

BRIEF COMMUNICATION

Homozygosity for a factor XII mutation in one female and one male patient with hereditary angio-oedema

A. S. Grumach^{1,*}, C. Stieber^{2,3,*}, C. L. Veronez⁴, N. Cagini⁴, R. N. Constantino-Silva¹, E. Cordeiro¹, M. M. Nöthen^{2,3}, J. B. Pesquero⁴ & S. Cichon^{2,3,5}

¹Outpatient Group of Recurrent Infections and Laboratory of Clinical Immunology, Faculty of Medicine ABC, Santo André, Brazil;

²Department of Genomics, Life & Brain Center, Institute of Human Genetics, University of Bonn, Bonn, Germany; ³Center for Rare Diseases Bonn, University Hospital of Bonn, Bonn, Germany; ⁴Department of Biophysics, Universidade Federal de São Paulo, São Paulo, Brazil; ⁵Division of Medical Genetics, Department of Biomedicine, University Hospital Basel, Basel, Switzerland

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Correspondence

Anete Sevciovic Grumach, MD, PhD, Center of Research, Faculty of Medicine ABC, Av Príncipe de Gales, 821 Santo André, 09060.650 São Paulo SP, Brazil.
Tel.: +55 11 983353860
Fax: +55 11 32845335
E-mail: asgrumach@gmail.com

*Authors contributed equally.

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Abstract

Hereditary angio-oedema (HAE) with normal C1 inhibitor is associated with heterozygous mutations in the factor XII gene (FXII-HAE). We report two Brazilian FXII-HAE families segregating the mutation c.983 C>A (p.Thr328Lys). In each family, one patient with a homozygous mutation was found. The homozygous female patient in family 1 displayed a severe phenotype. However, this falls within the clinical phenotype spectrum reported for heterozygous female mutation carriers. The homozygous male patient in family 2 also showed a severe phenotype. This finding is intriguing, as to our knowledge, it is the first such report for a male FXII-HAE mutation carrier. In the rare instances in which male mutation carriers are affected, a mild phenotype is typical. The present findings therefore suggest that homozygous FXII-HAE mutation status leads to a severe phenotype in females and males, and to an increased risk of manifest symptoms in the latter.

Hereditary angio-oedema (HAE) (OMIM 106100) is a rare autosomal dominant disease characterized by self-limiting oedema of the skin, the internal organs and the gastrointestinal and the laryngeal tracts. The classic forms, HAE types I and II, occur as a consequence of mutations in the *SERP-ING1* gene coding for the C1 inhibitor (C1-INH, C1-INH-HAE). HAE with normal C1-INH is a third form, which mainly affects women, and which is caused in some cases by mutations in the gene encoding the blood coagulation factor XII (FXII, FXII-HAE) (1–6). In these cases, elevated oestrogen levels appear to trigger episodes of swelling.

Due to the dominant inheritance and the low population frequency of *FXII* mutations, patients with FXII-HAE are

usually heterozygous mutation carriers (5–11). To date, no homozygous cases have been reported. For C1-INH-HAE, individuals with homozygous mutations in *SERP-ING1* have been reported in only two families (12, 13).

Here, we report the detection of one female and one male homozygous case in two Brazilian families segregating the most frequently observed mutation in FXII-HAE, Thr328Lys (c.983 C>A (p.Thr328Lys)).

Methods

The study was approved by the ethics committee of the Faculty of Medicine ABC (Santo André, SP, Brazil), and all participants provided written informed consent prior to inclusion. The procedure used to assign a FXII-HAE diagnosis is described elsewhere (1–3). DNA extraction and Sanger sequencing of exon 9 of *FXII* were performed in the

Abbreviations

C1-INH, C1 inhibitor; FXII, factor XII; FXII-HAE, hereditary angio-oedema with factor XII mutation; HAE, hereditary angio-oedema.

Department of Biophysics, Federal University of São Paulo, Brazil. The genotypes of two individuals from family 1 (including homozygous individual II-1) and 8 individuals from family 2 (including homozygous individual IV-17) were confirmed in the Institute of Human Genetics, University of Bonn, Germany.

Results

The pedigrees of the two Brazilian families segregating the Thr328Lys mutation are shown in Fig. 1. Table 1 shows the clinical phenotype of all affected individuals from the two families.

