



Clinical, biochemical, and genetic characterization of type III hereditary angioedema in 13 Northwest Spanish families

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ABSTRACT

Background: A new variant of hereditary angioedema has been reported during the last decade. Three main characteristics distinguish it from classic hereditary angioedema: normal C1 inhibitor activity, predominance in women, and different genetic alterations.

Objective: To assess the symptoms, laboratory findings, and treatment of a population with type III hereditary angioedema from Northwest Spain.

Methods: We studied 29 patients (26 female and 3 male) from 13 different families.

Results: The 26 female patients showed a similar clinical pattern to the classic forms of hereditary angioedema, and 22 of these patients had the estrogen-dependent phenotype. Three patients had a negative family history, and 1 of the parents was confirmed as an asymptomatic carrier in 2 of them. All had functional C1 inhibitor activity within the normal range in periods without high estrogen levels, but during attacks (in female patients) and pregnancy, activity decreased to below 50%. One male patient had normal C1 inhibitor activity during attacks, and he was initially diagnosed as having idiopathic angioedema. The C4 and antigenic C1 inhibitor levels were always normal. All studied patients had the c.1032C>A, Thr309Lys mutation in the factor XII gene. The mutation was also found in asymptomatic relatives: 5 of 6 men studied and 1 of 8 women studied.

Conclusion: Positive family history is a diagnosis criterion, but it could be lacking because there may be asymptomatic relatives, primarily males.

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Introduction

The classic forms of hereditary angioedema (HAE), types I and II, are caused by abnormal C1 inhibitor (C1-INH) function due to mutations in the gene encoding this inhibitor (*SERPINC1*). Type III HAE, also known as estrogen-related HAE or HAE with normal C1-INH function, is considered a novel type of HAE. The first report in the literature, by Warin et al,¹ dates back to 1986 and describes 2 sisters who experienced severe angioedema attacks only when taking oral contraceptives (OCs) and during pregnancy. Fourteen years later, Bork et al² and Binkley and Davis³ reported the first series of patients, and the molecular basis for the disease was described by Dewald and Bork⁴ and Cichon et al⁵ in 2006. Two different missense mutations in the gene

that codifies for coagulation factor XII (*FXII*) were only found in 20%⁶ to 24.5%⁷ of unrelated families with type III HAE (HAE-FXII). Moreover, a novel *FXII* gene mutation, a 72–base pair deletion (c.971_1018+24del72*), has been recently identified by Bork et al⁸ in a single family of Turkish origin. However, the genetic basis responsible for the disease in the remaining type III HAE families has yet to be found. Bork et al have proposed the denomination HAE-unknown⁷ for this variant of the disease.

Currently, several aspects of this novel type of HAE need to be clarified to improve diagnoses and therapeutic management. The aim of this study is to describe the clinical and biological characteristics and the therapeutic management of 29 patients from 13 different families with type III HAE. All families were from the Galicia region, located on the northern Atlantic coast of Spain, and they did not have an apparent familial relationship.

Methods

Study design

We conducted an analysis of patients with type III HAE studied by our group since 2000, when our first family (family 1) was

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described at the XXII National Congress of Spanish Society of Allergy and Clinical Immunology, around the same time the first cases of type III HAE were being described. All patients had experienced recurrent angioedema attacks unresponsive to corticosteroids and antihistamines, without associated urticaria, and with normal C1-INH measurements in basal periods (not so during high estrogen states and attacks). Estrogen influence was suspected in the index patients because they all presented with recurrent angioedema attacks exclusively or mainly in periods of exogenous estrogen intake and/or pregnancy.

Laboratory methods

C1-INH and C4 levels were measured by nephelometry. C1-INH function was quantified with a chromogenic assay (Berichrom, Siemens, Marburg, Germany). These determinations were assessed at the Reference Laboratory (Barcelona, Spain) with careful attention to sample handling for complement. In all cases, biological sampling was performed in basal periods and, when possible, also during attacks and in high estrogen states.

Genetic study

Genetic screening was performed in patients and in healthy relatives without angioedema symptoms. Blood samples were taken after written informed consent from the patients and their relatives, and approval was obtained from the institutional review board.

DNA extraction. DNA samples were extracted from peripheral blood mononuclear cells using Gentra Puregene BloodCore (Qiagen, Valencia, California) and following the manufacturer's instructions.

Genetic profile. Exon 9 of the *FXII* gene was screened by polymerase chain reaction and direct sequencing. Briefly, polymerase chain reactions were set up in a final volume of 30 μ L containing 0.5 μ g of genomic DNA, 2 mM deoxynucleotide triphosphates, 0.8 mM magnesium chloride, and 2.5 U of Taq DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany) and using the primers (10 pmol each) and cycling conditions previously described.⁵ Amplified products were purified from a 2% low melting agarose gel using the QIAquick gel extraction kit (Qiagen Inc, Valencia, California). The sequencing reactions were performed in 10 μ L, with a 5- to 20-pmol primer and using Big Dye terminator cycle sequencing v.3.1 (Applied Biosystems, Foster City, California). Sequencing products were precipitated in 65% ethanol, washed twice in 70% ethanol, and analyzed on an automatic sequencer (ABI-PRISM 3130; Applied Biosystems).

