

Letter to the Editor

Hereditary angioedema caused by the p.Thr309-Lys mutation in the *F12* gene: A multifactorial disease

To the Editor:

Hereditary angioedema (HAE) related to estrogens, nowadays identified as HAE with normal C1 inhibitor (C1-INH) function,¹ is a kind of bradykinin (BK)-induced angioedema. In some cases, a mutation has been detected in the *F12* gene (HAE-factor XII [HAE-FXII]).²⁻⁴

BK, the high-affinity ligand of type 2 BK receptor, is produced from high-molecular-weight kininogen upon cleavage by proteases (kallikrein, FXIIa, and plasmin),⁵ hereafter called kininogenases. BK is catabolized mainly by

metallopeptidases (kininases): angiotensin-converting enzyme (ACE), aminopeptidase P (APP), and carboxypeptidase N (CPN).^{2,4} BK level elevation produces an increase in endothelial permeability, vascular leakage, and angioedema.^{2,5} A schematic representation of BK generation and catabolism is shown in Fig 1 (reprinted with permission from Defendi et al⁶ [after modifications]).

We report data from studies performed on a large 3-generation Spanish family with a strictly estrogen-precipitating angioedema phenotype in an attempt to investigate factors that might affect the clinical expression of this disease.

The study was approved by the Research Ethical Committee of the Hospital Universitario La Paz, Madrid, Spain, and all the participants provided informed consent.