In family 1 (Fig. 1A), 10 members (4F:6M) were mutation carriers. Female II-1 was a homozygous mutation carrier. The remaining mutation carriers were heterozygous. All four female mutation carriers and four of six male mutation carriers were affected. The homozygous female (II-1) was 51 years of age and had presented with repeated episodes of severe subcutaneous oedema, abdominal pain and laryngeal oedema since the age of 20 years.

In family 2 (Fig. 1B), five members (4F:1M) were mutation carriers. Male IV-17 was a homozygous mutation carrier. The remaining mutation carriers were heterozygous. All mutation carriers were affected (Table 1). For three affected females in family 2, testing for the mutation was not possible. The homozygous male (IV-17) was 61 years of age and had presented with repeated episodes (on average once a month) of subcutaneous oedema of the face, extremities and genitalia, and abdominal pain since the age of 25 years. In addition, he reported five episodes of uvula oedema. All episodes of swelling were triggered by trauma.

Discussion

The detection and clinical characterization of individuals with homozygous *FXII* mutations are necessary to deter-

mine (i) whether homozygous mutation status is compatible with survival and (ii) whether homozygous and heterozygous carriers show differing clinical phenotypes. The homozygous female patient in family 1 displayed a severe phenotype, characterized by frequent episodes and a broad symptom spectrum. However, this clinical presentation falls within the clinical phenotype-spectrum reported for female heterozygous Thr328Lys mutation carriers (9, 14). Remarkably, the clinical phenotype of the homozygous male patient in family 2 was also severe. Available data suggest that a severe phenotype is unusual for affected male mutation carriers, although it must be noted that few affected male patients have been reported to date, and the clinical information provided in the respective reports has often been sparse (9–11, 14–16). Nonetheless, the fact that the male patient was affected at all is intriguing, as the majority of heterozygous males reported in the literature were unaffected (5, 14–16). A plausible hypothesis therefore is that in females and males, Thr328Lys homozygosity leads to a severe clinical presentation and that in males, it is associated with a higher risk of manifest disease (increased penetrance). Further research into patients with homozygous *FXII* mutations would therefore be of potential clinical value.

In family 1, a relatively large proportion of male mutation carriers (4/6) reported clinical symptoms. This contrasts with previous reports of FXII-HAE families, where male mutation carriers were rarely affected. Although a biological explanation for the variable penetrance in males remains a matter of speculation, differences in genetic background or environmental exposures may be responsible. For both C1-INH-HAE and FXII-HAE, previous reports have described a wide variation in disease severity among carriers of the same mutation (17, 18). Initial studies have suggested that disease severity may be influenced by the plasma activity of enzymes involved in kinin catabolism, such as aminopeptidase P, angiotensin-converting enzyme and carboxypeptidase P (18,