Case description

A detailed description of 29 patients with HAE from 13 unrelated families is provided in the eMethods.

Results

A total of 29 patients from 13 unrelated families were studied during a 12-year period. Most patients were female (26/29, 89.6%), with only 3 affected males, who belonged to the same family. One of these male patients (patient 3.2) had been previously diagnosed as having nonhistaminergic idiopathic angioedema. Most of the patients had recurrent angioedema with variable localization (face-hands-feet-abdomen-upper airway), except for 6 of them, who had exclusively facial attacks. The age at attack onset ranged from 14 to 55 years, with a mean age of 23.9 years.

Four of the index cases had no similar cases reported in their families initially (negative family history in 4 of 13 index cases). In the following years, a relative (family 9) began to experience attacks. In family 3, the first patient who started to have angioedema

attacks (patient 3.2) was not the index case because he was male, and several years passed until another member of the family began to have symptoms suggestive of type III HAE.

All the female patients, except one (patient 10.2), experienced angioedema attacks exclusively or mainly in high estrogen states as OC intake or pregnancy. Patient 10.2 presented with 10 attacks during 7 years in which she neither got pregnant nor received exogenous estrogens; however, her daughter (patient 10.1) experienced attacks exclusively during OC intake.

Complement determinations showed normal C4 and C1-INH levels in all patients during basal or high estrogen periods. Nevertheless, C1-INH activity was below 50% in 5 attacks (2 during pregnancy and 3 during OC intake) and in the 5 pregnancies tested but was found to be normal in 5 determinations performed during OC intake and during 2 attacks in the male patient.

The main clinical and laboratory findings for the study patients are summarized in Tables 1 and 2, respectively.

A total of 11 acute attacks in 4 patients were treated during the follow-up years. Intravenous tranexamic acid, 1,000 mg, was administered in 6 attacks, C1-INH concentrate, 1,000 U, in 2 attacks, and icatibant in 3 attacks. The results were variable (Table 3).

Follow-up data on 6 pregnancies of 3 patients are also given (Table 4). Oral tranexamic acid was effective as long-term prophylaxis treatment in the first pregnancy of patient 1.1. and the pregnancy of patient 7.1 (500–1,000 mg 3 times daily) because the patients did not experience any more attack after beginning the treatment (from the 17th and 12th gestation weeks, respectively). However, the attacks appeared despite long-term prophylaxis treatment in the last pregnancies of patient 1.1 (12 attacks with tranexamic acid, 500 mg 3 times daily) and patient 3.1 (27 attacks with tranexamic acid, 1,000 mg three times daily), although both patients reported that attacks were less severe.

Genetic studies revealed that a single, common mutation in the *FXII* gene (c.1032C>A, Thr309Lys) was segregating in all the affected families. All the patients underwent genetic study except for 2 of 6 patients from family 1 (Table 2). The results of the genetic studies on 14 healthy relatives (6 male and 8 female) are given in Table 5.

Discussion

Clinical heterogeneity is evident in patients with type III HAE. The diagnosis of this type of angioedema remains challenging because no specific assay is available except for genetic study, and even genetic data are not always diagnostically useful as proven in the literature.^{6,7} However, in all our families, whose index case presented with clinical symptoms suggestive of type III HAE (recurrent estrogen-related angioedema without urticaria, nonresponsive to corticosteroids and antihistamines, with normal C1-INH measurements in basal periods), the genetic study result was positive for the missense mutation (c.1032C>A, Thr309Lys) in the *FXII* gene. The other 2 mutations described by Bork et al in 2 families of German origin (c.1032C>G Thr309Arg)⁷ and in a family of Turkish origin (c.971_1018+24del72*),⁸ were not found in patients from our area. It is generally accepted that 3 patterns of HAE exist in women⁹: estrogen dependent (specific phenotype of this variant), estrogen sensitive (phenotype common to classical HAE), and estrogen independent. Most of our female patients had attacks exclusively in high estrogen states, and 3 patients also developed episodes outside those periods. Only 1 female patient has an estrogen-independent phenotype. This different phenotypic pattern is not explained by the genotype at the *FXII* locus because all of the patients carry the same genetic alteration. In their series, Duan et al¹⁰ demonstrated the involvement of other genetic polymorphisms associated with low levels of both aminopeptidase P and angiotensin I-converting enzyme, the major enzymes responsible for bradykinin degradation, and they speculated that these genetic

