 cesarean sections out of 35 deliveries; in all cases, the patients underwent prophylactic treatment with pdhC1INH immediately before delivery and no attacks were reported.

Our study has certain limitations. Information was obtained retrospectively through a questionnaire completed using clinical records and telephone interviews in the case of missing data. Due to the retrospective nature of the study, the information corresponded to pregnancies from several years earlier (up to 20 years in nearly half of the patients). It is also important to note that C1INH-HAE had not been previously diagnosed in 89 pregnancies, and in the majority of cases, a diagnosis was not made until several years later. Therefore, the patients may have underestimated the severity of symptoms or failed to associate them with C1INH-HAE. Nevertheless, the fact that most patients had not been diagnosed with C1INH-HAE before conception and did not receive any treatment during pregnancy provides interesting insights into the natural course of C1INH-HAE during pregnancy and labor.

In conclusion, after reviewing data from our series we can conclude that pregnancy appears to have a variable influence on the clinical expression of C1INH-HAE, and may differ from one patient to another and to a lesser extent, from one pregnancy to the next. Attacks tend to occur more frequently but they do not appear to increase in severity. pdhC1INH prophylaxis should be administered prior to cesarean delivery and is also highly recommended for vaginal delivery in patients with additional risks factors or severe C1INH-HAE symptoms during pregnancy or previous deliveries. pdhC1INH (2000 U) should always be available in the delivery room and during hospitalization. To improve outcomes in pregnant women with C1INH-HAE, it would be wise to maintain observation for 48 hours postpartum.

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### *Conflicts of Interest*

Dr Caballero has received sponsorship for educational purposes, has been paid for providing consultancy services, and has taken part in clinical trials sponsored by Jerini AG/Shire, CSL-Behring, Dyax Corp, Pharming NV, and Viropharma Pharmaceutical.

Dr Cimbollek has received sponsorship for educational purposes and has taken part in clinical trials sponsored by CSL-Behring, and Pharming NV.

Dr González-Quevedo has received speaker's fees from Jerini AG/Shire, has been paid for providing consultancy services to Viropharma Pharmaceutical, and has received sponsorship for educational purposes and taken part in clinical trials sponsored by CSL-Behring, Dyax Corp, and Pharming NV.

Dr Guilarte has received sponsorship for educational purposes, has been paid for providing consultancy services to Jerini AG/Shire, and has participated in clinical trials sponsored by Jerini AG/Shire and Pharming NV.

Dr. Ignacio Larco has taken part in a clinical trial sponsored by CSL-Behring.

Dr López-Serrano has received sponsorship for educational purposes, been paid for providing consultancy services, and taken part in clinical trials sponsored by Jerini AG/Shire, CSL-Behring, Dyax Corp, and Pharming NV.

Dr Marcos has been paid for providing consultancy services and received speaker's fees and sponsorship for educational purposes from Jerini AG/Shire.

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# The genetics of hereditary angioedema: A review

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## Introduction

While our understanding of hereditary angioedema (HAE) has evolved gradually since the condition was first described by Sir William Osler in 1888, it has been greatly enhanced in recent decades, particularly since the turn of the millennium, thanks to intensive research efforts, advances in laboratory techniques, increasing clinical observations, and improved understanding of the link between genetics and pathogenesis. HAE is characterized by recurrent non-pruritic, self-limiting subcutaneous or submucosal edema that can affect any part of the body (skin or mucous membranes or gastrointestinal and upper respiratory tract). Its severity and frequency varies among members of the same family and even within individual patients over time[1]. Though initially believed to be an exclusively monogenic disorder, it has been postulated that the clinical expression of HAE is influenced by other conditions or cofactors and there is increasing evidence of multiple gene involvement.

There are two types of HAE: HAE due to C1-inhibitor (C1-INH) deficiency (C1-INH-HAE) and HAE with normal C1-INH (nC1-INH-HAE). C1-INH-HAE is further divided into type I HAE, which accounts for approximately 90% of cases and is characterized by low levels of C1-INH, and type II HAE, which accounts for the remaining 10% of cases and is characterized by normal or elevated levels of dysfunctional C1-INH protein. nC1-INH-HAE, in turn, is caused by an alteration in the F12 gene in up to 25-30% of cases (FXII-HAE). In the remaining 70-75% of cases, the genetic basis is unknown (unknown-HAE or U-HAE). The description of nC1-INH-HAE has driven a new field of study investigating the genetic basis of HAE in relation to bradykinin receptors and enzymes that act on fibrinolysis and the contact system. Much of the recent research has focused on gene polymorphisms.

