

Hereditary Angioedema with Normal C1 Inhibitor and *F12* Mutations in 42 Brazilian Families



Camila Lopes Veronez, PhD^{a,*}, Adriana S. Moreno, PhD^{b,*}, Rosemeire Navickas Constantino-Silva, PhD^c, Luana S.M. Maia, MSc^b, Mariana P.L. Ferriani, MD, PhD^b, Fábio F.M. Castro, MD, PhD^d, Solange Rodrigues Valle, MD, PhD^e, Victor Koji Nakamura, BSc^a, Nathália Cagini, MSc^a, Rozana Fátima Gonçalves, MD, PhD^f, Eli Mansour, MD, PhD^g, Faradiba Sarquis Serpa, MD, PhD^h, Gabriela Andrade Coelho Dias, MD, PhDⁱ, Miguel Alberto Piccirillo, MD, PhD^j, Eliana Toledo, MD, PhD^k, Marli de Souza Bernardes, MD, PhD^l, Sven Cichon, MD, PhD^{m,n,o}, Christiane Stieber, MD, PhD^{p,q,r}, L. Karla Arruda, MD, PhD^b, João Bosco Pesquero, PhD^a, and Anete Sevciovic Grumach, MD, PhD^c *São Paulo, Ribeirão Preto, Santo André, Campinas, and São José do Rio Preto, SP, Brazil; Rio de Janeiro, RJ, Brazil; Belo Horizonte, MG, Brazil; Vitória, ES, Brazil; Londrina and Foz do Iguaçu, PR, Brazil; Basel, Switzerland; and Juelich and Bonn, Germany*

What is already known about this topic? Hereditary angioedema (HAE) with mutations in the *F12* gene (FXII-HAE) is a rare genetic disease with characteristics similar to those of angioedema with C1 inhibitor deficiency, which mainly affects female patients, increasing the expression and severity of symptoms.

What does this article add to our knowledge? The higher frequency of symptomatic male patients in the Brazilian population suggests that the gender penetrance previously described for FXII-HAE should be reconsidered. We report the highest number of families with FXII-HAE outside the European continent.

How does this study impact current management guidelines? Our results demonstrate the usefulness of genetic testing to diagnose patients presenting with clinical features of HAE with normal C1 inhibitor, including male patients, even in the absence of a family history or estrogen influence.

BACKGROUND: Hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) is a rare condition with clinical features similar to those of HAE with C1-INH deficiency. Mutations in

the *F12* gene have been identified in subsets of patients with HAE with normal C1-INH, mostly within families of European descent.

^aCenter for Research and Molecular Diagnostic of Genetic Diseases, Department of Biophysics, Federal University of São Paulo, São Paulo, SP, Brazil

^bDepartment of Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

^cDivision of Clinical Immunology, Faculdade de Medicina ABC, Santo André, SP, Brazil

^dDivision of Allergy and Clinical Immunology, School of Medicine of the University of São Paulo, São Paulo, SP, Brazil

^eFederal University of Rio de Janeiro, Cidade Universitária, Rio de Janeiro, RJ, Brazil

^fPrivate Allergy and Immunology Clinic, Belo Horizonte, MG, Brazil

^gDivision of Clinical Allergy and Immunology, School of Medicine, State University of Campinas, Campinas, SP, Brazil

^hSchool of Medicine of Santa Casa de Misericórdia of Vitória, Vitória, ES, Brazil

ⁱDivision of Allergy and Immunology, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

^jPrivate Allergy and Immunology Clinic, Londrina, PR, Brazil

^kSchool of Medicine, State University of São José do Rio Preto, São José do Rio Preto, SP, Brazil

^lPrivate Allergy and Immunology Clinic, Foz do Iguaçu, PR, Brazil

^mDivision of Medical Genetics, University Hospital Basel, Basel, Switzerland

ⁿDepartment of Biomedicine, University of Basel, Basel, Switzerland

^oInstitute of Neuroscience and Medicine (INM-1), Research Center Juelich, Juelich, Germany

^pInstitute of Human Genetics, University of Bonn School of Medicine & University Hospital of Bonn, Bonn, Germany

^qDepartment of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany

^rCenter for Rare Diseases Bonn, University Hospital of Bonn, Bonn, Germany

This work was supported by the São Paulo Research Foundation (FAPESP) (grant nos. 2011/24142-3, 2011/23439-2, 2013/02661-4, and 2014/27198-8). A.S.G. was supported by the Shire Research Program for Investigators.

Conflicts of interest: S. R. Valle is on the boards for Novartis, Sanofi, and CSL Behring and has received lecture fees from Novartis and Takeda. E. Mansour has received research and travel support from Shire; is on the CSL Behring board; and has received research support from Shire and CSL Behring. A. S. Grumach has received research support from São Paulo Research Foundation (FAPESP) (grant nos. 2011/24142-3, 2011/23439-2, 2013/02661-4, and 2014/27198-8) and Shire; has received travel support from Shire, CSL, Pharming, and Baxalta; is on the advisory boards for Shire, CSL, and LASID; has received consultancy fees from Shire, CSL, Pharming, Baxalta, and Viropharma; has received lecture fees from Shire, CSL, and Baxalta; and has received payment for developing educational presentations from Shire and CSL. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 2, 2017; revised August 29, 2017; accepted for publication September 22, 2017.

Available online November 8, 2017.

Corresponding author: Anete Sevciovic Grumach, MD, PhD, Clinical Immunology, Faculdade de Medicina ABC, Av Príncipe de Gales 821 - Santo André, 09060.650 SP, Brazil. E-mail: asgrumach@gmail.com.

* These authors contributed equally to this work.

2213-2198

© 2017 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2017.09.025>

Abbreviations used

ACEI- Angiotensin-converting enzyme inhibitor
 C1-INH- C1 inhibitor
 C1-INH-HAE- Hereditary angioedema with C1 inhibitor deficiency
 eOC- Estrogen-containing oral contraceptive
 FXII- Coagulation factor XII
 FXII-HAE- Hereditary angioedema with F12 gene mutations
 HAE- Hereditary angioedema
 HRT- Hormonal replacement therapy
 U-HAE- HAE with normal C1-INH of unknown cause

OBJECTIVES: Our aim was to describe clinical characteristics observed in Brazilians from 42 families with HAE and *F12* gene mutations (FXII-HAE), and to compare these findings with those from other populations.

METHODS: We evaluated a group of 195 individuals, which included 102 patients clinically diagnosed with FXII-HAE and their 93 asymptomatic relatives.

RESULTS: Genetic analysis revealed that of the 195 subjects, 134 individuals (77.6% females) carried a pathogenic mutation in *F12*. The T328K substitution was found in 132 individuals, and the c.971_1018+24del72 deletion was found in 2 patients. The mean age at onset of symptoms in patients with FXII-HAE was 21.1 years. The most common symptoms were subcutaneous edema (85.8% of patients), abdominal pain attacks (69.7%), and upper airway edema (32.3%). Of male individuals carrying *F12* mutations, 53.3% (16 of 30) were symptomatic. Compared with reports from Europe, fewer female patients (68.6%) reported an influence of estrogen on symptoms.