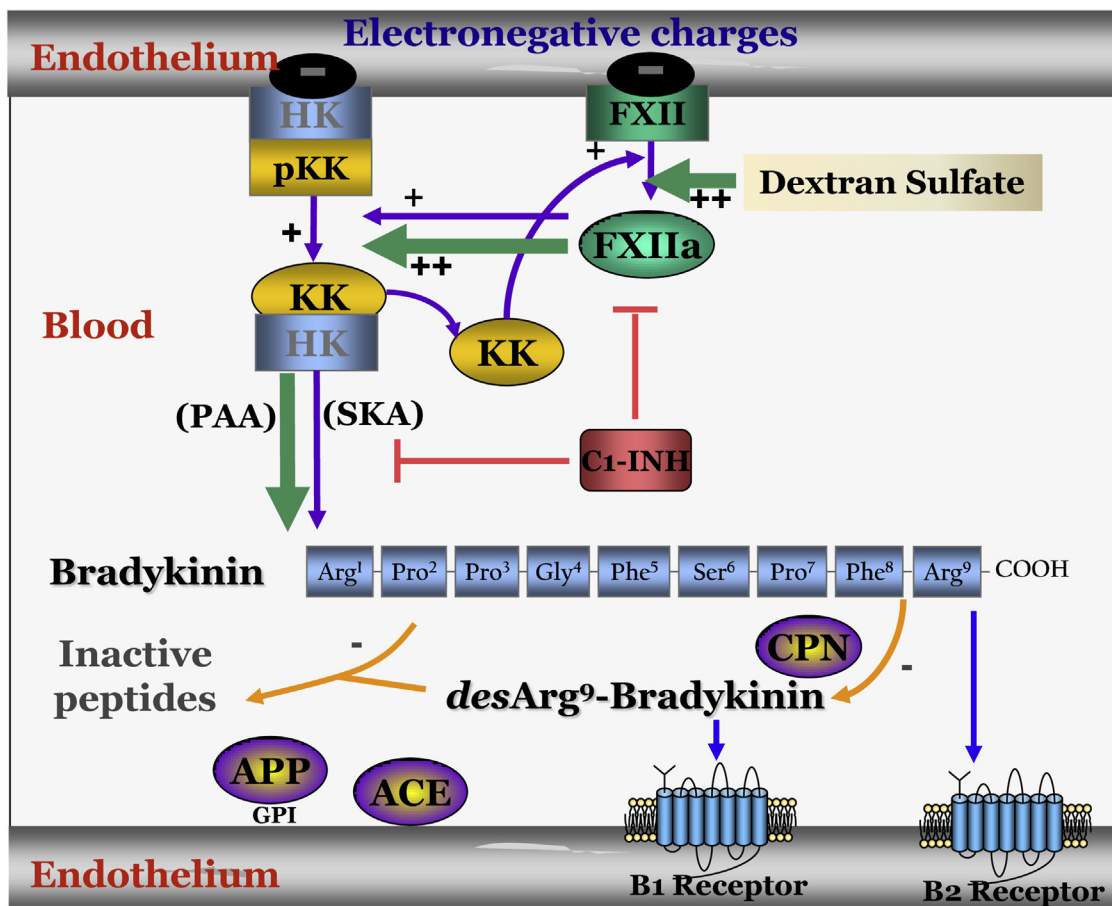


FIG 1. The kallikrein-kinin system: BK generation and catabolism. The kallikrein-kinin system consists of 3 plasma proteins that assemble when blood comes into contact with triggers, for example, negatively charged surfaces: FXII, plasma prekallikrein (PKK), and the nonenzymatic cofactor high-molecular-weight kininogen (HK); 80% of blood HK-PKK association is bound onto the endothelium. FXII and HK directly bind to polyanions; surface binding induces a conformational change in FXII, leading to limited proteolytic activity. Consecutively, activated FXII (FXIIa) cleaves PKK, generating plasma kallikrein (KK). In an amplified reaction, KK efficiently activates further FXII proenzyme molecules and cleaves its cofactor HK to yield BK, the B2 receptor ligand. This enzymatic activity is estimated in plasma by spontaneous kininogenase activity. The incubation on ice of plasma with dextran sulfate refers to full proenzyme activation, with subsequent measurement of the plasma PAA. Proenzyme activation and protease activities are under C1-INH control. The circulating CPN converts BK into *desArg*⁹-BK, the B1 receptor ligand, and the membrane-bound (glycosyl-phosphatidylinositol anchor, GPI) APP and ACE inactivate the receptor-binding capacity of both peptides. Reprinted with permission from Defendi et al⁶ (after modifications).

TABLE I. Complement values, SKA, PAA, SKA/PAA ratio, and kininase activity according to sex, the presence/absence of *F12* gene mutation, and the presence/absence of angioedema symptoms*

	Sex		Angioedema symptoms		F12 mutation	
	Female (n = 11)	Male (n = 9)	No (n = 15)	Yes (n = 5)	No (n = 9)	Yes (n = 11)
C4†						
Mean ± SE	21.12 ± 2.32	18.05 ± 2.65	19.06 ± 2.05	21.81 ± 3.41	18.66 ± 2.65	20.68 ± 2.37
Mean difference ± SE	3.06 ± 3.52		−2.74 ± 3.98		−2.02 ± 3.55	
P value	.449		.5403		.609	
C1-INH‡						
Mean ± SE	30.61 ± 2.47	32.1 ± 3.08	31.17 ± 2.32	31.24 ± 3.51	29.62 ± 2.9	32.41 ± 2.54
Mean difference ± SE	−1.48 ± 3.95		−0.07 ± 4.2		−2.79 ± 3.85	
P value	.732		.988		.522	
C1-INH function§						
Mean ± SE	106.38 ± 6.74	109.33 ± 7.63	113.49 ± 5.13	90.26 ± 8.66	108.58 ± 7.59	103.95 ± 6.8
Mean difference ± SE	−2.96 ± 10.18		23.23 ± 10.07		1.63 ± 10.19	
P value	.786		.082		.881	
SKA						
Mean ± SE	5.78 ± 1.70	7.91 ± 2.50	6.37 ± 1.73	5.80 ± 2.50	5.91 ± 2.48	6.67 ± 1.79
Mean difference ± SE	−2.13 ± 3.01		0.92 ± 3.04		−0.76 ± 3.03	
P value	.491		.766		.807	
PAA¶						
Mean ± SE	3428.06 ± 317.33	2863.86 ± 402.72	2949.3 ± 275.79	3899.97 ± 439.39	2627.29 ± 393.41	3546.77 ± 295.40
Mean difference ± SE	564.20 ± 512.73		−950.84 ± 518.77		−919.48 ± 491.97	
P value	.289		.088		.083	
SKA/PAA#						
Mean ± SE	0.18 ± 0.05	0.29 ± 0.07	0.24 ± 0.05	0.17 ± 0.07	0.29 ± 0.07	0.18 ± 0.05
Mean difference ± SE	−0.10 ± 0.09		0.07 ± 0.89		0.10 ± 0.09	
P value	.264		.433		.257	
APP**						
Mean ± SE	2.04 ± 0.14	1.91 ± 0.19	2.03 ± 0.14	1.91 ± 0.21	1.74 ± 0.18	2.13 ± 0.13
Mean difference ± SE	0.13 ± 0.24		0.12 ± 0.25		−0.39 ± 0.22	
P value	.602		.651		.104	
CPN††						
Mean ± SE	30.02 ± 1.50	31.17 ± 2.02	31.18 ± 1.41	28.68 ± 2.16	31.43 ± 2.07	29.93 ± 1.47
Mean difference ± SE	−1.15 ± 2.52		2.50 ± 2.58		1.50 ± 2.54	
P value	.654		.349		.565	
ACE‡‡						
Mean ± SE	48.27 ± 3.67	41.71 ± 4.61	44.25 ± 3.60	48.67 ± 5.09	51.50 ± 6.11	44.07 ± 3.27
Mean difference ± SE	6.56 ± 5.89		−4.42 ± 6.23		7.43 ± 6.93	
P value	.308		.505		.325	