## The genetics of C1-INH-HAE

### Mutations in the SERPING1 gene

C1-INH is a serine protease inhibitor that is a member of the serpin family, together with alfa-1-antitrypsin and antithrombin-III. Unlike other serpins, however, which have a single or small number of targets, C1-INH is a major inhibitor for several proteases. Its main function is to inhibit the complement system (C1r, C1s, mannose-binding-lectin-associated-serine-proteases: MASP-1, and MASP-2) and other activation cascades involved in bradykinin

production (factor XIIa, plasma kallikrein, and factor XIa from the contact/coagulation system; plasmin and tissue plasminogen activator from the fibrinolytic system)[2]. It is encoded by the human C1-inhibitor gene (Gene Bank X54486; Swiss-Prot P05155), also known as the SERPING1 gene (OMIM no. 606860; GenBank NM_000062.2), located on chromosome 11q12-q13.1[3,4]. The gene consists of eight exons (of which seven are protein-coding) and seven introns, distributed along a 17-Kd DNA segment. Exon 8 encodes the reactive center loop and the hinge region, which have an important role in protein function[5]. The SERPING1 gene presents unusually dense clusters of Alu repeats in its introns, and is accordingly strongly predisposed to rearrangements (deletions and duplications) and genetic instability[6-9]. De novo mutations are believed to exist in approximately 25% of all unrelated cases of C1-INH-HAE[10]. This high frequency of de novo mutations seems to be related to Alu sequences[7] and the existence of several mutation hotspots: the CpG dinucleotide at the end of exon 3 and exon 6, duplications of the ATG codon, and changes in the CpG dinucleotide of the reactive site (Arg444Cys and Arg 444Leu) due to different mechanisms[10].

More than 450 SERPING1 mutations are currently listed in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac)[11] and the HAE-specific HAEdb database (http://hae.enzim.hu/)[12]. Type II HAE is the result of point mutations in or near the reactive center loop that result in inactive C1-INH[13]. Up to 70% of patients with type II HAE have a mutation at Arg444 (the P1 residue) that produces dysfunctional C1-INH[14]. Mutations in type I HAE, however, are highly heterogeneous and are distributed throughout the gene. Up to 20% of the cases are the result of large rearrangements (partial deletions, and less frequently, partial duplications)[15]. The mutations most frequently observed are missense mutations (34%) and frameshift alterations and small indels (31%), followed by splice-site mutations (10%), nonsense mutations (7%), and regulatory mutations (1%)[16].

C1-INH-HAE has an autosomal dominant inheritance pattern with high penetrance, and most patients are heterozygous[17], although several cases of homozygosis[18-22] and two cases of gonadal mosaicism have been described[23,24]. Heterozygous patients have C1-INH plasma levels within 5-30% of normal values, rather than the 50% that might be expected. This seems to be the result not only of a synthesis defect but also of increased catabolism in patients with type I HAE[25], normal mRNA underexpression[26], or transinhibition of wild-type C1-INH translation by mutant mRNA and/or protein[27].

A significant correlation has been observed between HAE severity scores and baseline C1-INH activity (but not other complement components)[28]. This observation, however, contrasts with the findings of several studies that have failed to find such a correlation[29-33]. Many studies have attempted to establish a correlation between clinical phenotype and SERPING1 gene mutations, but the results have been contradictory, and most authors have not found any clear evidence of a relationship[31,32,34,35]. The largest study to date, conducted in 2014, investigated 256 patients from 117 unrelated families from 4 European countries, and found that missense mutations were associated with significantly later disease onset and a significantly lower probability of HAE attacks before the age of 10 years[36]. Onset before this age has been linked to a severe disease course. The study, however, did not evaluate other aspects of disease severity, such as laryngeal or abdominal attacks or frequency of episodes. A smaller study detected a significant relationship between missense mutations and clinical severity score and laryngeal and facial angioedema, but found no association with disease onset[37]. In our opinion, the lack of standardized criteria for classifying HAE severity makes it difficult to study the relationship between SERPING1 mutations and clinical phenotype. Nevertheless, the high variability in clinical expression between patients with the same mutation, and even in the same patient over time, led to the hypothesis that SERPING1 mutations alone might not be enough to explain the diversity of clinical expression.