CONCLUSIONS: Our study included a large number of patients with FXII-HAE, and, as the first such study conducted in a South American population, it highlighted significant differences between this and other study populations. The high number of symptomatic males and patients with estrogen-independent FXII-HAE found here suggests that male sex and the absence of a hormonal influence should not discourage clinicians from searching for *F12* mutations in cases of HAE with normal C1-INH. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1209-16)

Key words: Hereditary angioedema; Normal C1 inhibitor; *F12* mutations; Estrogen; Brazilian population

Hereditary angioedema (HAE) is a rare condition characterized by uncontrolled activation of the contact system with excessive bradykinin production and recurrent episodes of angioedema.¹ In most patients, HAE is associated with mutations in *SERPING1*, the gene encoding C1 inhibitor (C1-INH). C1-INH deficiency renders the contact system prone to activation, resulting in increased production of bradykinin. HAE with normal C1-INH was first described in 2000^{2,3} and, in subsets of patients,⁴⁻⁶ it has subsequently been associated with mutations in *F12*, the gene encoding coagulation factor XII (FXII-HAE).⁷⁻¹⁰ Patients are designated as cases of unknown HAE when they display clinical features of HAE and belong to families with no identified mutation in *SERPING1* or the *F12* gene.¹

Patients with HAE and normal C1-INH present clinical features that are similar to those of HAE caused by C1 inhibitor

deficiency (C1-INH-HAE), including subcutaneous, gastrointestinal, and laryngeal edema. However, different characteristics are also apparent in patients with FXII-HAE, including a lower frequency of abdominal pain attacks and a higher frequency of facial and tongue swelling, a higher number of symptomatic female patients, marked estrogen sensitivity, and delayed onset of disease.^{11,12} In addition, hemorrhagic lesions at the sites of angioedema, which develop 1 to 2 days after the onset of swelling, have been reported in HAE with normal C1-INH, but they have not yet been described in patients with C1-INH-HAE.^{11,13}

To date, all mutations described in patients with FXII-HAE are located in exon 9 of the *F12* gene, and affect a proline-rich region of coagulation factor XII. The missense mutations T328K and T328R are associated with a defect in O-linked glycosylation of FXII, which has been shown to increase contact activation of zymogen FXII. This loss of glycosylation ultimately increases activation of the kallikrein-kinin pathway, leading to higher levels of bradykinin, without affecting C1-INH binding to FXII.^{14,15} New pathways leading to excessive bradykinin formation in patients with HAE that involve plasmin activation of factor XII have been reported recently, which could have implications for diagnosis and treatment of the disease,^{16,17} although further studies are needed to confirm these mechanisms.

More than 60 families with FXII-HAE have been described worldwide; however, the prevalence of FXII-HAE in a South American population has not been established. A recent study estimated the prevalence of FXII-HAE to be 1:400,000.⁶ In the Brazilian population, few families with HAE with normal C1-INH have been reported. Those reported include 2 unrelated FXII-HAE families with members carrying a homozygous T328K mutation.¹⁸⁻²² In the present study, we describe the clinical characteristics of 134 Brazilian individuals with FXII-HAE, who are from 42 unrelated families, and compare their clinical features to those reported in patients from other countries.

METHODS

Subjects

After genetic testing, 102 Brazilian patients with recurrent episodes of subcutaneous edema without wheals, normal quantitative and/or functional C1-INH plasma levels, and no clinical response to high doses of antihistamines according to the criteria established by Cicardi et al¹ were included in this study. All patients who showed mutations in the *F12* gene known to be related to HAE were characterized as having FXII-HAE, a criterion for entering the study. Family history was a relevant factor for inclusion in the study; however, its absence was not an exclusion criterion. Clinical data regarding angioedema sites, triggering factors, and therapy were recorded using an established protocol (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Symptomatic and asymptomatic family members were also evaluated. Patients were referred to this study from 9 different medical centers in Brazil. Approval for this study was obtained from the Ethics Committee of the Federal University de São Paulo (CAAE: 04032912.6.0000.5505), the Clinical Hospital of Ribeirão Preto Medical School (HCRP 14000521/2012), and the School of Medicine of ABC (CAAE: 51896015.0.1001.0082). All participants provided informed consent.

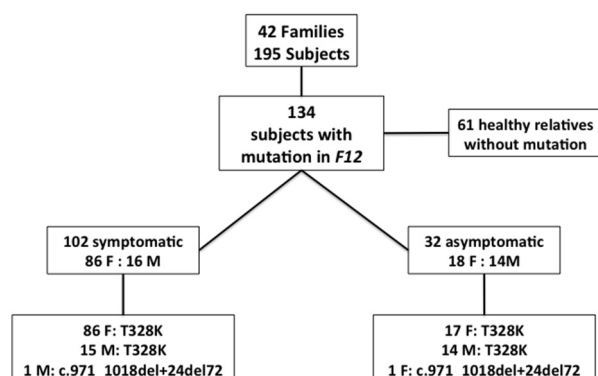


FIGURE 1. Distribution of FXII-HAE in Brazilian families. Of the 195 individuals analyzed from 42 families, 134 were found to have mutations in the *F12* gene. One hundred two *F12* mutation carriers were symptomatic and 32 were asymptomatic. The predominant mutation was T328K. F, Female; M, male.

Quantitative and functional C1-INH

Quantitative and functional C1-INH levels were estimated by nephelometry or radial immunodiffusion using a Human C1 Inactivator Binarid Radial Immunodiffusion Kit (The Binding Site Group Ltd, Birmingham, UK) and colorimetric enzyme assay using a Technochrom C1-INH kit (Technoclone GmbH, Vienna, Austria), respectively.

Genetics

Blood samples were collected in EDTA, and DNA was purified using an Illustra blood genomicPrep Mini Spin Kit (GE Healthcare UK Ltd, Buckinghamshire, UK). Exon 9 of the *F12* gene (NG_007568) was amplified by PCR using primers (forward, 5'-CTA CCG GAA CGT GAC T-3'; reverse, 5'-CAT CGA AGA CAG ACT CT-3') that covered its exon-intron boundaries. PCR amplification was performed using GoTaq Green Master Mix (Promega, Madison, Wis) and the following cycle parameters: denaturation at 95°C for 5 minutes and 35 amplification cycles at 95°C for 30 seconds, 54°C for 30 seconds and 45 seconds, and 72°C for 10 minutes. PCR fragments were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and sequenced using an ABI Prism 3500xl Genetic Analyzer sequencer and BigDye Terminator v3.1 reagent (Life Technologies, Foster City, Cali). Chromatograms were analyzed in Geneious Basic version 8.1.7 (<http://www.geneious.com>).²³

The polymorphism *F12-46C/T*, present in the promoter region of *F12* gene, was analyzed by sequencing with the following primers: forward (5'-GTG GGT ATT GTT GTA AGA TGC-3') and reverse (5'-CCA AGC TCA CCA GCA GGA AC-3').

Frequency of symptoms among females and males carrying F12 mutations

Of the 42 unrelated families studied, further analyses were performed on a subgroup of 20 families. Families selected for the subgroup analysis were those with at least 2 members with *F12* gene mutation, and with family pedigree data available. This analysis was performed to determine the frequency of symptomatic females and males and did not include 14 of 42 families with an index case only, and 8 of 42 families lacking accurate data on family pedigrees, including uncertainties in relatedness and clinical status. GenoPro

software version 2.5.3.9 (2011) was used to construct family pedigrees.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 6.04 for Windows, GraphPad Software, Inc, La Jolla, Calif; www.graphpad.com). The percentage of mutation-carrying patients was compared by sex using the Fisher exact test. Differences in the age of onset of disease according to sex, hormonal influence, and *F12-46C/T* genotype were compared by Mann-Whitney testing.

RESULTS

Genetic analyses

We performed genetic analyses of 195 individuals belonging to 42 unrelated families. One hundred two were symptomatic patients (86 females, 16 males) who presented pathogenic mutations in the *F12* gene that were previously associated with HAE, and 32 were asymptomatic relatives (18 females, 14 males) who were carriers of *F12* mutations. One hundred thirty-two patients (103 females, 29 males), distributed in 41 families, presented the pathogenic T328K mutation in the *F12* gene (Figure 1). Two siblings presented the deletion c.971_1018+24del72 (1 female, 1 male), and the homozygous T328K mutation was found in 2 unrelated patients (1 female, 1 male), as previously published.^{21,22}

The presence of the polymorphism *F12-46C/T* was analyzed in 38 patients with symptomatic T328K FXII-HAE, and 9 patients were identified as having the CT genotype.

Characteristics of the patients with FXII-HAE

Overall, we had information confirmed by family members on 450 individuals. Clinical features of symptomatic patients are presented and compared with those reported in other large FXII-HAE studied populations in Table I.^{6,13,24,25} For the genetic screening in the present study, exon 9 of *F12* was Sanger sequenced using methods from the studies presented in Table I. This approach allowed the detection of all 4 currently described mutations, including missense mutations, a deletion, and a duplication. Interestingly, 5 patients (12% of the 42 families) reported no family history.

Age at onset of symptoms

The mean age ($\pm$ SEM) at onset of symptoms was 21.1 years ($\pm$ 11.7), ranging from 2 to 68 years, for the 97 patients who could identify the age at which they experienced their first angioedema episode. The onset of clinical symptoms occurred before the age of 12 years in 10 of 97 (10.3%) patients, between the ages of 12 and 25 years in 70 of 97 (72.2%) patients, and after the age of 25 years in 17 of 97 (17.5%) patients (Figure 2, A). The mean age at the onset of first symptoms was 20.1 and 26.3 years for female and male patients, respectively (Figure 2, B). There were no differences in mean ages at onset of disease among patients with the promoter region genotype CC compared with those with genotype CT (18.2 and 21.3 years, respectively).