*Means and SE were estimated with a univariate mixed model. Three separate models that used sex, symptoms, and *F12* mutation as the main effect have been included.

†Reference values: 14.0–60.0 mg/dL.

‡Reference values: 16.0–33.0 mg/dL.

§Reference value: >50%.

||Reference values: 2.4–10.7 and 2.3–5.6 nmol/min/mL for female and male individuals, respectively.

¶Reference values: 2422–4560 and 2225–4273 nmol/min/mL for female and male individuals, respectively.

#Reference values: 0.07–0.29% and 0.07–0.17% for female and male individuals, respectively.

**Reference values: 1.36–3.09 and 1.29–2.48 nmol/min/mL for female and male individuals, respectively.

††Reference values: 26.2–41.6 and 23.9–36.4 nmol/min/mL for female and male individuals, respectively.

‡‡Reference values: 11–49 UI/L for female and male individuals.

The proband (patient A10-II.4), a 33-year-old woman, had a history of severe episodes of hand and face edema with dysphonia and dysphagia related to fertility treatment with *estradiol plus desogestrel-ethinylestradiol* over the previous 6 years. Estrogen therapy was suspended indefinitely. The patient remained symptom-free until the onset of each of her pregnancies.

The *F12* gene was screened according to previously described methodology in 19 first-degree relatives.⁷ Three sisters (A9-II.2, A11-II.8, and A12-II.6) and 1 niece (A16-III.1) described having angioedema episodes seemingly related to high estrogen levels (oral contraceptives and/or pregnancy). The patients' clinical symptoms were classified into asymptomatic, mild, moderate,

and severe according to an in-house score (see Table E1 in this article's Online Repository at www.jacionline.org). The missense p.Thr309Lys mutation was detected in all 5 female patients who had a phenotypic variant in which angioedema is exclusively precipitated by high estrogen levels (A10-II.4, A9-II.2, A11-II.8, A12-II.6, and A16-III.1) and in 6 asymptomatic relatives (see Fig E1 in this article's Online Repository at www.jacionline.org for family pedigree). All the *F12* gene mutation carriers were heterozygous for the mutation. Clinical characteristics of the mutation carriers are shown in Table E2 in this article's Online Repository at www.jacionline.org. Penetrance of the disease was 45.5% for all the mutation carriers, 62.5% for female carriers, and 0% for male carriers.

C4, antigenic, and functional C1-INH were measured as described.⁷ The kinin-formation pathway was investigated by measuring spontaneous kininogenase activity (SKA), corresponding to kallikrein amidase activity by using the PFRpNA peptide as previously performed,^{4,8} and prekallikrein activation ability (PAA) corresponding to the ability of plasma to develop the activatable proenzyme prekallikrein upon dextran sulfate incubation as described.⁹ Kinin catabolism was studied by measuring plasma APP, CPN, and ACE activity as reported.¹⁰ The presence of the SNP -2399A in the *XPNPEP2* gene was screened in 4 patients according to previously reported methods.¹¹

Complement data are shown in Table E3 in this article's Online Repository at www.jacionline.org. SKA, PAA, SKA/PAA ratio, and kinin catabolism enzymes activities are shown in Table E4 in this article's Online Repository at www.jacionline.org.