Table 1

Clinical data on 29 family members affected by type III hereditary angioedema

Patient No.	Sex/age, y	Age at onset, y	Family history of AE	Laryngeal edema	Abdominal attack	Trauma as triggering factor	AE exclusively in high estrogen states	AE mainly in high estrogen states	Family follow-up, y ^a
1.1	F/36	22	Yes	Yes	Yes (A)	No	Yes		11
1.2	F/61	29	Yes	No	Yes	Yes	Yes		
1.3	F/50	28	Yes	No	No	No	Yes		
1.4	F/56	35	Yes	No	No	No	Yes		
1.5	F/48	33	Yes	No	No	No	Yes		
1.6	F/52	21	Yes	No	No	No	Yes		3
2.1	F/24	21	No	Yes	Yes (A)	No		Yes	
3.1	F/35	20	Yes	Yes	Yes	Yes	Yes		
3.2	M/63	55	Yes	No	No	No	NA	NA	
3.3	M/32	28	Yes	No	No	No	NA	NA	
3.4	M/22	14	Yes	No	No	Yes	NA	NA	5
4.1	F/33	28	Yes	No	Yes	Yes	Yes		
4.2	F/58	21	Yes	No	No	No	Yes		
5.1	F/32	19	Yes	No	Yes	Yes	Yes		
5.2	F/23	16	Yes	No	Yes	No	Yes		
5.3	F/60	28	Yes	No	No	No	Yes		3
6.1	F/20	20	Yes	No	Yes	No	Yes		
6.2	F/77	22	Yes	Yes	No	No		Yes	
7.1	F/26	17	No	No	No	No	Yes		
8.1	F/30	15	No	No	No	No	Yes (?)		
9.1	F/26	23	Yes	No	No	No	Yes		4
9.2	F/23	22	Yes	No	No	No	Yes		
10.1	F/24	19	Yes	No	No	Yes	Yes		
10.2	F/53	44	Yes	No	No	No	No	No	
11.1	F/36	18	Yes	No	Yes	No	Yes		
11.2	F/53	17	Yes	No	No	No	Yes		2
12.1	F/33	15	Yes	No	No	No	Yes		
13.1	F/23	19	Yes	No	Yes	Yes	Yes		
13.2	F/62	25	Yes	Yes	No	Yes		Yes	

Abbreviations: A, white appendectomy; AE, angioedema; NA, not applicable.

^aTime since index case diagnosis.

variants might contribute to the diversity of clinical phenotypes. High estrogen levels increase expression of overactive mutant forms of FXII and suppress angiotensin I-converting enzyme and possibly aminopeptidase P, thus conditioning its role in this type of HAE.¹¹ In contrast to our findings, Bork et al^{12–14} did not find as strong estrogen influence as we did, and they concluded that the negative effect of estrogens is similar in all types of HAE.

Because all the patients who were described first were female, it was assumed that the clinical phenotype might be limited to the females. However, in recent years families with symptomatic males have been reported.^{6,15,16} In our series 3 symptomatic men from the same family have been studied. Interestingly, 1 of them (patient 3.2) was initially diagnosed as having nonhistaminergic idiopathic angioedema, and only the fact that his daughter later had recurrent angioedema with a type III HAE phenotype led us to perform genetic studies on both. We speculate that some patients diagnosed as having nonhistaminergic idiopathic angioedema might actually have type III HAE, and as long as no other relatives develop symptoms specific to this variant of HAE, the clinical diagnosis is difficult. In addition, and as reported in other males affected by type III HAE, male patient 3.2 (but not his 2 affected sons) had a different phenotypic expression of the disease with late onset (sixth decade of life), which is in contrast to the remaining patients, who presented with the disease in the second to fourth decades and had only facial angioedema. Five other female patients from our series also had exclusively facial attacks, as did 3 patients from a recently reported family of Arab origin.¹⁷ Bork et al¹⁸ have found facial swelling to be more frequent in this HAE variant.

A family history of angioedema may not be present in all cases, unlike what has been previously been described.^{6,18} In our HAE-FXII series, 3 patients had no family history of angioedema, and the diagnosis was suspected because of estrogen influence in the clinical expression of the disease. Genetic studies performed on 2 of the families demonstrated that 1 of their parents was an asymptomatic

carrier (the father of patient 2.1 and the mother of patient 7.1). Likewise, the *FXII* gene mutation was identified in 5 of 6 male asymptomatic relatives studied. In contrast, only 1 of the 8 asymptomatic female relatives studied presented the mutation (the mother of patient 7.1). Strikingly, patient 7.1 had 2 pregnancies and was treated with OCs for 3 months and never experienced angioedema attacks. Symptomatic patients are mostly female because males lack female-specific risk factors or have male-specific protective factors.¹⁶ In this novel type of HAE, additional factors are needed for its clinical expression, and it is predictable that healthy carriers may exist. Besides, we think that the follow-up time span is important, as reflected by the fact that 2 of our patients had no family history at the time of the first diagnosis (patient 3.2 and 9.1) and symptomatic relatives were only later identified.