Clinical symptoms

Most of the symptomatic patients presented subcutaneous swellings (85.8%) that affected the face (55.5%), lips (48.5%), periorbital area (15.1%), extremities (47.5%), genitals (11.1%), and other areas of the body (neck, ears) (7%). Attacks of abdominal pain were reported by 69 patients (69.7%), edema of upper airways affected 32 patients (32.3%), tongue swelling

TABLE I. Clinical and molecular characteristics of Brazilian patients with FXII-HAE compared with those in other populations

Characteristic	FXII-HAE Brazil (present study)	FXII-HAE France ²⁴	FXII-HAE Germany ⁶	FXII-HAE Spain ²⁵	FXII-HAE Spain ¹³
<i>F12</i> gene mutation carriers, n	134	57	104	35	35
Sex, n (%)					
Female	104 (77.6)	46 (81)	79 (76)	27 (77)	28 (80)
Male	30 (22.4)	11 (19)	25 (24)	8 (23)	7 (20)
Symptomatic, n (%)	102 (76.1)	19 (33)	35 (34)	6 (17)	13 of 35 (37)
Female	86 of 104 (82.7)	36 of 46 (78.3)	68 of 79 (86.1)	26 of 27 (96.3)	20 of 28 (71.4)
Male	16 of 30 (53.3)	2 of 11 (18.2)	1 of 25 (4)	3 of 8 (37.5)	2 of 7 (28.6)
Families, n	42	24	23	13	9
Family history, n (%)	37 (88)	16 (67)	22 (96)	12 (92)	6 (67)
Age at disease onset (y)	n = 97	n = 38	n = 61	n = 29	n = 16 F; n = 2 M
Mean $\pm$ SD	21.1 $\pm$ 11.7	21	20.3 $\pm$ 9.2	23.9 $\pm$ 8.9	19.9 F; 61.5 M
Median (range)	18 (2-68)	22 (8-40)	NA (1-65)	21 (14-55)	NA (14-28 F; 55-68 M)
Mutation (carriers/families)					
T328K	132/41	57/24	92/19	35/13	35/9
T328R	—	—	5/2	—	—
c.971_1018+24del72	2/1	—	7/2	—	—
Location of swelling, n (%)	n = 99	n = 38	n = 61	n = 29	n = 16*
Subcutaneous	85 (85.8)	35 (92)	61 (100)	NA	NA
Extremities	47 (47.5)	(87)	30 (50)	NA	10 (62.5)
Face	55 (55.5)	(91)	61 (100)	NA	12 (75)
Lips	48 (48.5)	NA	NA	NA	14 (87.5)
Eyes	15 (15.1)	NA	NA	NA	10 (62.5)
Genitals	11 (11.1)	NA	9 (15)	NA	5 (31.2)
Tongue	12 (12.1)	NA	20 (33)	NA	7 (43.8)
Abdominal	69 (69.7)	(80)	40 (66)	10 (35)	14 (87.5)
Larynx	32 (32.3)	NA	15 (25)	5 (17)	14 (87.5)
Death from asphyxiation, n/total of symptomatic	1/102	0	2/69	NA	NA
Triggering factors, n (%)					
Unknown	20 (20.2)	NA	NA	NA	NA
Stress	48 (48.5)	NA	NA	NA	9 (56.3)
Trauma	47 (47.5)	NA	NA	8 (28)	9 (56.3)
Hormones	59 (68.6) [†]	34 (94)	48 of 58 (83)	25 (96)	13 (81.3)
Pregnancy	12 (20.3) [‡]	NA	43 of 92 (47)	NA	8 (61.5)
Estrogen-containing oral contraceptives	49 (83) [‡]	NA	43 of 48 (90)	NA	12 (92.3)
Surgery	6 (6)	NA	NA	NA	NA
Dental intervention	8 (8)	NA	NA	NA	3 (18.7)
Pressure	8 (8)	NA	NA	NA	9 (56.3)
Weather changes	2 (2)	NA	NA	NA	NA

NA, Not available.

*Analysis of 16 symptomatic females, excluding 4 paucisymptomatic females.¹⁴[†]Percentage of hormone-related events in relation to the total number of symptomatic female patients (86 Brazilian, 36 French,²⁴ 58 German,⁶ 26 Spanish,²⁵ and 20 Spanish¹³).[‡]Pregnancy occurrence and estrogen-containing oral contraceptive use (percentage calculated from the total number of patients who indicated a hormonal influence on their symptoms, n = 56).

was reported by 12 patients (12.1%), and uvula swelling was experienced by 2 patients (2%). Three patients presented edema of extremities only, and 9 presented edema affecting the lips and/or tongue only. Eight patients reported abdominal pain attacks only, and 1 patient reported laryngeal edema and no other symptoms. One patient reported spontaneous bruising preceding attacks of abdominal pain that disappeared just after edema was established. These lesions are not similar to hemorrhagic lesions or ecchymoses limited to the sites of the angioedema, which were described in some patients with HAE with normal C1-INH by Bork et al¹¹ and Piñero-Saavedra

et al.¹³ Coagulopathy was not identified or manifested in any of our patients with FXII-HAE.

Triggering factors

Stress and trauma were associated with swelling episodes in 48.5% and 47.5% of the patients, respectively (Table I). A hormonal influence was suspected in 59 of 86 symptomatic female patients (68.6%), with estrogen-containing oral contraceptives (eOCs) being the presumed hormonal trigger in most cases (49 of 59 [83%]) and exclusively in 43 of the 59 (72.8%) female patients (Table I). In 2 patients, the attacks were reported to worsen during menses, and, in

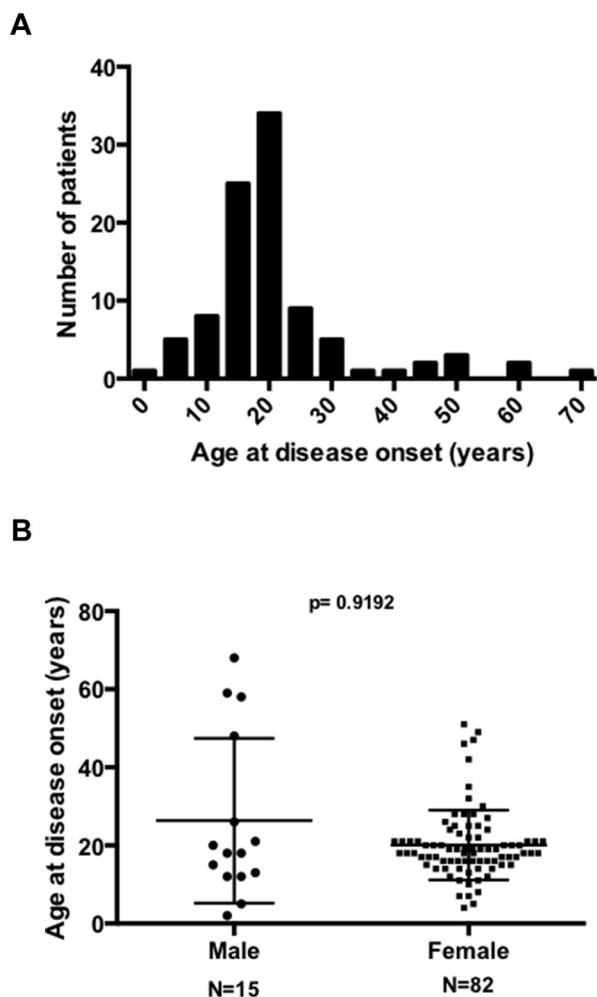


FIGURE 2. Age at disease onset in patients with FXII-HAE. Age (mean $\pm$ SEM) at disease onset in Brazilian patients with FXII-HAE was 21.1 years ($\pm$ 11.7) years, ranging from 2 to 68 years (A). The age (mean $\pm$ SEM) at onset of symptoms did not differ between male and female patients (B). Error bars in (Figure 2, B) indicate the SD.

1 patient, estrogen-containing hormonal replacement therapy (HRT) triggered the onset of HAE symptoms.