The association between C4, C1-INH, C1-INH function, SKA, PAA, SKA/PAA, APP, CPN, and ACE and the expression of clinical symptoms, sex, or the *F12* gene mutation was studied by ANOVA by using mixed models. The models included the clinical symptoms, sex, or the *F12* gene mutation as a main effect and the subject as a random effect (Table I). The estimated main effect for sex, clinical symptoms, or presence of *F12* gene mutation was not statistically significant for the studied variables. Nevertheless, it is worth noting that the estimated C1-INH function mean was lower in symptomatic patients ($P = .082$), and PAA was higher in patients with clinical symptoms and those with *F12* gene mutation ($P = .088$ and $P = .083$, respectively), which might indicate an activation of the contact system.

Three out of 11 patients with *F12* gene mutation had a decrease in at least 1 of the studied BK catabolic enzymes. All of them were women: 2 had moderate HAE-FXII disease severity, and 1 had severe HAE-FXII disease. APP activity was low in 2 of 5 (40%) symptomatic patients, but not in any of the 13 asymptomatic ones, which indicates some association ($P = .06$). No association was observed between CPN activity and clinical expression. The SNP -2399A of *XPNPEP2* gene was not detected in the index case (A10-II4) or in symptomatic (A9-II2 and A11-II8) and asymptomatic (A3-III4) individuals.

Individuals who had not been treated for angioedema caused by C1-INH deficiency were shown to have a significant inverse relationship among APP and CPN activity and disease severity.¹⁰ This association was also observed in an HAE-FXII family in which 3 female symptomatic patients carried the SNP -2399A in the *XPNPEP2* gene, which had been associated with decreased APP.¹¹ In our study, plasma, APP, and CPN activities were found not to be significantly associated with the clinical manifestations in this family. However, decreased plasma APP was found only in symptomatic carriers and in none of the asymptomatic carriers. Moreover, none of the symptomatic *F12* gene mutation carriers had the SNP -2399A in the *XPNPEP2* gene.

Based on the results of kininase activity and blind to disease clinical activity, the laboratory tried to predict the risk of developing severe angioedema for each patient, regardless of the presence of *F12* gene mutation (Table E4). The laboratory prediction matched the presence of clinical symptoms in 8 of 11 patients with the *F12* gene mutation (kappa index = 0.42; $P = .09$), which indicates moderate agreement.

As far as we know, mutations in the *F12* gene have been described as causative for HAE-FXII^{2,3} and the presence of the SNP -2399A in the *XPNPEP2* gene has been identified as a possible aggravating factor.¹¹ This together with differences found in

the clinical expression of the disease have led us to postulate that this disease is actually multifactorial and the mutation in the *F12* gene is a prerequisite for the expression of disease symptoms. Other factors may have protective or aggravating effects on clinical features.

While our data affirm the important role played by estrogens as precipitating clinical expression in HAE-FXII, they are by no means the only factors. A low level of kininase activity could be an aggravating factor.

The low penetrance of the disease phenotype in this family suggests that there must be some unknown protective factor that prevents the development of angioedema.

Based on the results of this study with a limited number of subjects, enzymatic activities (metallopeptidases, SKA or PAA) could not be recommended as the exclusive predictive parameters of clinical severity in HAE-FXII. Further studies are needed to identify additional factors that contribute to the variability in clinical expression.

We are indebted to Mrs Françoise Csopaki for her skillful technical assistance.

