

Hereditary angioedema with normal C1-INH with *versus* without specific *F12* gene mutations

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Abstract

Background: Hereditary angioedema with normal C1-INH may be linked to specific mutations in the coagulation *factor 12* (FXII) gene (HAE-FXII) or mutations in genes that are still unknown (HAE-unknown). To assess the differences in transmission and inheritance, clinical features, and laboratory parameters between patients with HAE-FXII and HAE-unknown.

Methods: Sixty-nine patients with HAE-FXII from 23 unrelated families and 196 patients with HAE-unknown from 65 unrelated families were studied.

Results: Both HAE-FXII and HAE-unknown are inherited as autosomal-dominant traits with incomplete penetrance. The male to female ratio was 1 : 68 in HAE-FXII and 1 : 6.3 in HAE-unknown. The maternal to paternal transmission ratio was 35 : 14 for HAE-FXII and 109 : 12 for HAE-unknown. Mean age at onset of clinical symptoms was 20.3 years in patients with HAE-FXII and 29.6 years in patients with HAE-unknown. The incidence of asphyxiation due to angioedema was similar for HAE-FXII and HAE-unknown. Oral contraceptives and pregnancies had a significantly higher impact on HAE-FXII than on HAE-unknown. Slightly decreased C1-INH activity and C4 concentration were observed in more patients with HAE-FXII than HAE-unknown. Tests for FXI and FXII activity, plasminogen activator inhibitor 1, and activated partial thromboplastin time showed variability but no significant differences between the groups. No abnormalities were found for C1-INH protein, C1q, alpha2-macroglobulin, antithrombin III, and angiotensin-converting enzyme. In families with HAE-FXII, the number of female offspring with *F12* mutations was significantly increased and that of male offspring was significantly decreased.

Conclusions: HAE-FXII and HAE-unknown differ in various respects, including gender distribution, genetics, symptoms, and estrogen impact.

Angioedema is a clinical symptom of various hereditary and nonhereditary conditions (1), including hereditary angioedema (HAE) due to C1 inhibitor (C1-INH) deficiency (HAE-C1-INH). HAE-C1-INH is a rare disorder that is caused by mutations in the gene encoding C1-INH (*SERPINC1*), resulting in low plasma levels of functionally active C1-INH. As a consequence, faulty, uninhibited activation of the kallikrein-kinin system can lead to the release of bradykinin, thereby causing recurrent episodes of swelling of various organs (2, 3).

In 2000, a new type of HAE was described that was not associated with a deficiency of C1-INH and was termed 'HAE with normal C1-INH' (HAEnCI) or 'HAE type III' (4). Between 2006 and 2013, four different mutations were identified in the gene coding for coagulation factor XII (FXII; *F12* gene) in patients affected by HAEnCI: 2 missense mutations, a large deletion and a duplication (5–8). These mutations cause a change in the amino acid sequence of the proline-rich region of the FXII protein and are linked to a subtype of HAEnCI which was termed 'HAEnCI with

mutations in the *F12* gene' or 'HAE-FXII' (5, 8). HAE-FXII is inherited as an autosomal-dominant trait (9).

HAEnCI has been reported in numerous patients since its first description (10–12), but *F12* gene mutations were detected in only 24.5% of the affected families whereas in the majority of HAEnCI families with two or more patients, the genetic background is still unknown ('HAEnCI without mutations in the *F12* gene' or 'HAE-unknown') (9).

The aim of this study was to investigate the differences and similarities in inheritance, in clinical features including gender, age at onset, affected organs, the impact of estrogen, and in laboratory findings between patients with HAE-FXII and patients with HAE-unknown.