In 1 patient, symptoms began at the age of 68 years, after antihypertensive therapy with the angiotensin-converting enzyme inhibitor (ACEI) enalapril was initiated. He presented with recurrent episodes of angioedema of the face, lips, tongue, and genitals, which did not resolve with the withdrawal of ACEI and lasted for 5 years. A thorough clinical investigation included a molecular analysis of the *F12* gene, which revealed the presence of the c.971_1018+24del72 mutation. This mutation was also identified in the patient's asymptomatic sister.²² Treatment with tranexamic acid resulted in a marked reduction in the frequency and severity of angioedema episodes. Twenty symptomatic patients (20.2%) could not identify the factors precipitating angioedema.

HAE TREATMENT

Management of patients with FXII-HAE focused on the discontinuation of potential trigger factors, including eOCs,

HRT, or ACEI, and providing drug therapy, as recently reported by Bork et al.²⁶ eOC was discontinued in 44 female patients who reported worsening of symptoms with these medications, and 27 switched to progestin as a contraceptive medication and/or were prescribed progestin as a treatment. In 1 patient who had symptoms that began after HRT was initiated, withdrawal of this medication led to little improvement, and the patient required danazol for control of her symptoms. One male patient had persistent and frequent angioedema episodes, despite withdrawal of ACEI treatment; he was subsequently treated with tranexamic acid. Nine patients with acute attacks were treated with icatibant, and 4 patients were treated with plasma-derived C1-INH. All 13 patients showed a good response, with a marked reduction in the duration and intensity of attacks. Treatment modalities and clinical outcomes in our patients with FXII-HAE are presented in Table II.

Analysis of the frequency of symptoms according to sex within family pedigrees

Overall, relevant information was provided for 450 individuals by family members. A subgroup of 20 families, comprising 419 individuals (230 females, 189 males) with known clinical statuses, was selected for a pedigree analysis (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). Genetic screening was performed on 195 of 450 individuals in the cohort and on 164 of 419 individuals in the selected subgroup. Of the 164 individuals in the selected subgroup who were genetically analyzed, 118 were females and 46 were males, indicating a female:male ratio of 2.5:1. Females and males showed similar percentages of *F12* gene mutations, with 77 of 118 (65%) and 29 of 46 (63%) for females and males, respectively. Among females with *F12* gene mutations in the selected subgroup, 62 of 77 (80.5%) were symptomatic, whereas 15 of 29 (51.7%) males presented HAE symptoms. The frequency of symptomatic female and male patients within the overall group was 86 of 104 (82.7) and 16 of 30 (53.3%), respectively, similar to that of the 20-family subgroup. An example of a family pedigree is shown in Figure 3.

DISCUSSION

In the present study, we described the clinical features of Brazilian patients with FXII-HAE from 42 families, who were evaluated at 9 HAE reference centers coordinated by the Brazilian Study Group on Hereditary Angioedema (GEBRAEH). Since the first report of this disease in Germany, several families presenting FXII-HAE have been identified in France, Spain, Italy, the United Kingdom, Luxemburg, Turkey, Australia, and Morocco,²⁷⁻³⁸ with T328K as the most commonly associated mutation. In South America, few Brazilian families presenting FXII-HAE have been reported previously.¹⁸⁻²² Our results from Brazilian patients were compared with those from other large FXII-HAE cohorts in France, Germany, and Spain,^{6,13,24,25} and they suggested a higher proportion of symptomatic male patients and less dependence on estrogen influence in our patients with FXII-HAE.

The reasons for these significant differences within the studied populations are unclear. There are data to support the hypothesis that mutation p.Thr328Lys originated from a common founder.⁸ In keeping with this, we did not identify any FXII *de novo* mutations in our patients, and, to our knowledge, there are no reports of FXII *de novo* mutations leading to FXII-HAE in

TABLE II. Treatment modalities and outcomes of patients with FXII-HAE

Treatment	Procedure/Drug	Female (n)	Male (n)	Outcome
Discontinuation of triggering factors	Withdrawal of eOC	44	NA	Complete control or reduction in the number/duration of attacks
	Withdrawal of HRT	1	NA	No effect; required danazol
	Withdrawal of ACEI	0	1	No effect; required tranexamic acid
Long-term prophylaxis	Progestin	27	NA	Complete control or reduction in number/duration of attacks
	Tranexamic acid	30	2	
	EACA	1	1	
	Danazol	7	0	
	Oxandrolone	4	0	
Acute therapy	Icatibant	9	0	Marked reduction in the duration and intensity of attacks
	pdC1-INH	4	0	

EACA, Epsilon amino caproic acid; NA, not applicable; *pdC1-INH*, plasma-derived C1 inhibitor concentrate.

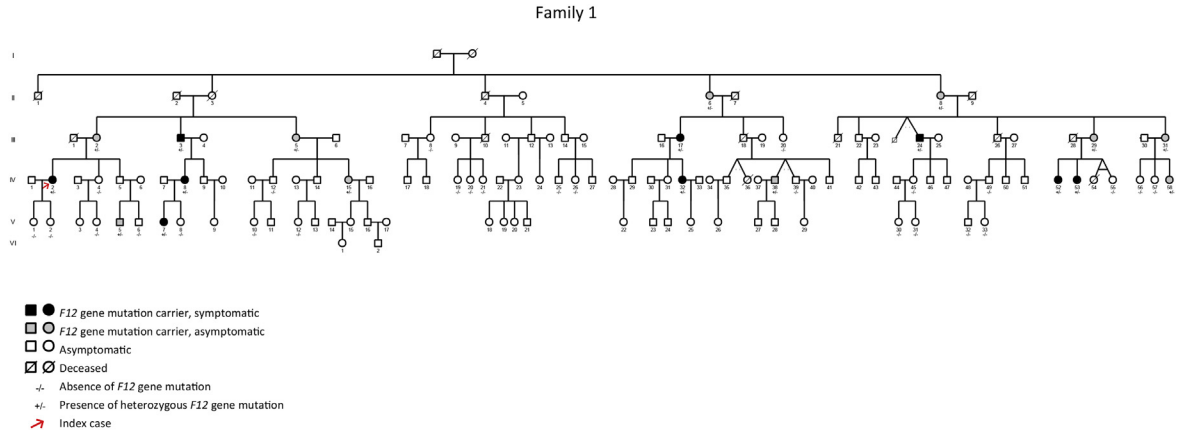


FIGURE 3. Pedigree of a large family with FXII-HAE. The index case (III-2) first developed symptoms at age 46 years after starting HRT. Her diagnosis prompted the genetic analysis of 45 of 96 family members, leading to the identification of a total of 19 family members with the T328K mutation. Of these, 9 (7 females, 2 males) were symptomatic. II.3 and IV.5 are obligatory carriers in the family pedigree.

families from other countries. The Brazilian population has a mixture of native, European, and African ancestry; however, a previous study analyzing 934 individuals from the 4 most populous regions of Brazil revealed that European ancestry was predominant, ranging from 60.6% to 77.7%.³⁹ It would be interesting to perform a large genetic study to track the origin of the Brazilian patients with FXII-HAE and to compare their ethnic makeup with that of these patients in a European population; however, this was beyond the objectives of the present study. Although the existence of different genetic markers in the European and Brazilian populations may account for our results, we could not rule out a role for phenotype-modifying environmental factors in the expression of the disease.

The proportion of patients reporting a hormonal influence on their symptoms in the present study (68.6%) was lower than that previously described in French (94%), German (83%), and Spanish studies (96% and 81.3%).^{6,13,24,25} Estrogen has been recognized as a regulator of the expression of genes encoding crucial proteins in the bradykinin release pathway, including the *F12* gene, but this regulation is not completely understood.⁴⁰⁻⁴² A study of the polymorphism *F12*-46C/T (rs1801020), located in the promoter region of the *F12* gene, was performed in 258 patients with C1-INH-HAE. The T allele, associated with

decreased FXII plasma levels,⁴³ was also associated with a delay in disease onset and to less need for long-term treatment.⁴⁴ In the present study, no association was found between this polymorphism and mean age at disease onset.