This new variant of HAE was initially characterized by normal levels of C1-INH and C4, even during attacks.^{2,19–23} However, Bouillet et al²⁴ and others^{6,15} found C1-INH activity to be normal or near normal in symptom-free periods but lower during contraceptive treatment in 67% of the female patients tested. Picone et al²⁵ described 2 pregnant patients who also had transient deficiencies of C1-INH function. Our patients also had C-INH activity within a normal or near-normal range outside high estrogen periods. This C1-INH activity decreased (below 50%) during the attacks (5 attacks tested) and in high estrogen states during pregnancy (5 pregnancies tested) but not with combined contraceptive treatment (5 patients tested). Two patients (patients 5.1 and 5.2) had normal C1-INH function during contraceptive treatment but exhibited decreases during attacks. Male patient 3.2 was the only one with conserved C1-INH function during attacks. In all cases, C1-INH and C4 levels remained within the normal range. This is the first report, to our knowledge, of patients with type III HAE in which a transient deficiency of C1-INH activity is demonstrated during attacks. Perhaps C1-INH binding to bradykinin-forming enzymes during swelling attacks leads to temporary C1-INH depletion. Thus, we

Table 2
Laboratory data on 29 family members affected by type III hereditary angioedema^a

Patient No.	Basal C4, mg/dL	HEE C4, mg/dL	Attack C4, mg/dL	Basal C1-INH activity, %	HEE C1-INH activity, %	Attack C1-INH activity, %	Attack C1-INH protein, mg/dL	Mutation c.1032C>A, Thr309Lys
1.1	28	26.8 (G) 30.9 (G)	39.6	83	64 (first G) 12 (third G) <10 (third G)	34 (first G)	21 (first G)	Yes
1.2	30	ND	ND	87	ND	ND	ND	Yes
1.3	30.3	ND	ND	83	ND	ND	ND	ND
1.4	23	ND	ND	85	ND	ND	ND	Yes
1.5	ND	26 (OC)	ND	ND	98 (OC)	ND	ND	ND
1.6	25.6	ND	ND	109.62	ND	ND	ND	Yes
2.1	13.2	ND	ND	87	ND	ND	ND	Yes
	12.8			62				
3.1	24	ND	31.2	93	ND	40 (second G)	27	Yes
3.2	25.2	NA	26.2	127	NA	104	ND	Yes
			27.9			121	30	
3.3	25.9	NA	ND	109	NA	ND	ND	Yes
3.4	18.3	NA	ND	90.42	NA	ND	ND	Yes
4.1	28.4	ND	ND	124	ND	ND	ND	Yes
4.2	55.7	ND	ND	119	ND	ND	ND	Yes
5.1	ND	25.2 (OC)	24	ND	99 (OC)	50 (OC)	23	Yes
5.2	ND	21.2 (OC)	21.8	ND	70.8 (OC)	44 (OC)	23	Yes
5.3	24.2	ND	ND	131	ND	ND	ND	Yes
6.1	21	24	24	99	ND	30 (OC)	31	Yes
6.2	29	ND	ND	91	ND	ND	ND	Yes
7.1	21.9	24.1 (G)	ND	108	13 (G) 37 (G)	ND	ND	Yes
8.1	21.8	ND	ND	120	ND	ND	ND	Yes
9.1	16	ND	ND	116	ND	ND	ND	Yes
9.2	11.3	ND	ND	86	ND	ND	ND	Yes
10.1	ND	18	ND	ND	91 (OC)	ND	ND	Yes
10.2	34	ND	ND	114	ND	ND	ND	Yes
11.1	ND	19.8 (G)	ND	ND	47.27 (G)	ND	ND	Yes
11.2	16	ND	ND	114	ND	ND	ND	Yes
12.1	30	ND	ND	114	ND	ND	ND	Yes
13.1	23.4	23 (OC)	ND	112	117 (OC)	ND	ND	Yes
13.2	30	ND	ND	120	ND	ND	ND	Yes

Abbreviations: Basal, outside high estrogen states (gestation or exogenous estrogen intake) and outside attacks; C1-INH, C1 esterase inhibitor; G, gestation; HEE, high estrogen states; NA, not applicable; ND, not determined; OC, oral contraceptives.

^aNormal ranges are as follows: C4 protein, 15 to 40 mg/dL; C1-INH activity, 70% to 130%; C1-INH protein, 22 to 45 mg/dL.

propose avoiding the term *HAE with normal C1-INH function* when referring to this new variant of HAE, and we suggest that type III HAE, proposed by Bork et al,² would be the most appropriate nomenclature.