Carmen Gómez-Traseira, MD^{a,b}

Alberto López-Lera, PhD^{b,c,d}

Christian Drouet, PhD^e

Margarita López-Trascasa, PhD^{b,c,d}

Elia Pérez-Fernández, MSc^{b,f}

Bertrand Favier, PhD^e

Nieves Prior, MD^{a,b}

Teresa Caballero, MD, PhD^{a,b,d}

From ^athe Allergy Department, Hospital Universitario La Paz, Madrid, Spain; ^bHospital La Paz Health Research Institute (IdiPAZ), Madrid, Spain; ^cthe Immunology Unit, Hospital Universitario La Paz, Madrid, Spain; ^dBiomedical Network Research on Rare Diseases-U754 (CIBERER), Madrid, Spain; ^eUniversité Joseph Fourier Grenoble, GREPI/AGIM CNRS FRE 3405, Centre d'Exploration de l'Angioedème, CHU de Grenoble, Grenoble, France; and ^fthe Biostatistics section, Research Unit, Hospital Universitario La Paz, Madrid, Spain. E-mail: carmengomez.tras@gmail.com.

T.C. is an investigator from the IdiPAZ program for promoting research activities (2009). A.L.-L. is a researcher from the CIBERER program. The study was partially funded by a grant from the Ministerio de Ciencia e Innovación (grant no. PI09/00122) awarded to M.L.-T. (PI) and by a grant from the European Communities (grant no. EU FP6 Erare program) awarded to C.D. (PI).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Zuraw BL, Bork K, Binkley KE, Banerji A, Christiansen SC, Castaldo A, et al. Hereditary angioedema with normal C1 inhibitor function: consensus of an international expert panel. *Allergy Asthma Proc* 2012;33:145-56.
2. Caballero T, Baeza ML, Cabañas R, Campos A, Cimbollek S, Gómez-Traseira C, et al. Spanish consensus on the diagnosis, management and treatment of angioedema mediated by bradykinin, part I: classification, epidemiology, pathophysiology, genetics, clinical symptoms and diagnosis. *J Investig Allergol Clin Immunol* 2011;21:333-47.
3. 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.
4. Cichon S, Martin L, Christian H, 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.
5. Kaplan AP, Ghebrehewet B. The plasma bradykinin-forming pathways and its interrelationships with complement. *Mol Immunol* 2010;47:2161-9.
6. Defendi F, Charignon D, Csopaki F, Ponard D, Drouet C. Actualités biologiques sur les angioédèmes à kinines. *Rev fr Laboratoires* 2012;42:55-68.
7. 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.
8. 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.
 9. Kluft C. Determination of prekallikrein in human plasma: optimal conditions for activating prekallikrein. *J Lab Clin Med* 1978;91:83-95.
 10. Drouet C, Désormeaux A, Robillard J, Ponard D, Bouillet L, Martin L, et al. Metalloproteinase activities in hereditary angioedema: effect of androgen prophylaxis on plasma aminopeptidase P. *J Allergy Clin Immunol* 2008;121:429-33.
 11. 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.