Methods

Patients and families

Data from patients with HAE-FXII or HAE-unknown were collected both prospectively and retrospectively. In families with HAE-FXII, both affected patients and asymptomatic potential carriers of the *F12* gene mutation were analyzed. HAE-FXII patients with a negative family history were identified as described elsewhere (13) and included. In families with HAE-unknown, analysis was restricted to symptomatic patients with at least one additional family member in a different generation with recurrent angioedema. Sporadic angioedema patients with a negative family history were excluded because the genetic basis of their disease could not be determined. All participants initially underwent a thorough clinical examination during which a detailed, standardized medical and family history was obtained. In particular, the frequency and intensity of the symptoms were assessed, and the patients were asked to identify all organs that had been affected by their angioedema since its first manifestation. In addition, the impact of estrogen exposure was recorded, focusing on estrogen-containing oral contraceptives (OCs), hormonal replacement therapy (HRT), and pregnancies.

After the first consultation, patients were seen or contacted by telephone every 4–6 months during the first year and every 12 months thereafter. Data on HAEnCI were collected from the patients' medical history and from clinical examinations as well as follow-up records. This study was conducted in the Angioedema Outpatient Service at the University of Mainz, Germany (AOS).

Mutation analyses

HAE-specific *F12* mutations and mutations of the *SERPINE1* gene were identified as described elsewhere (7, 14).

Laboratory analysis

Tests of the contact/kallikrein–kinin, complement, coagulation, and fibrinolytic systems were performed on citrated plasma samples collected during latent periods. Protein levels of C1-INH antigen, C4, and C1q were measured by radial

immunodiffusion. C1-INH activity (reference range 70–130%) was determined using the chromogenic substrate C₂H₅CO-Lys (ε-Cbo)-Gly-Arg-pNA (Technochrom® C1-INH, Technoclone, Vienna, Austria). Alpha2-macroglobulin (A2M), anti-thrombin III (AT III), angiotensin-converting enzyme (ACE), FXI, FXII, plasminogen activator inhibitor 1 (PAI-1), and activated partial thromboplastin time (aPTT) were determined with routine methods.

Presentation of results

The two groups, HAE-FXII and HAE-unknown, were compared using binomial tests, chi-square tests, and t-tests on the per-patient basis (when cells <5 patients were involved, Fisher's exact test was utilized). The chi-square contribution of single cells was used to indicate whether a cell had significantly more (+) or less (–) observations than expected. No statistical tests on the per-attack basis were performed because attacks are usually clustered within patients. If a category was empty in both groups, only the nonempty categories were taken for the test. T-tests were performed assuming equal variances. Laboratory findings were categorized into normal, decreased, and increased, and the categories were compared with chi-square tests between HAE-FXII vs HAE-unknown. This seemed to be clinically more relevant than simply performing t-tests over means. Significance level alpha was set to 0.05 (two-tailed). Statistics were performed using STATA (version 12, StataCorp, College Station, Texas, USA). As a large amount of data is presented, of the inferential statistics only *P*-values are reported.

Results

Patients and families

A total of 265 patients from 88 unrelated families with clinical symptoms of HAEnCI were identified (Fig. 1). These included 69 of 265 (26.0%) patients from 23 of 88 (26.1%) families with HAE-FXII and 196 of 265 (74.0%) patients from 65 of 88 (73.9%) families with HAE-unknown. HAE-FXII families had 1–8 symptomatic members (Table 1). In 5 of 23 (21.7%) families, there was only one affected patient. The majority of HAE-unknown families had two or three affected patients, but there were three families with 10 symptomatic members (Table 1).

Mutation analyses

F12 gene

Three different mutations of the *F12* gene could be identified across the 23 families with HAE-FXII. While members of the majority of families (19/23) had a p.Thr328Lys missense mutation, a p.Thr328Arg missense mutation occurred in 2 of 23 families, and a large deletion (c.971_1018+24del 72) in exon 9 and intron 9 of the *F12* gene was reported in the remaining 2 of 23 families.

