

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 hyper-estrogenic 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 hyper-estrogenic states or are of Western European or African origin.

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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

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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) deletion⁶ and an 18-bp

duplication⁷ 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

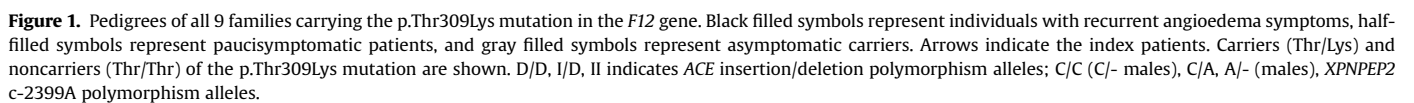
Associations between polymorphisms and clinical conditions were evaluated using the Fisher exact test. Data were analyzed using the Open Epi software, version 3.03, for Windows (www.OpenEpi.com). $P < .05$ was considered statistically significant.

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 questioning 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.



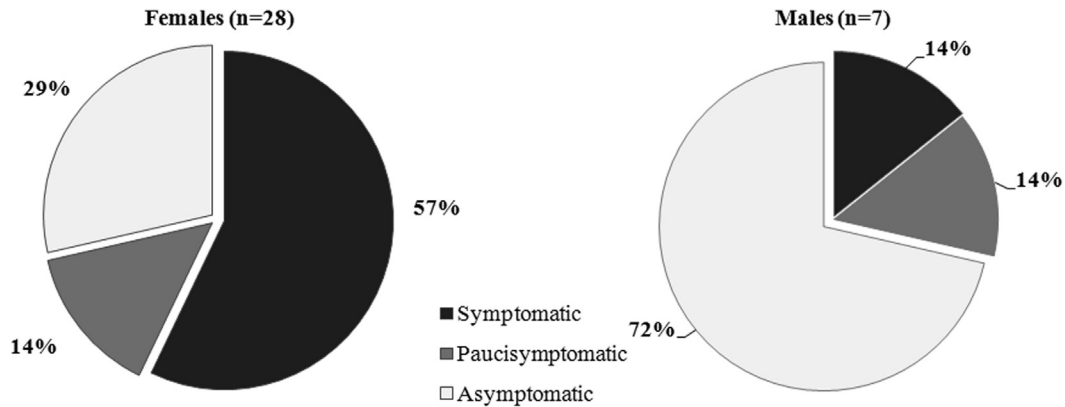


Figure 2. Distribution of clinical phenotypes.

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 Paucisymptomatic 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.

^aData 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 subintract 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.

^aNo 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-HAE²⁹ 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 *XPNPPE2* Genes^a

Gene	Total (n = 24)	Symptomatic (n = 14)	Paucisymptomatic (n = 4)	Asymptomatic (n = 6)
Insertion/deletion (rs1799752) <i>ACE</i> 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) <i>XPNPPE2</i> 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

^aData 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 al³⁵ 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 inter-patient 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-HAE²⁸ 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 al²⁸ 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 Binkley³⁸ suggested that the *ACE* insertion/deletion polymorphism—the major *ACE* gene locus that influences serum ACE concentration¹⁴—and the APP-regulatory polymorphism—*XPNPPE2* 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 Binkley³⁸ 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.

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