Our data suggest that the current major criterion for the diagnosis of FXII-HAE, family history, should be only one of the criteria for suspecting a case of FXII-HAE. Overall, family history was reported in only 58% of patients with HAE with normal C1-INH; conversely, 42% did not have a family history. This may be because of the high proportion of asymptomatic carriers with FXII mutations in families of patients with FXII-HAE, ranging from 23.9% in our own study to as much as 83% in European studies.^{13,24,25} Moreover, we found that it was very important to carefully ask about family history, particularly about the presence of mild symptoms or symptoms that might not prompt clinical suspicion of HAE, including recurrent episodes of abdominal pain. There is a debate between countries on when to genotype patients clinically suspected of HAE with normal C1-INH, and this may account for the variable frequency of detection of FXII mutations.⁴⁵ Our data indicate that the absence of a family history should not prevent a practicing physician from pursuing diagnosis of FXII-HAE by genetic testing.

Considering the lack of specific biochemical assays to detect FXII-HAE and the high frequency of asymptomatic carriers of *F12* gene mutations, a genetic study of the relatives of patients with FXII-HAE is recommended, especially before starting treatment with eOC, HRT, and/or ACEI. Indeed, sequencing of exon 9 of the *F12* gene is a fast and objective method to diagnose the disease, and it may help guide treatment in uncertain cases. In one example, genetic screening of a single family following diagnosis of the index case, who was participating in the present study, allowed the identification of a total of 19 individuals bearing the *F12* gene mutation T328K who had not been previously diagnosed with HAE. In addition to the index case (Figure 3), 8 patients were symptomatic (6 females, 2 males). Four were paucisymptomatic, including the 2 men; however, 4 patients presented recurrent episodes of moderate to severe abdominal pain and/or subcutaneous angioedema in the lips, hands, or feet that requires many visits to emergency departments or unscheduled doctors' consultations. Specific treatments comprising withdrawal of eOC or progestin and tranexamic acid therapy were prescribed for 3 patients, 1 of whom received icatibant for an acute episode of abdominal pain, and one 9-year-old girl with recurrent abdominal attacks who was treated with tranexamic acid showed marked clinical improvement.

Limitations to this study are as follows: (1) The precise level of penetrance could not be established because of the lack of information on relatives of some of our study participants. Genetic analysis of 164 individuals with consistent clinical information indicated that 65% carried *F12* mutations; there was no difference by sex. However, 80.5% of females carrying *F12* gene mutations were symptomatic compared with 51.7% of males. Interpretation of these data may be limited by the fact that we tested 2.5 times more females than males in this study, despite inviting all susceptible individuals to be genetically screened. Nevertheless, in the 4 studies listed in Table I, a total of 8 symptomatic FXII-HAE males in 69 families were reported,^{6,13,24,25} whereas 16 symptomatic FXII-HAE males in 42 families were identified in the present study. (2) Only patients with HAE with normal C1-INH and mutations in the *F12* gene (FXII-HAE) were included in our study. We did not study patients with HAE with normal C1-INH of unknown cause (U-HAE) because of the very small number of patients who fulfilled the criteria for this clinical condition. It would have been interesting to compare frequencies and characteristics of FXII-HAE and U-HAE among Brazilian patients; however, several factors prevented this analysis, including uncertain family histories, lack of data on the response to 4-fold doses of antihistamines, and inconsistent laboratory results. Recent reports identifying mutations in genes encoding angiopoietin-1⁴⁶ and plasminogen⁴⁷ in individuals with a family history of U-HAE highlight the rapid advances in identifying genetic variants of HAE that allow a prediction of clinical outcomes, such as potentially severe tongue swelling in patients with HAE with a mutation in the plasminogen gene.⁴⁷ (3) Although this cohort of patients with FXII-HAE was large enough to allow for clinical characterization, sample size may have been a limitation in identifying associations between polymorphisms and clinical symptoms.

Our study determined clinical features in a large cohort of patients with FXII-HAE from 42 unrelated families that allowed for a comparison with European FXII-HAE cohorts described in

the literature. Findings of a larger number of symptomatic males and less estrogen influence in triggering symptoms in female patients than found in previous studies suggest that FXII-HAE is not a disease that only affects women as initially believed and that the disease should be considered in men with recurrent angioedema episodes. These differences between our population and others may be used to increase physicians' awareness and encourage them to search for a precise diagnosis by screening for genetic variants of the *F12* gene, and recently identified mutations in angiopoietin-1 and plasminogen genes in patients with HAE with normal C1-INH. Another contribution of our study is its demonstration of the importance of active genetic screening of families with FXII-HAE to identify both asymptomatic carriers of *F12* gene mutations and symptomatic patients for whom the diagnosis of HAE had not been considered.

Acknowledgements

We thank Eduardo Costa, MD, PhD, from the Allergy and Immunology Division at the State University of Rio de Janeiro for providing blood samples from patients with HAE. We are grateful to Priscila Amorin Nicolicht from the Department of Biophysics at Universidade Federal de São Paulo (UNIFESP) and Mrs Marina Mendonça Dias from Ribeirão Preto Medical School, University of São Paulo, for technical assistance.

REFERENCES

1. Cicardi M, Aberer W, Banerji A, Bas M, Bernstein JA, Bork K, et al. Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. *Allergy* 2014; 69:602-6.
2. Bork K, Barnstedt SE, Koch P, Traupe H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* 2000;356:213-7.
3. Binkley KE, Davis A III. Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema. *J Allergy Clin Immunol* 2000;106:546-50.
4. 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;124:129-34.
5. Vitrat-Hinck V, Gompel A, Dumestre-Perard C, Boccon-Gibod I, Drouet C, Cesbron JY, et al. Type III hereditary angio-oedema: clinical and biological features in a French cohort. *Allergy* 2010;65:1331-6.
6. Bork K, Wulff K, Witzke G, Hardt J. Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations. *Allergy* 2015;70:1004-12.
7. 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;343:1286-9.
8. Cichon S, Martin L, Hennies HC, Müller F, Van Driessche K, Karpushova A, et al. Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. *Am J Hum Genet* 2006;79:1098-104.
9. Bork K, Wulff K, Meinke P, Wagner N, Hardt J, Witzke G. A novel mutation in the coagulation factor 12 gene in subjects with hereditary angioedema and normal C1 inhibitor. *Clin Immunol* 2011;141:31-5.
10. Kiss N, Barabas E, Varnai K, Halasz A, Varga LA, Prohászka Z, et al. Novel duplication in the F12 gene in a patient with recurrent angioedema. *Clin Immunol* 2013;149:142-5.
11. Bork K, Gül D, Hardt J, Dewald G. Hereditary angioedema with normal C1 inhibitor: clinical symptoms and course. *Am J Med* 2007;120:987-92.
12. Bork K. Hereditary angioedema with normal C1 inhibitor. *Immunol Allergy Clin North Am* 2013;33:457-70.
13. Piñero-Saavedra M, González-Quevedo T, Saenz de San Pedro B, Alcaraz C, Bobadilla-González P, Fernández-Vieira L, et al. Hereditary angioedema with F12 mutation: clinical features and enzyme polymorphisms in 9 Southwestern Spanish families. *Ann Allergy Asthma Immunol* 2016;117:520-6.
14. Bork K, Wulff K, Hardt J, Witzke G, Lohse P. Characterization of a partial exon 9/intron 9 deletion in the coagulation factor XII gene (F12) detected in two Turkish families with hereditary angioedema and normal C1 inhibitor. *Hemophilia* 2014;20:e372-5.