In all but one family (22/23), the specific *F12* mutation was detected in two or more family members and a *de novo*

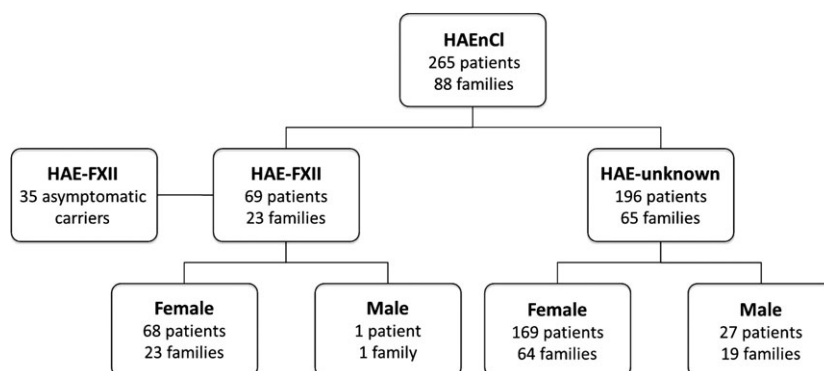


Figure 1 Patient cohort with hereditary angioedema with normal C1 inhibitor. HAE-FXII = hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) and HAE-specific mutation in the *F12* gene;

HAE-unknown = HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene.

mutation could be ruled out. In the remaining family, the carrier of the mutation was adopted and the biological family history unavailable.

All patients with HAE-FXII had one of the above-mentioned *F12* gene mutations, whereas none of these mutations were found in any of the patients with HAE-unknown.

SERPING1 gene

Sequencing of the *SERPING1* gene in 18 patients with HAE-FXII from 12 families and 10 patients with HAE-unknown from 10 families did not reveal any pathogenic mutation, thereby ruling out C1-INH deficiency as a cause of the clinical symptoms.

Table 1 Number of families with 1–10 symptomatic members in 23 families (69 patients) with HAE-FXII and 65 families (196 patients) with HAE-unknown

	HAE-FXII Number of families N = 23	HAE-unknown Number of families N = 65
Number of symptomatic patients per family		
1	5	n.a.
2	8	36
3	4	15
4	1	6
5	1	5
6	2	0
7	1	0
8	1	0
9	0	0
10	0	3

HAE-FXII, hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) and HAE-specific mutation in the *F12* gene; HAE-unknown, HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene; N, total number of families with the respective condition; n.a., not applicable, patients were not included because heritability could not be determined.

Gender

Overall, in both HAE-FXII and HAE-unknown, significantly more women than men were clinically affected by the disease ($P \leq 0.001$ and $P \leq 0.001$) (Fig. 1).

Per-patient analysis

Among the 69 patients with HAE-FXII, all but one patient was female, resulting in a male to female ratio of 1 : 68 (Fig. 1). For HAE-unknown, this ratio was 1 : 6.3, with a significantly higher percentage of affected men with HAE-unknown (13.8%) than with HAE-FXII (1.4%) ($P \leq 0.004$).

Per-family analysis

In 22 of 23 (95.7%) families with HAE-FXII, only women were affected. The remaining family included one male patient and five female patients. In 46 of 65 (70.8%) families with HAE-unknown, all patients were female. In 1 of 65 (1.5%) families, only men (father and son) had symptoms, and in 18 of 65 (27.7%) families, both men and women were affected.

Inheritance pattern

The mode of inheritance could be determined in all HAE-FXII families by mutation analysis and in 59 of 65 (90.8%) families with HAE-unknown by pedigree analysis.

In the remaining 6 of 65 (9.2%) HAE-unknown families, symptomatic patients belonged to the same generation (siblings) and mutation data of individuals from different generations were not available. The results of these analyses showed an autosomal-dominant inheritance for both HAE-FXII and HAE-unknown.

Penetrance

HAE-FXII

In 23 HAE-FXII families, 104 carriers with a specific *F12* gene mutation were identified, including 69 (66.3%) patients

with clinical symptoms and 35 (33.7%) asymptomatic carriers of the mutation. This phenotypic variation suggests incomplete penetrance of the *F12* gene. Of 79 female carriers, 68 were symptomatic (86.1%), whereas of 25 male carriers, only one was affected (4%), indicating that penetrance was also gender specific.