<http://dx.doi.org/10.1016/j.jaci.2013.04.032>

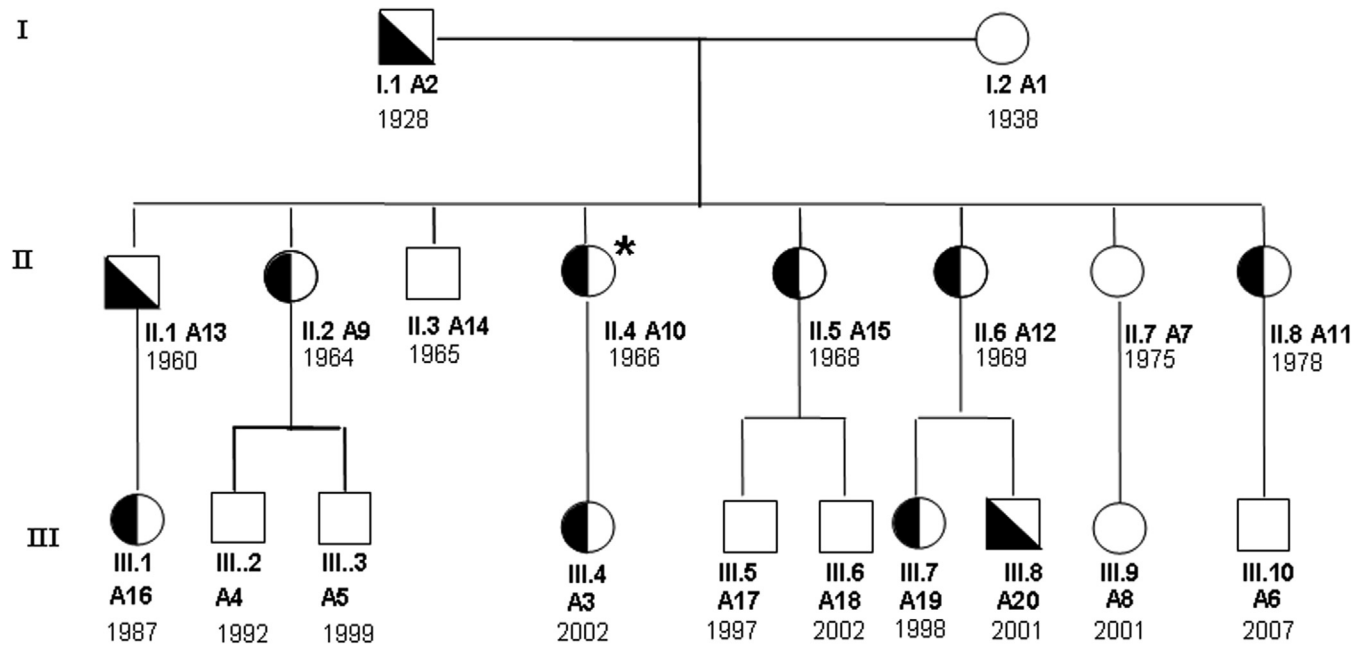


FIG E1. Family pedigree: heterozygous *F12* gene mutation carriers have a semisolid mark and unaffected progeny are not filled in at all. The index case is indicated by an asterisk.

TABLE E1. HAE-FXII severity score (questions referred to patient's lifetime)

Asymptomatic (0)	Never angioedema episodes
Mild (1)	≤6 non-life-threatening angioedema episodes per year in the symptomatic period without any emergency assistance requirement
Moderate (2)	• ≤6 non-life-threatening angioedema episodes per year in the symptomatic period with at least 1 emergency assistance requirement - or • >6 non-life-threatening angioedema episodes per year in the symptomatic period without any emergency assistance requirement.
Severe (3)	• A life-threatening angioedema episode per year in the symptomatic period - or • >6 non-life-threatening angioedema episodes per year in the symptomatic period with at least 1 emergency assistance requirement

TABLE E2. Clinical features in carriers of *F12* gene mutation

Patient	Generation	Sex	Age at onset of angioedema (y)	Angioedema attack sites*	Clinical severity†	Symptoms with possible precipitating factors				
						Fertility therapy	OC intake	Pregnancy	ACE inhibitors	Triggers
A10-II.4 (index)	Second	F	27	Face, uvula, upper airway, limbs	Severe	Angioedema (<i>estradiol + desogestrel-ethinylestradiol</i>)	NR	Angioedema	NR	Dental procedures
A9-II.2	Second	F	28	Uvula, tongue, upper airway, limbs	Moderate	NR	Angioedema (<i>desogestrel-ethinylestradiol</i>)	2 normal pregnancies	NR	OTI and general anesthesia during cesarean delivery. Dental procedure.
A11-II.8	Second	F	22	Face, uvula, upper airway, abdominal, limbs	Moderate	NR	Angioedema (<i>gestodene-ethinylestradiol</i>)	Angioedema	NR	Unknown (spontaneous)
A12-II.6	Second	F	15	Face, limbs	Mild	NR	Angioedema (<i>desogestrel-ethinylestradiol</i>)	2 normal pregnancies	NR	Microtrauma (dancing)
A16-III.1	Third	F	22	Limbs	Mild	NR	Angioedema (<i>gestodene-ethinylestradiol</i>)	NA	NR	Microtrauma (carrying a suitcase, typing on computer)
A2-I.1	First	M	NA	NA	Asymptomatic	NA	NA	NA	NR	NA
A13-II.1	Second	M	NA	NA	Asymptomatic	NA	NA	NA	NR	NA
A15-II.5	Second	F	NA	NA	Asymptomatic	NA	Tolerated (<i>desogestrel-ethinylestradiol</i>)	2 normal pregnancies	NR	NA
A3-III.4	Third	F	NA	NA	Asymptomatic	NA	NR	NA	NR	NA
A19-III.7	Third	F	NA	NA	Asymptomatic	NA	NR	NA	NR	NA
A20-III.8	Third	M	NA	NA	Asymptomatic	NA	NA	NA	NR	NA

The drug prescribed as fertility treatment or OC is shown in *italics* and in brackets; the symptoms presented with possible precipitating factors are in **boldface**.