15. Björkqvist J, de Maat S, Lewandrowski U, Di Gennaro A, Oschatz C, Schönig K, et al. Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III. *J Clin Invest* 2015;125:3132-46.
16. Joseph K, Tholanikunnel BG, Wolf B, Bork K, Kaplan AP. Deficiency of plasminogen activator inhibitor 2 in plasma of patients with hereditary angioedema with normal C1 inhibitor levels. *J Allergy Clin Immunol* 2016;137:1822-9.
17. de Maat S, Björkqvist J, Suffritti C, Wiesenekker CP, Nagtegaal W, Koekman A, et al. Plasmin is a natural trigger for bradykinin production in patients with hereditary angioedema with factor XII mutations. *J Allergy Clin Immunol* 2016;138:1414-23.
18. Stieber C, Grumach AS, Cordeiro E, Constantino-Silva RN, Barth S, Hoffmann P, et al. First report of a FXII gene mutation in a Brazilian family with hereditary angio-oedema with normal C1 inhibitor. *Br J Dermatol* 2015;173:1102-4.
19. Moreno AS, Valle SO, Levy S, França AT, Serpa FS, Arcuri HA, et al. Coagulation factor XII gene mutation in Brazilian families with hereditary angioedema with normal C1 inhibitor. *Int Arch Allergy Immunol* 2015;166:114-20.
20. Moreno AS, Maia LS, Palhas PB, Dias MM, Muglia VF, Castelli EC, et al. Genetic analysis as a practical tool for diagnosis of hereditary angioedema with normal C1 inhibitor: a case report. *J Investig Allergol Clin Immunol* 2016;26:57-9.
21. Grumach AS, Stieber C, Veronez CL, Cagini N, Constantino-Silva RN, Cordeiro E, et al. Homozygosity for a factor XII mutation in one female and one male patient with hereditary angio-oedema. *Allergy* 2016;71:119-23.
22. Veronez CL, Serpa FS, Pesquero JB. A rare mutation in F12 gene in a patient with ACE inhibitor-induced angioedema. *Ann Allergy Asthma Immunol* 2017;118:743-5.
23. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 2012;28:1647-9.
24. Deroux A, Boccon-Gibod I, Fain O, Pralong P, Ollivier Y, Pagnier A, et al. Hereditary angioedema with normal C1 inhibitor and factor XII mutation: a series of 57 patients from the French National Center of Reference for Angioedema. *Clin Exp Immunol* 2016;185:332-7.
25. Marcos C, López Lera A, Varela S, Liñares T, Alvarez-Eire MG, López-Trascasa M. Clinical, biochemical, and genetic characterization of type III hereditary angioedema in 13 Northwest Spanish families. *Ann Allergy Asthma Immunol* 2012;109:195-200.e2.
26. Bork K, Wulff K, Witzke G, Hardt J. Treatment for hereditary angioedema with normal C1-INH and specific mutations in the F12 gene (HAE-FXII). *Allergy* 2017;72:320-4.
27. Bouillet L, Ponard D, Rousset H, Cichon S, Drouet C. A case of hereditary angio-oedema type III presenting with C1-inhibitor cleavage and a missense mutation in the F12 gene. *Br J Dermatol* 2007;156:1063-5.
28. Martin L, Raison-Peyron N, Nöthen MM, 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;120:975-7.
29. Bell CG, Kwan E, Nolan RC, Baumgart KW. First molecular confirmation of an Australian case of type III hereditary angioedema. *Pathology* 2008;40:82-3.
30. Prieto A, Tórnero 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;64:284-6.
31. 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;123:906-10.
32. Nagy N, Greaves MW, Tanaka A, McGrath JA, Grattan CE. Recurrent European missense mutation in the F12 gene in a British family with type III hereditary angioedema. *J Dermatol Sci* 2009;56:62-4.
33. Hentges F, Hilger C, Kohnen M, Gilson G. Angioedema and estrogen-dependent angioedema with activation of the contact system. *J Allergy Clin Immunol* 2009;123:262-4.
34. Picone O, Donnadiu 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;2010:957507.
35. Baeza ML, Rodríguez-Marco A, Prieto A, Rodríguez-Sainz C, Zubeldia JM, Rubio M. Factor XII gene missense mutation Thr328Lys in an Arab family with hereditary angioedema type III. *Allergy* 2011;66:981-2.
36. Gómez-Traseira C, López-Lera A, Drouet C, López-Trascasa M, Pérez-Fernández E, Favier B, et al. Hereditary angioedema caused by the p.Thr309Lys mutation in the F12 gene: a multifactorial disease. *J Allergy Clin Immunol* 2013;132:986-9.
37. Mansi M, Zanichelli A, Coerezza A, Suffritti C, Wu MA, Vacchini R, et al. Presentation, diagnosis and treatment of angioedema without wheals: a retrospective analysis of a cohort of 1058 patients. *J Intern Med* 2015;277:585-93.
38. Firinu D, Bafunno V, Vecchione G, Barca MP, Manconi PE, Santacrose R, et al. Characterization of patients with angioedema without wheals: the importance of F12 gene screening. *Clin Immunol* 2015;157:239-48.
39. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One* 2011;6:e17063.
40. Chen LM, Chung P, Chao S, Chao L, Chao J. Differential regulation of kinyogen gene expression by estrogen and progesterone in vivo. *Biochim Biophys Acta* 1992;1131:145-51.
41. Citarella F, Misiti S, Felici A, Farsetti A, Pontecorvi A, Fantoni A. Estrogen induction and contact phase activation of human factor XII. *Steroids* 1996;61:270-6.
42. Madeddu P, Emanueli C, Song Q, Varoni MV, Demontis MP, Anania V, et al. Regulation of bradykinin B2-receptor expression by oestrogen. *Br J Pharmacol* 1997;121:1763-9.
43. Kanaji T, Okamura T, Osaki K, Kuroiwa M, Shimoda K, Hamasaki N, et al. A common genetic polymorphism (46 C to T substitution) in the 5'-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. *Blood* 1998;91:2010-4.
44. Speletas M, Szilágyi Á, Csuka D, Koutsostathis N, Psarros F, Moldovan D, et al. F12-46C/T polymorphism as modifier of the clinical phenotype of hereditary angioedema. *Allergy* 2015;70:1661-4.
45. Craig TJ, Bernstein JA, Farkas H, Bouillet L, Boccon-Gibod I. Diagnosis and treatment of bradykinin-mediated angioedema: outcomes from an angioedema expert consensus meeting. *Int Arch Allergy Immunol* 2014;165:119-27.
46. Bafunno V, Firinu D, D'Apolito M, Cordisco G, Loffredo S, Leccese A, et al. Mutation of angiotensinogen-1 gene associates with a new type of hereditary angioedema. *J Allergy Clin Immunol* 2018;141:1009-17.
47. Bork K, Wulff K, Steinmüller-Magin L, Braenne I, Staubach-Renz P, Witzke G, et al. Hereditary angioedema with a mutation in the plasminogen gene. *Allergy* 2018;73:442-50.



UNIVERSIDADE FEDERAL DE SÃO PAULO
ESCOLA PAULISTA DE MEDICINA
DEPARTAMENTO DE BIOFÍSICA



UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
DEPARTAMENTO DE CLÍNICA MÉDICA

Initial evaluation for HAE patients

Personal Information

Code _____

Date: ____/____/____

Name: _____

Birth: ____/____/____ Age: _____ Nationality: _____

Ancestry:

☐ European: _____ ☐ Amerindian ☐ Afrodescendant ☐ Oriental: _____

Race:

☐ White ☐ Black ☐ Brown ☐ Yellow ☐ Indigenous ☐ Other: _____

MD requester: _____ Institution: _____

e-mail: _____

CLINICAL DATA

Age at onset of symptoms: _____

☐ Asymptomatic

Mean frequency of attacks before treatment: _____

Angioedema and associated symptoms (during entire life)

☐ Hands	☐ Lips	☐ Abdominal pain	☐ Other
☐ Feet	☐ Eyes	☐ Abdominal distension	☐
☐ Arms	☐ Tongue	☐ Diarrhea	☐
☐ Legs	☐ Uvula	☐ Nausea	☐
☐ Genitals	☐ Larynx	☐ Vomit	☐
☐ Face	☐ Dyspnea	☐ Urticaria	☐
☐ Neck	☐ Dysphagia	☐	☐
☐ Ears	☐ Erythema Marginatum	☐	☐

Rua Pedro de Toledo, 669 - 9º andar-fundos
CEP: 04039-032 São Paulo - SP Brasil
Tel.: +55 11 5576-4848 ramal 1999 / 1314
e-mails: jbpsquero@gmail.com; ca_veronez@yahoo.com.br

Av. Bandeirantes, 3900 - Anexo A, Bloco B, 2º andar sala 54
CEP: 14049-900 Ribeirão Preto - SP Brasil
Tel.: +55 16 33153279/ 33150218
e-mails: angioedemah@gmail.com; karla@fmrp.usp.br

FIGURE E1. Protocol for acquisition of clinical information on patients with FXII-HAE and family members. Information was obtained on demographic characteristics and clinical data including age at disease onset; frequency, severity, and location of HAE symptoms; diagnosis and treatment of HAE; trigger factors, comorbidities, laboratory tests, and family history. ER, Emergency room; FFP, fresh frozen plasma.