HAE-unknown

As the genetic basis of HAE-unknown is still unknown, asymptomatic carriers of the disease could not be identified. A pedigree analysis, however, showed that in 21 cases, symptoms had skipped a generation, indicating that there were asymptomatic carriers and that penetrance was incomplete for HAE-unknown as well.

Transmission

Phenotype analysis

The pedigree analysis from symptomatic or asymptomatic mothers or fathers revealed that the majority of offspring had inherited HAE-FXII or HAE-unknown from their mothers as evidenced by maternal to paternal inheritance ratios of 35 : 14 (2.5 : 1) for HAE-FXII and 109 : 12 (9.1 : 1) for HAE-unknown (Table 2). Accordingly, in both HAE-FXII and HAE-unknown, the number of women who passed on the disease to their offspring was significantly higher compared with men (HAE-FXII: $P \leq 0.006$; HAE-unknown: $P \leq 0.001$).

Genotype analysis of HAE-FXII

Among the 104 symptomatic and asymptomatic carriers, the specific *F12* gene mutation was detected in significantly more women than men, resulting in a male to female ratio of 25 : 79 ($P \leq 0.001$). Further analysis of the transmission rate of the *F12* gene mutation by male and female carriers showed gender-specific differences in heritability. The mutation was passed on by maternal and paternal transmission to more than half of the daughters (68.3% and 60.9%, respectively) and less than half of the sons (31.1% and 9.1%, respectively) (Table 3).

Clinical symptoms

Sufficient and reliable data on clinical symptoms could be obtained from 61 patients with HAE-FXII and 178 patients with HAE-unknown.

Age at onset

The mean age [SD] at onset of clinical symptoms was significantly lower ($P \leq 0.001$) for patients with HAE-FXII (20.3 years [9.2 years]) than for patients with HAE-unknown (29.6 years [16.5 years]) (Fig. 2A and B). The age at initial symptom manifestation ranged from 1 to 65 years for HAE-FXII, with the majority of patients becoming symptomatic during the first two decades of life, and from 1 to 80 for HAE-unknown, with more than half of the patients developing initial symptoms during a later stage of life.

Table 2 Maternal and paternal transmission of HAE-FXII and HAE-unknown to symptomatic offspring (phenotype analysis)*

Route of transmission of disease	HAE-FXII Number of symptomatic offspring (%)			HAE-unknown Number of symptomatic offspring (%)		
	Sons	Daughters	Total	Sons	Daughters	Total
Maternal	1 (2.9)	34 (97.1)	35 (100.0)	15 (13.8)	94 (86.2)	109 (100.0)
Paternal	0	14 (100.0)	14 (100.0)	3 (25.0)	9 (75.0)	12 (100.0)

HAE-FXII, hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) and HAE-specific mutation in the *F12* gene; HAE-unknown, HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene.

*First generation of families was excluded because the gender of the transmitters was unknown.

According to binomial tests, the difference between proportions of maternal and paternal heritage was statistically significant for HAE-FXII in females ($P = 0.006$), HAE-unknown in males ($P = 0.008$), and HAE-unknown in females ($P = 0.001$).

Table 3 Transmission of *F12* gene mutations from heterozygous parents to daughters and sons in families with HAE-FXII (genotype analysis of offspring)

Route of transmission	Number of daughters (%)				Number of sons (%)			
	Total	Carriers	Noncarriers	<i>P</i> -value*	Total	Carriers	Noncarriers	<i>P</i> -value*
Total	83 (100)	55 (66.3)	28 (33.7)	Not tested	56 (100)	15 (26.8)	41 (73.2)	Not tested
Maternal	60 (100)	41 (68.3)	19 (31.7)	≤ 0.006	45 (100)	14 (31.1)	31 (68.9)	≤ 0.016
Paternal	23 (100)	14 (60.9)	9 (39.1)	≤ 0.405	11 (100)	1 (9.1)	10 (90.9)	≤ 0.012

HAE-FXII, hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) and HAE-specific mutation in the *F12* gene; HAE-unknown, HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene.