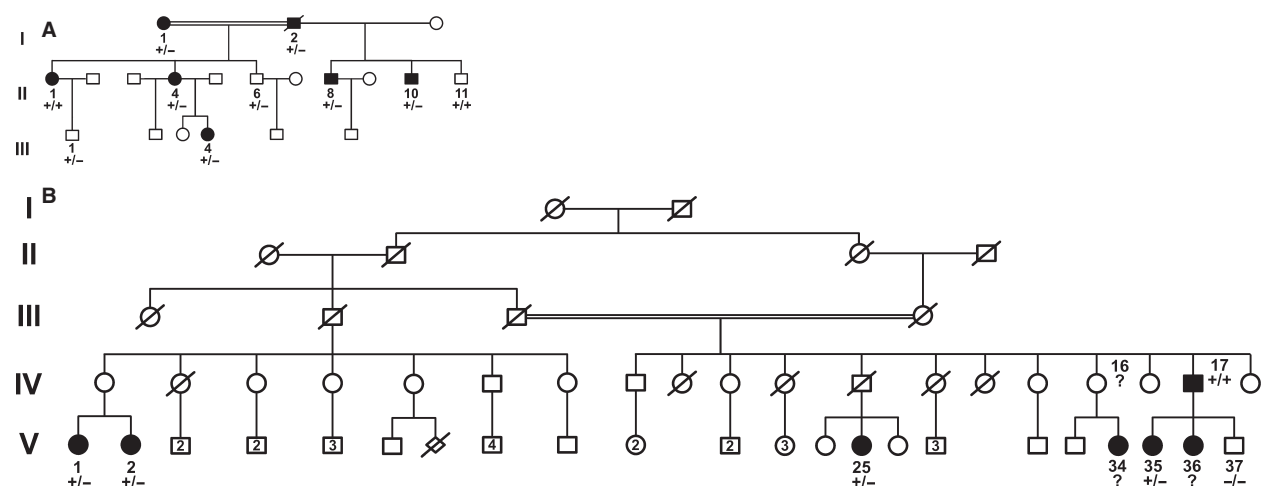


Figure 1 Pedigrees of the HAE-FXII families. A – Family 1. B – Family 2. Affected patients are indicated by black shading. The genotype of the *FXII* gene mutation Thr328Lys is indicated by the following: -/- (homozygous wild type, C/C); +/- (heterozygous for mutation, C/A); +/+ (homozygous for mutation, A/A); ? = not genotyped. Double lines represent familial consanguinity.

Table 1 Clinical characteristics of individuals from the two FXII-HAE families

Genealogy	Age [years]	Gender	FXII mutation	Symptom	Age at onset	Triggering factor					Symptom			
						Stress	Trauma	Hormones	Dental therapy	Without known trigger	Subcutaneous	Abdominal	Laryngeal	
Family 1														
I-1	69	F	+/-	+	16	-	-	-	-	+	+	+	+	+
I-2	76	M	+/-	+	U	-	-	-	-	+	-	+	+	+
II-1	51	F	+/+	+	20	+	-	+	+	-	+	+	+	+
II-4	49	F	+/-	+	16	+	-	+	+	-	+	+	+	+
II-6	43	M	+/-	-	-	-	-	-	-	-	-	-	-	-
II-8	35	M	+/-	+	12	-	-	-	-	+	-	+	-	-
II-10	37	M	+/-	+	U	-	-	-	-	+	-	+	-	-
II-11	29	M	+/-	+	26	-	-	-	-	+	-	-	+	+
III-1	28	M	+/-	-	-	-	-	-	-	-	-	-	-	-
III-4	22	F	+/-	+	18	-	+	+	-	+	+	+	-	-
Family 2														
IV-16	63	F	U	+	51	+	+	+	-	+	+	-	-	-
IV-17	61	M	+/+	+	25	-	+	-	-	+	+	+	+	+
V-1	52	F	+/-	+	28	+	+	+	-	+	+	+	+	+
V-2	48	F	+/-	+	25	-	-	-	+	+	+	-	-	-
V-25	28	F	+/-	+	20	+	+	+	-	+	+	-	-	-
V-34	29	F	U	+	20	-	-	+	-	+	+	+	-	-
V-35	31	F	+/-	+	28	+	+	+	-	+	+	+	-	-
V-36	28	F	U	+	20	+	+	+	-	+	+	+	+	+
unknown.														

U, unknown.

19). These factors are also candidates for influencing penetrance in male mutation carriers.

The molecular mechanisms underlying oedema formation in patients with the FXII mutation Thr328Lys are the subject of ongoing research. Originally, the mutation was associated with increased enzymatic activity (5). However, this observation has not been explained at the functional level, as amino acid position 328 (309 in the mature FXII protein) is located outside the enzymatic domain. Another potential molecular mechanism is altered binding of C1INH to FXII-328Lys, as suggested by an investigation of *in silico* modelling data (20). A very recent *in vitro* and *in vivo* (mouse model) functional study has shed more light on the precise molecular mechanism. Björkqvist et al. (21) showed that Thr328Lys results in a loss of O-linked glycosylation at this amino acid residue. This in turn leads to increased susceptibility for contact-mediated autoactivation of zymogen FXII in mutation carriers compared with controls, resulting in excessive activation of the bradykinin-forming kallikrein-kinin pathway (21). In this study, FXII-driven coagulation and the ability of C1-INH to bind and inhibit activated FXII were not affected by the mutation (21). Against the background of this functional evidence, we hypothesize that Thr328Lys homozygosity leads to even higher plasma bradykinin levels than heterozygosity, thus resulting in a more severe disease phenotype.

In summary, the present study extends current knowledge of FXII-HAE. Identification and evaluation of the first reported individual with homozygous *FXII* mutations indicates that although homozygosity for the reported mutation is compatible with survival, it leads to a severe phenotype in females and males and to a higher risk of manifest disease in males. Further research is warranted to elucidate the factors that modify the phenotypic effect of *FXII* mutations both within, and between, families.

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Conflict of interest

ASG has worked as consultant and speaker for Shire and CSL. No other conflicts of interest.

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