Currently, no treatment for type III HAE has been established, but there is increasingly successful experience in attack treatment with C1-INH concentrate in 64 attacks,^{6,7,26} tranexamic acid in 1 attack,²⁰ icatibant in 3 abdominal attacks,²⁷ and ecallantide in one facial attack.²⁸ In contrast, C1-INH concentrate was not effective in 12 reported attacks.^{2,7} In our patients, we obtained clearly favorable results in 2 of 3 patients treated with icatibant, 1 of 2 patients treated with C1-INH concentrate, and 1 of 6 patients treated with intravenous tranexamic acid. We also prescribed tranexamic acid

as a prophylactic treatment during pregnancy with variable success, as has been previously reported.^{2,6,7,29} Danazol^{2,7,21} and progesterone⁷ have been successfully used in nonpregnant patients. In a recent report,²⁶ C1-INH concentrate was used as long-term prophylaxis during pregnancy at a dose of 500 U, twice monthly. Regarding short-term prophylactic treatment, only 5 cases have been reported, and all of these patients were treated with C1-INH.^{6,25,26} None of our patients without short-term prophylactic treatment had symptoms during the 6 deliveries nor did any of the remaining female patients (retrospective data). Because attacks may be triggered by dental procedures or intubation, as with classic HAE, patients with type III HAE must also be treated before these procedures. However, the drug of choice and the indication of

Table 3
Treatment in acute attacks

Patient No.	Period (gestational/no gestational)	Attack location	Drug (dose)	Resolution time ^a
1.1	First gestation	Upper airway	IV tranexamic acid (1,000 mg)	3 h
	Second gestation	Upper airway	IV tranexamic acid (1,000 mg)	48 h
	Third gestation	Face	C1-INH concentrate (1,000 U)	48 h
		Upper airway	C1-INH concentrate (1,000 U)	4 h
2.1	No gestation	Upper airway	Icatibant (30 mg)	< 1 h
3.1	Second gestation	Lip	IV tranexamic acid (1,000 mg)	> 6 h
		Upper airway	IV tranexamic acid (1,000 mg)	> 6 h
		Face	IV tranexamic acid (1,000 mg)	> 6 h
3.2	(Male)	Face	Icatibant (30 mg)	6 days (as without attack treatment)
		Face	IV tranexamic acid (1,000 mg)	5 days (as without attack treatment)
		Face	Icatibant (30 mg)	24 h

Abbreviations: C1-INH, C1 esterase inhibitor; IV, intravenous.

^aComplete resolution of symptoms.

Table 4
Follow-up of pregnant patients

	Patient 1.1			Patient 3.1			Patient 7.1 (only pregnancy)
	First pregnancy	Second pregnancy (miscarriage at 19 weeks)	Third pregnancy	First pregnancy	Second pregnancy		
Offspring sex	Male	Unknown	Male	Female	Male	Male	
No. of attacks	4	4	13	4	31	8	
First attack, gestation week	10	7	7	Unknown	8	8	
Last attack, gestation week	17 (before beginning prophylaxis treatment)	16 (before beginning prophylaxis treatment)	30	Unknown	38	12 (before beginning prophylaxis treatment)	
Localization of attacks (No. of attacks)	Foot Abdomen Hand Upper airway	Hand (2) Abdomen Upper airway	Foot (7) Hand (4) Face Upper airway	Face (4)	Foot (12) Face (7) Upper airway (6) Hand (5) Knee	Face (4) Hand (3) Foot (1)	
Long-term prophylaxis treatment dose (gestation week at beginning of treatment)	Tranexamic acid, 500 mg/8–12 h (17)	Tranexamic acid, 500 mg/8–12 h (16)	Tranexamic acid, 500 mg/8 h (7)	None	Tranexamic acid, 1,000 mg/8 h (16)	Tranexamic acid, 1,000 mg/8 h (12)	
Delivery	No attack, no short-term prophylaxis	No attack, no short-term prophylaxis	No attack, no short-term prophylaxis	No attack, no short-term prophylaxis	No attack, no short-term prophylaxis	No attack, no short-term prophylaxis	

Table 5

Genetic study in 14 asymptomatic relatives

Sex	Positive genetic study (c.1032C>A, Thr309Lys)	Negative genetic study
Male	Father of patient 2.1 (obligate carrier) Father of patient 6.1 (obligate carrier) Father of patient 9.1 (obligate carrier) Father of patient 13.1 (obligate carrier) Grandfather of patient 13.1 (obligate carrier)	Brother of patient 7.1
Female	Mother of patient 7.1 (obligate carrier) ^a	Two sisters of patient 1.1 Daughter of patient 1.4 Mother of patient 2.1 Sister of patient 2.1 Sister of patient 9.1 Mother of patient 9.1

short-term prophylactic treatment throughout the patient's life in estrogen-dependent HAE (with and without high estrogen states) have not been clearly established in the literature.