*Expected arrangements of the genotypes in autosomal-dominant inheritance: The risk to be carrier is 50% for both daughters and sons.

Binomial tests for equality (i.e., a ratio 50/50) were performed.

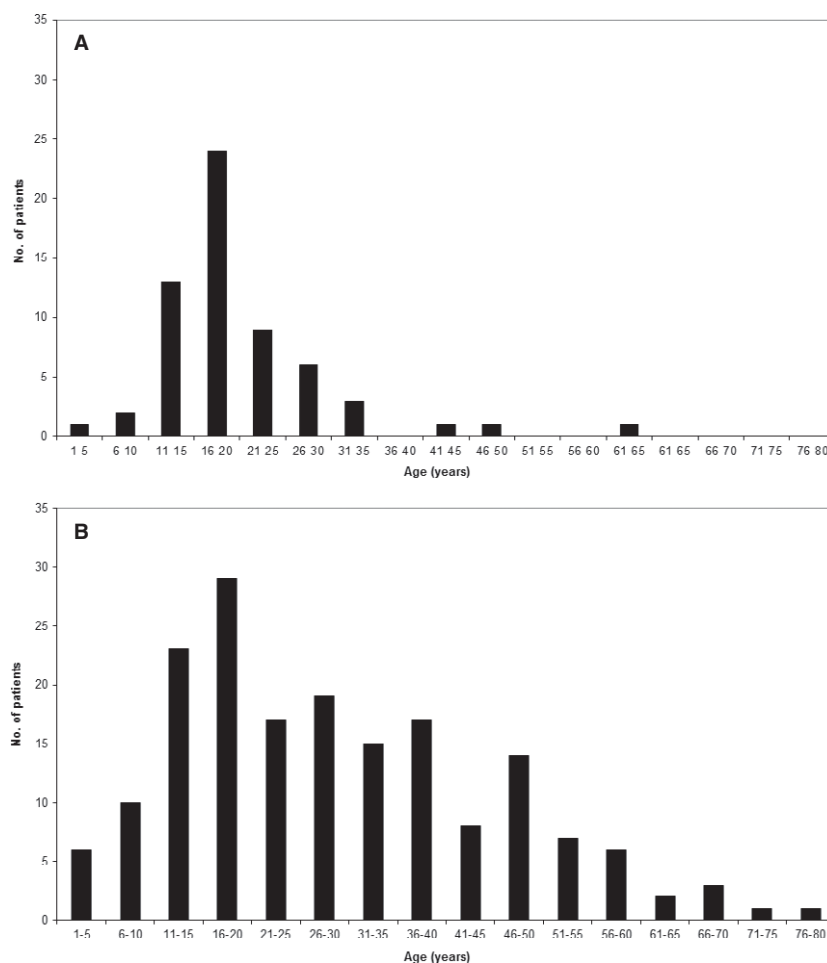


Figure 2 Age at onset of clinical symptoms in (A) 61 patients with HAE-FXII and (B) 178 patients with HAE-unknown.

Affected organs

The majority of patients, but significantly more HAE-FXII patients than HAE-unknown patients, experienced skin edema including swelling of the face, hand, feet, and genitals (Table 4). Abdominal attacks occurred in a significantly higher and tongue swellings in a significantly lower proportion of patients with HAE-FXII than with HAE-unknown (Table 4). In 10 patients with HAE-unknown but in no patient with HAE-FXII, clinical symptoms were restricted to tongue swellings.

The total number of symptomatic years was 931 in the patients with HAE-FXII and 3409 in patients with HAE-unknown. The mean number of attacks per symptomatic years was 10.7 in patients with HAE-FXII and 13.7 in HAE-unknown. In a subset of 49 patients with HAE-FXII and 89 patients with HAE-unknown, data about the duration of swelling were available. The mean attack duration in HAE-FXII and HAE-unknown was for skin swellings 3.3 days (SD 1.6 days; range 6 hours–8 days) and 2.1 days (SD 1.4 days; range 6 hours–10 days), for abdominal attacks 2.7 days (SD 1.5 days; range 12 hours–7 days) and 2.2 days

(SD 1.4 days; range 5 hours–7 days), and for tongue swellings 1.8 days (SD 0.9 days; range 12 hours–3 days) and 1.7 days (SD 1.4 days; range 4 hours–7 days).