Certain polymorphisms accompanying pathogenic mutations in the SERPING1 gene have been proposed as possible indicators of a severe phenotype in C1-INH-HAE, although no correlation has been found to date for the p.V480M polymorphism (c.1438G>A, rs4926)[38,39] and contradictory results have been reported for the polymorphism c.-21T>C in exon 2[30,33,40]. The distribution of SERPING1 alterations has been observed to vary among different countries with Caucasian populations[36], suggesting that additional factors such as epigenetic changes or environmental influences (e.g., hormones, radiation, dietary habits) might contribute to SERPING1 gene abnormalities and/or expression in at least some cases. More studies of this type, but with larger populations, are needed.

No evidence of SERPING1 mutations has been found in up to 10% of C1-INH-HAE families with typical clinical manifestations of HAE and diminished C4 and C1-INH levels and activity, giving rise to the hypothesis that the disease could, in some cases, be due to alterations to intronic or untranslated regions that may modify SERPING1 expression or other factors that result in increased post-translational consumption of C1-INH[36].

## Other alterations

Much of the current evidence suggests that alterations to the SERPING1 gene are not the only factor that determines the clinical expression of C1-INH-HAE. Other genetic variations investigated to date include polymorphisms

in the angiotensin-converting enzyme (ACE) gene. ACE is the main enzyme responsible for bradykinin degradation, and a defect in this degradation has been attributed a potential role in the clinical expression of HAE. The I/D polymorphism, which is the most relevant polymorphism in the ACE gene[41], is responsible for up to 47% of enzyme levels, but no evidence has been found for a link between clinical HAE manifestations and this polymorphism. No evidence has been found either for an association with polymorphisms investigated to date in certain cell receptors, such as the two known receptors for bradykinin (polymorphisms 58C/T and 181C/T in BDKRB1 gene and 669C/G and 1098G/C in BDKR2 gene), which is a key mediator of vascular permeability[42].

Alterations to the F12 gene, which encodes coagulation factor FXII, have also been studied because of their role in the kinin system. The functional promoter polymorphism 46C/T, for instance, was significantly associated with delayed disease onset –independently of the accompanying SERPING1 gene mutation— and individuals with this polymorphism did not need long-term treatment[43]. F12-46C/T carriage has, therefore, been postulated as an independent modifier of C1-INH-HAE severity.

The lectin pathway (LP) is a component of the innate immune defense formed by several pattern recognition molecules that form complexes with MASP-1 and MASP-2, which cleave C4 and C2. Mannose-binding lectin (MBL) is a component of this LP and because of its ability to activate the complement system, it might have an influence on HAE pathophysiology. Studies investigating possible links, however, have not found clinical HAE expression to be associated with either MBL plasma levels and their capacity for complement activation[44] or polymorphisms that influence these levels[42].

Peripheral blood mononuclear cells (PBMCs) have also been analyzed, but no evidence has been found for a common altered PBMC expression pattern or for differential gene expression in PBMCs[45].

Emotional and physical stress both influence the hypothalamic-pituitary-adrenal axis, triggering the release of minerals and glucocorticoids. The N363S (rs6195) polymorphism in exon 2 of the gene encoding glucocorticoid receptor has been linked to an increase in glucocorticoid sensitivity. As emotional stress has been recognized as a common trigger of angioedema attacks, the possible involvement of this polymorphism in the clinical manifestations of C1-INH-HAE has also been investigated, but no association has been found between disease severity and the carriage or non-carriage of this polymorphism[46].

In a recent study, 15 genes associated with the contact system were investigated by next-generation sequencing in 23 members of a Brazilian family, 9 of whom had HAE symptoms[47]. Although some genetic alterations (p.Ile197Met –HMWK-, p.Glu298Asp –NOS3-, and p.Gly354Glu –B2R) were found in almost all the symptomatic patients, suggesting a possible influence on symptom expression, they were also found in some of the asymptomatic individuals, highlighting the need for more studies.

Finally, newly circulating extracellular microRNAs have been postulated as potential predictors of HAE attack frequency in a small population studied[48], potentially opening up a new path of research in this field.

## Genetics of nC1-INH-HAE

Patients with nC1-INH-HAE have no deficiencies in C1-INH levels or activity and no alterations in the SERPING1 gene. The underlying pathophysiology of nC1-INH-HAE remains unknown, although the role of contact pathway dysregulation is gaining increasing recognition.