In conclusion, our 29 patients and 14 asymptomatic relatives from 13 different families in a Spanish region (<3,000,000 inhabitants) had the same molecular basis for the disease, with mutations in the *FXII* gene (c.1032C>A, Thr309Lys). However, they had variable clinical expression: symptomatic men vs asymptomatic men, younger vs older age of onset, exclusively estrogen dependent vs estrogen sensitive and/or estrogen independent, exclusively facial attacks vs variable attack locations, and normal vs reduced C1-INH activity during attacks. These facts led us to hypothesize that other individual factors might influence the expression of the disease. Further studies will be necessary to elucidate additional genetic and nongenetic determinants responsible for the clinical heterogeneity of this novel form of HAE and thus help establish definitive diagnostic criteria.

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References

- [1] Warin RP, Cunliffe WJ, Greaves MW, Wallington TB. Recurrent angioedema: familial and oestrogen-induced. *Br J Dermatol*. 1986;115:731–734.
- [2] Bork K, Barnstedt SE, Koch P, Traupe H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet*. 2000;356:213–217.
- [3] Binkley KE, Davis A. Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema. *J Allergy Clin Immunol*. 2000;106:546–550.
- [4] Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. *Biochem Biophys Res Commun*. 2006;343:1286–1289.
- [5] Cichon S, Martin L, Hennies HC, et al. Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. *Am J Hum Genet*. 2006;79:1098–1104.
- [6] Vitrat-Hincky V, Gompel A, Dumestre-Perard C, et al. Type III hereditary angioedema: clinical and biological features in a French cohort. *Allergy*. 2010;65:1331–1336.
- [7] Bork K, Wulff K, Hardt J, Witzke G, Staubach P. Hereditary angioedema caused by missense mutations in the factor XII gene: clinical features, trigger factors and therapy. *J Allergy Clin Immunol*. 2009;124:129–134.
- [8] Bork K, Wulff K, Meinke P, Wagner N, Hardt J, Witzke G. A novel mutation in the coagulation factor XII gene in subjects with hereditary angioedema and normal C1-inhibitor. *Clin Immunol*. 2011;141:31–35.
- [9] Bouillet L. Hereditary angioedema in women. *Allergy Asthma Clin Immunol*. 2010;6:17.
- [10] Duan QL, Binkley K, Rouleau GA. Genetic analysis of factor XII and bradykinin catabolic enzymes in a family with estrogen-dependent inherited angioedema. *J Allergy Clin Immunol*. 2009;123:906–910.
- [11] Binkley K. Factor XII mutations, estrogen-dependent inherited angioedema, and related conditions. *Allergy Asthma Clin Immunol*. 2010;6:16.
- [12] Bork K. Hereditary angioedema with normal C1 inhibition. *Curr Allergy Asthma Rep*. 2009;9:280–285.
- [13] Bork K, Fischer B, Dewald G. Recurrent episodes of skin angioedema and severe attacks of abdominal pain induced by oral contraceptives or hormone replacement therapy. *Am J Med*. 2003;114:294–298.