F, Female; M, male; NA, not applicable; NR, not required; OC, oral contraceptive; OTI, orotracheal intubation.

*Upper airway edema was clinically manifested by dysphonia and dyspnea.

†According to severity HAE-FXII score described in [Table E1](#).

TABLE E3. Complement study in each subject of the studied family during estrogen exposure-free intervals (except samples indicated with an asterisk)

Patient	<i>F12</i> gene mutation	C4 (14.0-60.0 mg/dL)	C1-INH (16.0-33.0 mg/dL)	C1-INH function (>50%)
A10-II.4 (index case)	Yes	30.9*† 41.2	32.5*† 51.1	62*† 79
A2-I.1 (father)	Yes	18.5	32.9	>120
A1-I.2 (mother)	No	15.3	20.9	93
A13-II.1	Yes	24.3	37.5	>120
A9-II.2	Yes	19.4	31.3	77
A14-II.3	No	32.2	51.9	74
A15-II.5	Yes	23.3	40.7	>120
A12-II.6	Yes	15.9	22.8	>120
A7-II.7	No	21.1	24.5	127
A11-II.8	Yes	23.4* 22.9	24.6* 36.4	70* 73
A16-III.1	Yes	10.7	20.0	>120
A4-III.2	No	12.7	22.0	106
A5-III.3	No	24.4	26.9	109
A3-III.4	Yes	24.1	26.9	108
A17-III.5	No	9.5	24.0	>120
A18-III.6	No	18.3	37.1	>120
A19-III.7	Yes	10.6	29.5	>120
A20-III.8	Yes	17.3	35.1	>120
A8-III.9	No	25.0	26.7	137
A6-III.10	No	5.3	21.5	78

Normal ranges are indicated in parentheses.

*The sample was obtained during pregnancy.

†The sample was obtained during an acute attack.

TABLE E4. Clinical symptoms, results of enzymatic assays, and prediction of severe angioedema attack risk in 20 individuals from 3 generations