### Mutations in the F12 gene

Approximately 25% of patients with nC1-INH-HAE have a mutation in the F12 gene[49]. This leaves 75% of patients with familial nC1-INH-HAE with no known genetic basis. The mutations described to date are located in exon 9 and intron 9, on chromosome 5q33-qter (OMIM no. 610619). The most frequent mutation, which is found in the majority of patients, results in a threonine-to-lysine substitution (c.983C>A, p.Thr309Lys) in the secreted zymogene protein (also referred to as p.Thr328Lys with the addition of the leader protein)[49]. Another mutation predicting a threonine-to-arginine substitution (c.983C>G, p.Thr309Arg; also referred to as p.Thr328Arg with the addition of the leader protein) in the same codon has been described in two families[49]. There has also been a report of an 18-bp duplication and a 72-bp deletion in this same proline-rich region. The 18-bp duplication (c.892_909dup) is caused by the repeated presence of 6 amino acids (p.298–303)[50], while the 72-bp deletion (c.971_1018 + 24del72) causes a loss of 48 bp in exon 9 (coding amino acids 324-340) and a loss of 24 bp in intron 9, including the splice site of exon 9[51]. These deletions do not cause a reading frameshift or a premature stop codon, and the FXII catalytic domain remains preserved[52].

We do not yet fully understand how these genetic alterations contribute to the pathophysiology of nC1-INH-HAE. One initial theory proposed was that the p.Thr309Lys mutation might be associated with increased FXII enzymatic activity[53], but this was not confirmed in a subsequent study[54] and is no longer believed to be the case. p.Thr309Lys (T309K) and p.Thr309Arg (T309R) have both been studied in in vivo and in vitro mouse models and been observed to result in a loss of O-liked glycosylation of the amino acid residue. This loss increased the susceptibility

of FXII zymogen autoactivation, leading to excessive activation of bradykinin formation through the kallikrein-kinin pathway[55]. These mutations have also been found to result in an accelerated activation of FXII by plasmin, a natural activator of the contact system[56]. Furthermore, this activation is not completely regulated by C1-INH regulation, possibly explaining why HAE occurs in patients with normal C1-INH levels and activity. It has also been hypothesized that variations in the plasminogen activation system could be related to disease expression and/or activity, but more studies are needed[56,57].

nC1-INH-HAE presents an autosomal dominant inheritance pattern but has a very low penetrance, particularly in males (over 90% of male carriers are asymptomatic compared with 40% of females)[58]. Mutations in the F12 gene are mainly heterozygous. There has just been one report of 2 Brazilian patients from unrelated families who had a p.Thr309Lys mutation in homozygosity[59].

As occurs with C1-INH deficiency, no relationship has been observed between F12 mutations and clinical expression in heterozygous patients with FXII-HAE. The observation of a severe phenotype in the two homozygous patients described to dates suggests that this genotype might be associated with severe disease expression, but further research is needed.

### Other genetic alterations

Polymorphisms in bradykinin-degrading enzymes, which result in altered enzyme levels or activity, have also been studied in nC1-INH-HAE. In an initial study, the I allele of the I/D polymorphism of the ACE gene and the A allele of the XPNPEP2 gene were found in three symptomatic patients from a family with FXII-HAE[60], suggesting a possible influence on phenotype. This theory, however, was not confirmed in a recent study, which found no correlation with disease expression or severity[61].

Recently, a missense mutation in the angiopoietin-1 gene (ANGPT1, c.807G>T, p.A119S), associated with a reduced ability to bind the natural receptor "tunica interna endothelial cell kinase-2" (TIE2) of the ANGPT1 p.A119S variant, has been described in a family with U- HAE[62], adding a further research field.

## Conclusions

The original hypothesis that HAE was an exclusively monogenic disease is losing weight. Growing understanding of the pathophysiology of HAE has triggered the search for genetic alterations at other points of the contact system and related systems, with evidence increasingly pointing to the involvement of multiple genes. The lack of a standardized system for measuring disease severity constitutes a clear obstacle. Recently missense mutations in the SERPING1 gene[36] and the 46C/ T polymorphism in the F12 gene[43], have been both associated with later disease onset (and hence less severe disease) in Caucasian patients with C1-INH-HAE. These results are promising but should be regarded with caution as they cannot explain the high variability of clinical presentation in carriers of the same mutation or even in the same patient over time. Studies are increasingly investigating the role of epigenetic and environmental factors in this variability. In the case of nC1-INH-HAE, detection of genetic alterations is essential for an accurate diagnosis, as there are no biological markers or clear clinical or laboratory diagnostic criteria.

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