- [14] Bork K. Hereditary angioedema with normal C1 inhibitor activity including hereditary angioedema with coagulation factor XII gene mutations. *Immunol Allergy Clin N Am*. 2006;26:709–724.
- [15] Martin L, Raison-Peyron N, Nöthen M, Cichon S, Drouet C. Hereditary angioedema with normal C1 inhibitor gene in a family with affected women and men is associated with the p.Thr328Lys mutation in the F12 gene. *J Allergy Clin Immunol*. 2007;120:975–977.
- [16] Bork K, Gül D, Dewald G. Hereditary angio-oedema with normal C1 inhibitor in a family with affected women and men. *Br J Dermatol*. 2006;154:542–545.
- [17] Baeza ML, Rodríguez-Marco A, Prieto A, Rodríguez-Sainz, Zubeldia JM, Rubio M. Factor XII gene missense mutation Thr328Lys in an Arab family with hereditary angioedema type III. *Allergy*. 2011;66:981–982.
- [18] Bork K, Gül D, Hardt J, Dewald G. Hereditary angioedema with normal C1 inhibitor: clinical symptoms and course. *Am J Med*. 2007;120:987–992.
- [19] Binkley KE, Davis AE. Estrogen-dependent inherited angioedema. *Transfus Apher Sci*. 2003;29:215.
- [20] Serrano C, Guilarte M, Tella R, et al. Oestrogen-dependent hereditary angioedema with normal C1 inhibitor: description of six new cases and review of pathogenic mechanisms and treatment. *Allergy*. 2008;63:735–741.
- [21] Martin L, Degenne D, Toutain A, Ponard D, Watier H. Hereditary angioedema type III: an additional French pedigree with autosomal dominant transmission. *J Allergy Clin Immunol*. 2001;107:747–748.
- [22] Herrmann G, Schneider L, Krieg T, Hunzelmann N, Sharfetter-Kochanek K. Efficacy of danazol treatment in a patient with the new variant of hereditary angio-edema (HAE III). *Br J Dermatol*. 2004;150:157–158.
- [23] Prieto A, Tornero P, Rubio M, Fernández-Cruz E, Rodríguez-Sainz C. Missense mutation Thr309Lys in the coagulation factor XII gene in a Spanish family with hereditary angioedema type III. *Allergy*. 2009;64:284–286.
- [24] Bouillet L, Ponard D, Drouet C, Jullien D, Massot C. Angioedema and oral contraception. *Dermatology*. 2003;206:106–109.
- [25] Picone O, Donnadieu AC, Brivet FG, Boyer-Neumann C, Frémeaux-Bacchi V, Frydman R. Obstetrical complications and outcome in two families with hereditary angioedema due to mutation in the F12 gene. *Obstet Gynecol Int*. 2010;957507.
- [26] Bouillet L, Ponard D, Rousset H, Cichon S, Drouet C. A case of hereditary angio-oedema type III presenting with C 1-inhibitor cleavage and a missense mutation in the F 12 gene. *Br J Dermatol*. 2007;156:1045–1092.
- [27] Bouillet L, Boccon-Gibod I, Ponard D, et al. Bradykinin receptor 2 antagonist (icatibant) for hereditary angioedema type III attacks. *Ann Allergy Asthma Immunol*. 2009;103:448.
- [28] Cronin JA, Maples KM. Treatment of an acute attack of type III hereditary angioedema with ecallantide. *Ann Allergy Asthma Immunol*. 2012;108:61–62.
- [29] Fiz Matías J, Ferrer Cerón SM, García Pérez C, Marcos Vidal JM. Obstetric analgesia for a woman with type III hereditary angioneurotic edema. *Rev Esp Anestesiol Reanim*. 2007;54:253–254.

eMethods

Family 1

This was our first family described (oral communication at the XXII Spanish Society of Allergology and Clinical Immunology Congress, September 2000). Patient 1.1, her mother (patient 1.2), and 4 maternal aunts (patients 1.3, 1.4, 1.5, and 1.6) presented with recurrent episodes of angioedema with variable localization (face-hands-feet-abdomen). During an 11-year follow-up, all patients remained asymptomatic by avoiding estrogen treatment, except patient 1.1, who had recurrent attacks during her 3 pregnancies. The second pregnancy ended in miscarriage at the fifth month because of spina bifida and severe cardiac and cerebral malformations. She has remained symptom free for 18 months since her last delivery.

Family 2

Patient 2.1 sought treatment because of several skin swellings and 2 unexplained abdominal episodes requiring hospitalization. In the first episode, she unnecessarily underwent an appendectomy. In the second, 1 month later, she was diagnosed as having abundant ascites and intestinal wall swelling, demonstrated by computed tomography. The patient had begun taking oral contraceptives (OCs) 4 days before her first abdominal episode, and they were withdrawn after the second one. In the following 6 years, she presented with several angioedema episodes located on the hands, face, and abdomen but with low intensity and frequency, except for an episode of throat swelling with dyspnea that required treatment 2 years ago.

Family 3

Patient 3.1 had recurrent angioedema 1 month after beginning OC use. After a dental procedure, she developed a facial edema. The episodes stopped after discontinuation of contraception use and reappeared in her 2 pregnancies. The patient has remained symptom free since her last pregnancy 2 years ago.

Her father (patient 3.2) was also studied 2 years before because of recurrent angioedema. He has presented with episodes annually during the last 8 years, always located on his face, of 1-week duration. Our initial diagnosis was idiopathic nonhistaminergic angioedema. This patient had taken neither testosterone blockers nor angiotensin-converting enzyme inhibitors. In the last 3 years, he has received angiotensin II type receptor antagonists without worsening.

Patient 3.3, the brother of patient 3.1, has recurrent episodes of edema on his left hand and feet at a frequency of 3 to 4 episodes per year. They disappear spontaneously within 24 hours. Patient 3.4, another brother of patient 3.1, presents with hand edemas after local trauma, of 48-hour duration, and foot sole edemas after exercise. These brothers are not taking any drug that influences angioedema development.

Family 4

Patient 4.1 had recurrent episodes of angioedema on her face, hands, feet, and abdomen. One of the facial attacks was after a dental procedure. The attacks appeared monthly for 4 years while she was taking OCs. In the last 3 years, after stopping the treatment, she has remained asymptomatic.