UNIVERSIDADE FEDERAL DE SÃO PAULO
ESCOLA PAULISTA DE MEDICINA
DEPARTAMENTO DE BIOFÍSICA



UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
DEPARTAMENTO DE CLÍNICA MÉDICA

Location and frequency of symptoms in the PAST YEAR

Location	Frequency of symptoms			
	None	< 6 times/year	6-11 times/year	> 1 time/month
Hands				
Feet				
Arms				
Legs				
Genitals				
Face				
Neck				
Ears				
Lips				
Eyes				
Tongue				
Uvula				
Larynx				
Dyspnea				
Dysphagia				
<i>Erythema Marginatum</i>				
Abdominal pain				
Abdominal distension				
Nausea				
Vomit				
Diarrhea				

Have you had prophylaxis in the last year? () yes () no Medication:

Dose:

Mean duration of attacks

Without prophylaxis:

☐ 1 day ☐ 2 days ☐ 3 - 5 days ☐ 5 days ☐ > 5 days

With prophylaxis:

☐ 1 day ☐ 2 days ☐ 3 - 5 days ☐ 5 days ☐ > 5 days

Rua Pedro de Toledo, 669 - 9º andar-fundos
CEP: 04039-032 São Paulo - SP Brasil
Tel.: +55 11 5576-4848 ramal 1999 / 1314
e-mails: jbpesquero@gmail.com; ca_veronez@yahoo.com.br

Av. Bandeirantes, 3900 - Anexo A, Bloco B, 2º andar sala 54
CEP: 14049-900 Ribeirão Preto - SP Brasil
Tel.: +55 16 33153279/ 33150218
e-mails: angioedemah@gmail.com; karla@fmrp.usp.br

FIGURE E1. (CONTINUED).



UNIVERSIDADE FEDERAL DE SÃO PAULO
ESCOLA PAULISTA DE MEDICINA
DEPARTAMENTO DE BIOFÍSICA



UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
DEPARTAMENTO DE CLÍNICA MÉDICA

Severity of attacks

MILD	Discomfort noticed, but no disruption of normal daily activity
MODERATE	Discomfort sufficient to reduce or affect normal daily activity
SEVERE	Inability to work or perform daily activity. Emergency treatment: conservative, substitutive (C1-INH, FFP) Emergency treatment: invasive (intubation, tracheotomy)

Severity of attacks **BEFORE** treatment:

☐ Mild
☐ Moderate
☐ Severe

Severity of attacks **AFTER** treatment, **IN THE PAST YEAR**:

☐ Asymptomatic
☐ Mild
☐ Moderate
☐ Severe

Trigger Factors (before treatment)

☐ Stress ☐ Trauma ☐ Infections ☐ Dental Procedures ☐ Pregnancy ☐ Intercourse ☐ Exercise

☐ Food (specify): _____

☐ Medicine (specify): _____

☐ Surgeries (specify): _____

☐ Contraceptive pills or hormonal reposition (specify): _____

☐ Other: _____

Prodrome:

☐ Yes ☐ No

Describe: _____

How long before the attacks?

☐ < 2 hs ☐ 2-24 hs ☐ Days _____

Rua Pedro de Toledo, 669 - 9º andar-fundos
CEP: 04039-032 São Paulo - SP Brasil
Tel.: +55 11 5576-4848 ramal 1999 / 1314
e-mails: jbpesquero@gmail.com; ca_veronez@yahoo.com.br

Av. Bandeirantes, 3900 - Anexo A, Bloco B, 2º andar sala 54
CEP: 14049-900 Ribeirão Preto - SP Brasil
Tel.: +55 16 33153279/ 33150218
e-mails: angioedemah@gmail.com; karla@fmrp.usp.br

FIGURE E1. (CONTINUED).



UNIVERSIDADE FEDERAL DE SÃO PAULO
ESCOLA PAULISTA DE MEDICINA
DEPARTAMENTO DE BIOFÍSICA



UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
DEPARTAMENTO DE CLÍNICA MÉDICA

DIAGNOSIS

Have already been diagnosed with HAE? ☐ Yes ☐ No When?

Other diagnosis:

TREATMENT OF ATTACKS

- ☐ Treatment at home ☐ ER. How many times? _____
☐ Hospitalization. How many times? _____
☐ Intensive Care Unit, how many times? _____
☐ Invasive procedures. How many times? _____
☐ Intubation ☐ Laparotomy
☐ Tracheostomy
☐ Hydration ☐ Epinephrine ☐ Fresh frozen plasma
☐ Pain killers ☐ Tranexamic acid ☐ Icatibant (Firazyr®)
☐ Sedation ☐ E-aminocaproic ☐ C1-INH replacement (Berinert®, Cynriz®)
☐ Antihistamines ☐ Danazol ☐ _____
☐ Corticosteroid ☐ _____

PROPHYLAXIS

Drug	Dose	Administration Frequency
☐ Tranexamic acid		
☐ Danazol		
☐ Aminocaproic acid		
☐ Oxandrolone		
☐ C1 Inhibitor		
☐ Progestin		
☐ Other		

Pregnancy

☐ Yes ☐ No

Intercurrences _____

Rua Pedro de Toledo, 669 - 9º andar-fundos
CEP: 04039-032 São Paulo - SP Brasil
Tel.: +55 11 5576-4848 ramal 1999 / 1314
e-mails: jbpesquero@gmail.com; ca_veronez@yahoo.com.br

Av. Bandeirantes, 3900 - Anexo A, Bloco B, 2º andar sala 54
CEP: 14049-900 Ribeirão Preto - SP Brasil
Tel.: +55 16 33153279/ 33150218
e-mails: angioedemah@gmail.com; karla@fmrp.usp.br

FIGURE E1. (CONTINUED).



UNIVERSIDADE FEDERAL DE SÃO PAULO
ESCOLA PAULISTA DE MEDICINA
DEPARTAMENTO DE BIOFÍSICA



UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
DEPARTAMENTO DE CLÍNICA MÉDICA

Comorbidities

☐ Cancer
 ☐ Diabetes
 ☐ Hypertension
 ☐ Thyroid disease
 ☐ Heart disease

☐ Asthma
 ☐ Rhinitis
 ☐ Drug allergies
 ☐ Other: _____

Drugs in use

Drug	Specify	Dose
☐ Angiotensin-converting enzyme inhibitors (ACEI)		
☐ Non-steroidal anti-inflammatories		
☐ Angiotensin receptor blockers (ARBs)		
☐ Oral contraceptives		
☐ Other		

Biochemistry Measurements (without prophylaxis)

Exams	Data	Results	Reference value	Measured during attacks (yes or no)
C1-INH quantitative	___/___/___			
C1-INH functional	___/___/___			
C4	___/___/___			
C1q	___/___/___			
C1-INH quantitative	___/___/___			
C1-INH functional	___/___/___			
C4	___/___/___			
C1q	___/___/___			
C1-INH quantitative	___/___/___			
C1-INH functional	___/___/___			
C4	___/___/___			
C1q	___/___/___			
	___/___/___			
	___/___/___			
	___/___/___			
	___/___/___			

Rua Pedro de Toledo, 669 - 9º andar-fundos
CEP: 04039-032 São Paulo - SP Brasil
Tel.: +55 11 5576-4848 ramal 1999 / 1314
e-mails: jbpsquero@gmail.com; ca_veronez@yahoo.com.br

Av. Bandeirantes, 3900 - Anexo A, Bloco B, 2º andar sala 54
CEP: 14049-900 Ribeirão Preto - SP Brasil
Tel.: +55 16 33153279/ 33150218
e-mails: angioedemah@gmail.com; karla@fmrp.usp.br

FIGURE E1. (CONTINUED).