Patient	Generation	Year of birth	Sex	Angioedema symptoms*	F12 gene mutation	Sampling date	Active disease†	SKA‡	PAA§	SKA/PAA	APP activity¶	CPN activity#	ACE activity**	Severe angioedema risk according to laboratory assessment††
A10-II.4 (index)	2	1966	F	Severe	Yes	2007	No	4.0	3575	0.11	1.44	33.2	ND	Protected
						2009/11	No	3.4	6388 (↑)	0.05 (↓)	2.35	28.9	58.0 (↑)	Protected
A2-I.1 (father)	1	1928	M	Asymptomatic	Yes	2007	No	5.5	2441	0.22	1.47	27.3	ND	Protected
						2009/03	No	18.4	2144	0.85	1.69	26.2	24.0	Protected
						2009/11	No	31.0	2435	0.13	1.76	28.2	41.0	Protected
A1-I.2 (mother)	1	1938	F	Asymptomatic	No	2007	No	12.0 (↑)	3469	0.34 (↑)	1.45	29.7	ND	Protected
						2009/03	No	16.1 (↑)	2919	0.55 (↑)	1.53	32.7	34.0	Protected
						2009/11	No	4.7	2986	0.16	1.67	32.6	64.0 (↑)	Protected
A13-II.1	2	1960	M	Asymptomatic	Yes	2009/03	No	2.2 (↓)	3983	0.06	1.83	39.5 (↑)	23.0	Protected
						2009/11	No	3.9	4497 (↑)	0.09	1.68	38.0 (↑)	43.0	Protected
A9-II.2	2	1964	F	Moderate	Yes	2007	No	5.1	3236	0.16	1.25 (↓)	21.3 (↓)	ND	Severe disease
						2009/11	No	3.3	4442	0.07	1.71	24.9 (↓)	48.0	Possible susceptibility
A14-II.3	2	1965	M	Asymptomatic	No	2009/03	No	3.6	4410 (↑)	0.81	1.55	45.1 (↑)	47.0	Protected
						2009/09	No	4.4	4675 (↑)	0.09	1.51	37.7 (↑)	61.0 (↑)	Protected
A15-II.5	2	1968	F	Asymptomatic	Yes	2009/03	No	2.0 (↓)	3267	0.06	2.91	31.2	31.2	Protected
						2009/11	No	3.6	3121	0.12	2.92	27.7	55.0 (↑)	Protected
A12-II.6	2	1969	F	Mild	Yes	2009/11	No	2.7	4075	0.07	2.48	27.5	54.0 (↑)	Protected
A7-II.7	2	1975	F	Asymptomatic	No	2007	No	4.1	2974	0.14	1.37	33.9	ND	Protected
A11-II.8	2	1978	F	Moderate	Yes	2007	Yes† (performed during pregnancy)	21.4 (↑)	3384	0.63 (↑)	1.24 (↓)	27.3	ND	Severe disease
						2009/11	No	3.4	4020	0.09	1.94	31.0	47.0	Protected
A16-III.1	3	1987	F	Mild	Yes	2009/03	No	5.3	3341	0.16	2.22	37.0	29.0	Protected
						2009/11	No	4.3	2533	0.17	2.19	27.8	56.0 (↑)	Protected
A4-III.2	3	1992	M	Asymptomatic	No	2007	No	3.4	2313	0.15	2.05	23.1 (↓)	ND	Possible susceptibility
A5-III.3	3	1999	M	Asymptomatic	No	2007	No	4.8	2692	0.18 (↑)	2.02	26.8	ND	Protected
A3-III.4	3	2002	F	Asymptomatic	Yes	2007	No	4.0	3029	0.13	2.31	34	ND	Protected
						2009/11	No	4.2	3971	0.11	2.64	45.3 (↑)	50.0 (↑)	Protected
A17-III.5	3	1997	M	Asymptomatic	No	ND	No	ND	ND	ND	ND	ND	ND	ND
A18-III.6	3	2002	M	Asymptomatic	No	ND	No	ND	ND	ND	ND	ND	ND	ND
A19-III.7	3	1998	F	Asymptomatic	Yes	2009/03	No	1.4 (↓)	2772	0.05 (↓)	2.62	29.7	49.0	Protected
						2009/11	No	5.6	2845	0.2	2.41	28.3	66.0 (↑)	Protected
A20-III.8	3	2001	M	Asymptomatic	Yes	2009/03	No	1.7 (↓)	3580	0.05 (↓)	2.62	30.1	53.0	Protected
						2009/11	No	6.5 (↑)	3616	0.18 (↑)	2.45	26.0	77.0 (↑)	Protected
A8-III.9	3	2001	F	Asymptomatic	No	2007	No	4.1	2460	0.17	1.98	29.7	ND	Protected
A6-III.10	3	2007	M	Asymptomatic	No	2007	No	10.2 (↑)	1964 (↓)	0.57 (↑)	4.15 (↑)	21.3 (↓)	ND	Possible susceptibility

F, Female; M, male; ND, not done.

*Clinical severity was assessed according to criteria given in Table E1.

†Active disease refers to the presence of angioedema episodes in relation to high estrogens levels at the time the blood samples were taken.

‡Reference values: 2.4-10.7 and 2.3-5.6 nmol/min/mL for female and male individuals, respectively.

§Reference values: 2422-4560 and 2225-4273 nmol/min/mL for female and male individuals, respectively.

||Reference values: 0.07%-0.29% and 0.07%-0.17% for female and male individuals, respectively.

¶Reference values: 1.36-3.09 and 1.29-2.48 nmol/min/mL for female and male individuals, respectively.

#Reference values: 26.2-41.6 and 23.9-36.4 nmol/min/mL for female and male individuals, respectively.

**Reference values: 11-49 UI/L for female and male individuals.

††Severe angioedema risk refers to subject's classification as protected or susceptible to develop severe angioedema attacks according to results of APP, CPN, and ACE activities.