Her mother (patient 4.2) had several edema episodes on her hands and face during her 5 pregnancies and 1 episode of facial edema after 1 month of starting OC use. She has been asymptomatic since her last pregnancy 30 years ago. She has 5 brothers and 1 sister, and only her sister had 1 episode of edema during pregnancy.

Family 5

Patient 5.1 sought treatment because of edema involving her face, uvula, and soft palate 3 hours after an upper endoscopy and facial edema 4 hours after a dental procedure. She also presented with recurrent edema episodes located on her face and pharyngeal area and monthly abdominal episodes. She started taking OCs 1 year before the beginning of the attacks. She has remained asymptomatic in the last 18 months after stopping OC therapy.

Patient 5.2, the sister of patient 5.1, reported recurrent episodes of edema after beginning use of OCs. The attacks affected her face, hands, and feet, with a frequency of 1 or 2 episodes per year. During her 2 pregnancies, she presented with several episodes of feet and facial angioedema and 1 episode of tongue swelling. At the age of 21 years, 5 months after reinitiation of OC use, she was admitted to the hospital twice in 1 month because of abdominal attack and experienced monthly skin episodes. She has remained asymptomatic during the last 15 months after stopping OC use.

Patient 5.3, the mother of patients 5.1 and 5.2, had recurrent lip angioedema when she was pregnant. Other affected relatives, not included in this study, were the grandmother, aunt, and 1 female cousin of patient 5.1.

Family 6

Patient 6.1 presented with recurrent hand and foot swelling and 3 episodes of edema involving her face and pharyngeal area. She was admitted to the hospital because of abdominal pain. Eight months before her first episode, she had started taking OCs. In the last year, she has been treated with progestogens and remains asymptomatic.

Patient 6.2, the paternal grandmother of 6.1, reported episodes of hand swelling during her 4 pregnancies. When she was 47 years old, not pregnant, and not receiving OC treatment, she was admitted to hospital because of angioedema involving her face and hands, dyspnea, and pharyngeal stridor. At this time, she reported several other episodes on her face and hands. After menopause, she has remained asymptomatic, despite treatment with an angiotensin II receptor antagonist.

Family 7

Patient 7.1 experienced recurrent episodes of swelling on her hands, feet, elbows, and face. All the attacks appeared exclusively when she took OCs and during her only pregnancy. She has remained asymptomatic for the last 2 years since beginning prophylactic treatment in the 12th week of pregnancy and after delivery (18 months ago) without treatment.

Family 8

Patient 8.1 presented with 5 angioedema episodes. Two occurred during OC treatment, 1 occurred during pregnancy, and the patient did not remember the remaining 2 episodes. All attacks were located on her face, affecting the lips, eyelids, and uvula. Both her parents are deceased.

Family 9

Patient 9.1 sought treatment because of 3 episodes of facial angioedema 1½ months after starting OC use. The treatment was changed to progestogens, and she has remained asymptomatic for 4 years. Two years later, 1 of her 2 sisters (patient 9.2) presented with a facial attack 2 months after having a vaginal contraceptive device implanted. She is avoiding exogenous estrogens and has been asymptomatic for the last 2 years.

Family 10

Patient 10.1 has presented with 15 angioedema episodes, affecting the facial area since 2005. All her episodes have been related to

OC treatment, and she has remained asymptomatic by avoiding this for the last year. Her mother, patient 10.2, experienced 10 episodes, affecting her lips, eyelids, and fingers from 2001 to 2007. She has never received exogenous estrogens.

Family 11

Patient 11.1 experienced recurrent episodes of swelling of her face, forearm, and abdomen. All the attacks appeared exclusively when she took OCs and in her single pregnancy. During her pregnancy, she experienced 10 abdominal attacks and 1 episode that affected the bilateral aspect of the soles of her feet. Her mother, patient 11.2, had presented with only 3 facial episodes during her 3 pregnancies.

Family 12

Patient 12.1 presented with recurrent edema attacks located on the facial area, hands, and feet. Her episodes were related to OC intake and 2 pregnancies. In the last 20 months, after stopping OC

use, she has remained asymptomatic. She states that her mother and 2 female cousins had swelling episodes.

Family 13

Patient 13.1 sought treatment because of recurrent lip swellings triggered by minor traumas and unexplained abdominal pain episodes. The symptoms appeared 1 year after starting OC therapy. She has been symptom free for the last 2 years by avoiding estrogens.

This patient's great-aunt (patient 13.2) also experienced angioedema attacks in the lips, fingers, and upper airway since her first pregnancy. After delivery, she continued experiencing angioedema attacks until she underwent a hysterectomy and oophorectomy at the age of 47 years. She has never received OCs. The patient reports that 2 additional relatives living in Colombia (a daughter and an aunt), not included in this study, have also experienced edema attacks.