UNIVERSIDADE FEDERAL DE SÃO PAULO
ESCOLA PAULISTA DE MEDICINA
DEPARTAMENTO DE BIOFÍSICA



UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO
DEPARTAMENTO DE CLÍNICA MÉDICA

Family History

Do you have relatives with HAE symptoms:

☐ Yes ☐ No

Who? _____

Relatives diagnosed with HAE by physicians:

☐ Yes ☐ No

Who? _____

FAMILY PEDIGREE

■● Symptomatic male/female

□○ Asymptomatic male/female

Registered in ABRANGHE:

☐ Yes ☐ No

DIAGNOSTIC HYPOTHESIS

☐ HAE type I

☐ HAE type II

☐ HAE with normal C1-INH

☐ Angioedema due to acquired C1-INH deficiency

☐ ACEI induced angioedema

☐ Idiopathic histaminergic angioedema

☐ Idiopathic non-histaminergic angioedema

☐ _____

Observations:

Rua Pedro de Toledo, 669 - 9º andar-fundos
CEP: 04039-032 São Paulo - SP Brasil
Tel.: +55 11 5576-4848 ramal 1999 / 1314
e-mails: bpesquero@gmail.com; ca_veronez@yahoo.com.br

Av. Bandeirantes, 3900 - Anexo A, Bloco B, 2º andar sala 54
CEP: 14049-900 Ribeirão Preto - SP Brasil
Tel.: +55 16 33153279/ 33150218
e-mails: angioedemah@gmail.com; karla@fmrp.usp.br

FIGURE E1. (CONTINUED).

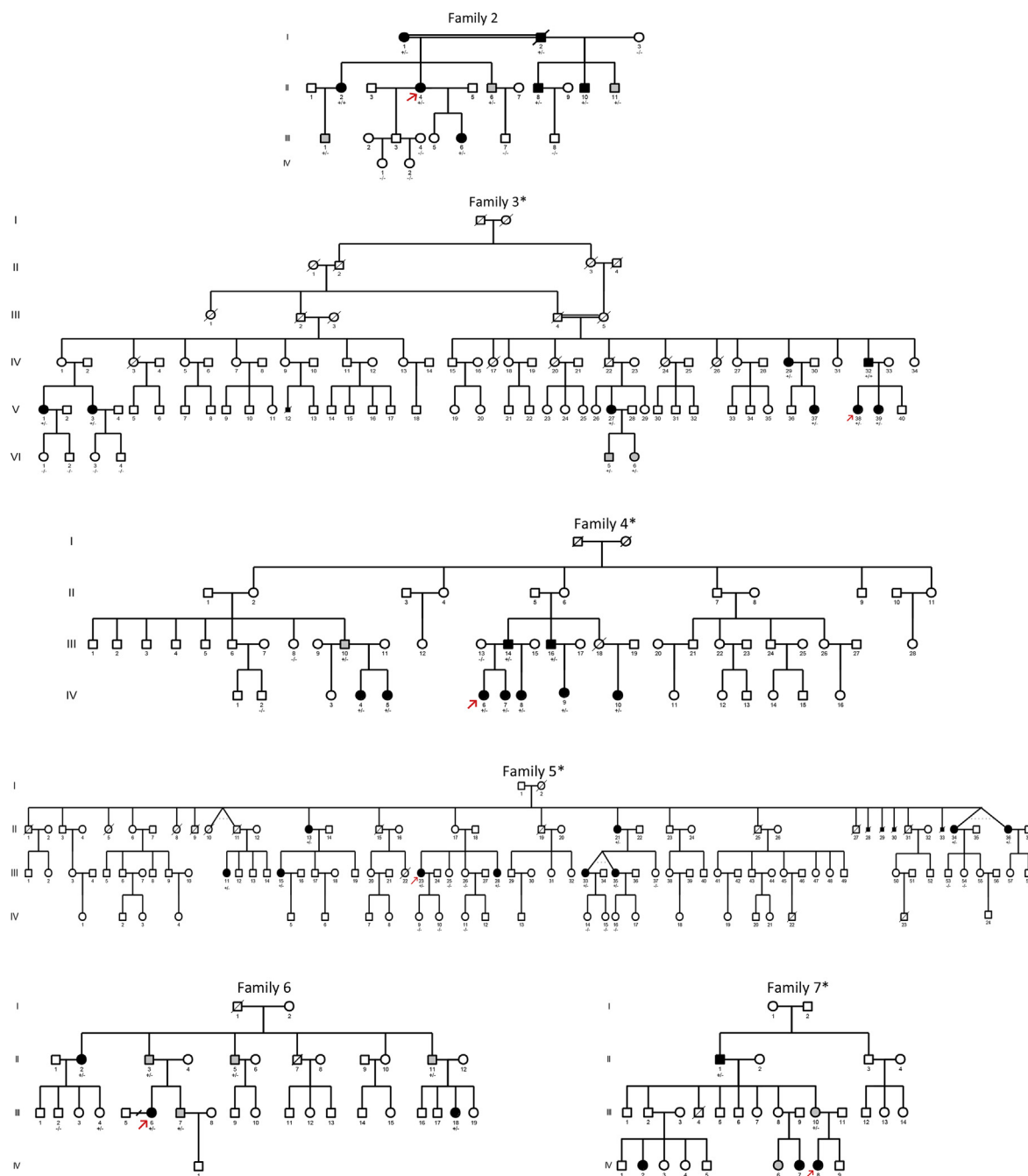


FIGURE E2. Pedigrees of 19 families with FXII-HAE. Nineteen families from whom consistent data on family pedigrees were available underwent further analysis. This subgroup of 20 families comprised 419 individuals with information available and 164 underwent genetic analysis. Homozygous *F12* gene mutation T328K was present in family 2 (patient II.2) and family 3 (patient IV.32).²¹ *The following obligatory carriers were identified by analysis of the family pedigree: II.2, II.3, III.2, III.4, III.5, IV.1, IV.22, and V.40.

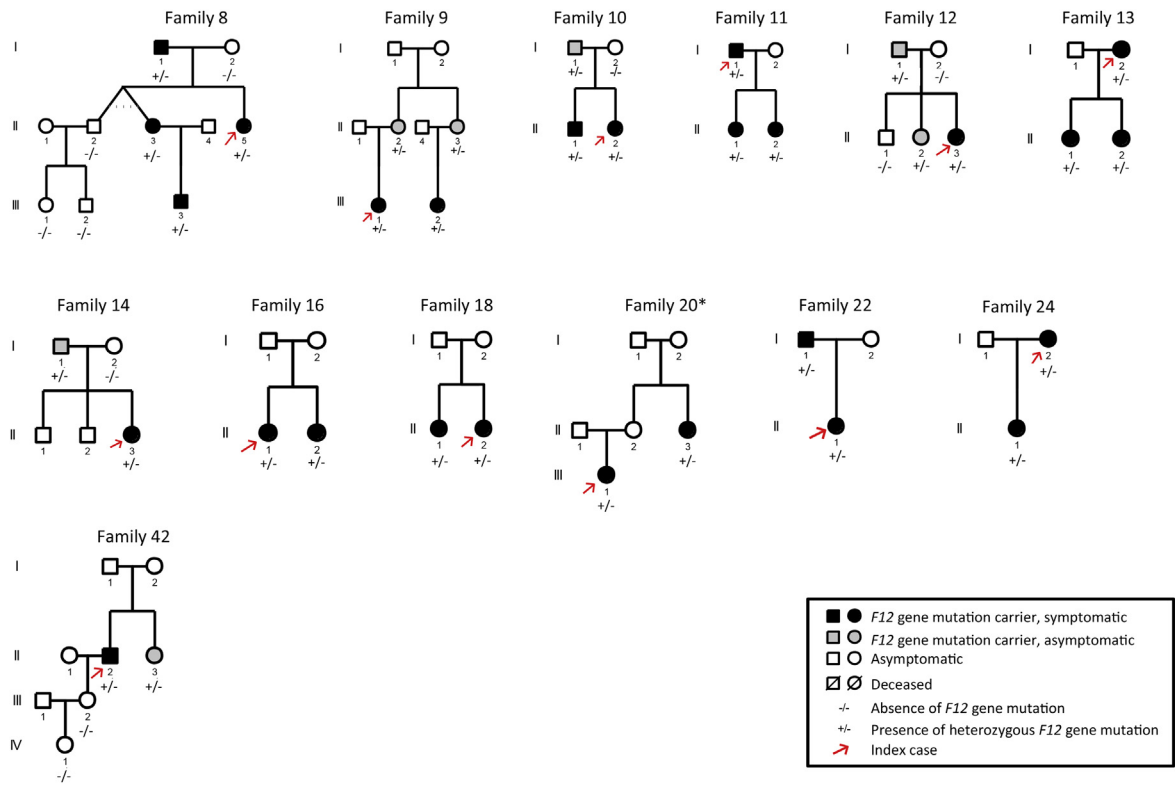


FIGURE E2. (CONTINUED).

Reproduced with permission of copyright owner. Further reproduction prohibited without permission.