Death from asphyxiation

A total of 7 of 265 patients, all female, died from asphyxiation due to upper airway obstruction induced by angioedema. Thereof 2 of 69 (2.9%) patients with HAE-FXII died at the ages of 38 and 42, respectively, and 5 of 196 (2.6%) patients with HAE-unknown died at the ages of 34, 36, 36, 47, and 67, respectively. One patient died by a laryngeal attack after the diagnosis 'HAE-unknown' had been made. She asphyxiated during a hospital stay because of pancreatitis. The other six patients asphyxiated before the diagnosis was established.

Impact of oral contraceptives, hormonal replacement therapy, and pregnancies

Reliable data from high-estrogen periods were obtained from 61 women with HAE-FXII and 154 women with

Table 4 Sites and numbers of attacks during the course of disease for 61 patients with HAE-FXII and 178 patients with HAE-unknown

Affected organs	HAE-FXII		HAE-unknown		Difference between groups* <i>P</i> -value
	Number of patients† (%)	Number of attacks (%)	Number of patients† (%)	Number of attacks (%)	
Total	61 (100)	9969 (100)	178 (100)	46 844 (100)	Not tested
Skin					
Skin (total)	61 (100)	5846 (58.6)	157 (88.2)	27 410 (58.5)	≤0.003
Face	61 (100)	2231 (22.4)	153 (86.0)	18 205 (38.9)	≤0.001
Hands/arms	30 (49.2)	2258 (22.7)	58 (32.6)	4637 (9.9)	≤0.020
Feet/legs	31 (50.8)	1325 (13.3)	52 (29.2)	3957 (8.4)	≤0.002
Genitals	9 (14.8)	122 (1.2)	20 (11.2)	401 (0.9)	≤0.468
Other organs					
Tongue (total)	20 (32.8)	86 (0.9)	91 (51.1)	7117 (15.2)	≤0.013
Abdomen (total)	40 (65.6)	3947 (39.6)	74 (41.6)	11 927 (25.5)	≤0.001
Larynx (total)	15 (24.6)	90 (0.9)	32 (18.0)	390 (0.8)	≤0.262

HAE-FXII, hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) and HAE-specific mutation in the *F12* gene; HAE-unknown = HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene.

*All tests on the per-patient basis.

†Numbers of patients do not add up to 100% because most patients had swellings at various body sites during the course of disease.

HAE-unknown. Fifty-eight of 61 (95.1%) women with HAE-FXII and 144 of 154 (93.5%) women with HAE-unknown had experienced at least one such period during their lives.

No influence of estrogen on disease severity was observed in a significantly higher ($P \leq 0.05$) percentage of women with HAE-unknown than women with HAE-FXII (Table 5). A significantly higher percentage of women with HAE-FXII than with HAE-unknown experienced exacerbation or initial onset of symptoms upon OC intake ($P \leq 0.05$). Clinical symptoms started or exacerbated during significantly more pregnancies in women with HAE-FXII than with HAE-unknown ($P \leq 0.05$) (Table 5). The proportion of women who had clinical symptoms only during periods of OCs, HRT, and pregnancy, was significantly higher for HAE-FXII than for HAE-unknown ($P \leq 0.05$).

Laboratory findings

Laboratory data were obtained for 24 patients with HAE-FXII and 33 patients with HAE-unknown (Table 6). There was no significant difference in the percentage of patients with HAE-FXII and patients with HAE-unknown with respect to decreased C1-INH activity (in slightly more patients with HAE-FXII), increased FXI and FXII activities, decreased PAI-1 activity, and shortening of the aPTT. A significantly higher percentage of patients with HAE-FXII than patients with HAE-unknown had C4 levels outside the normal range (16.6% and 0%, respectively; $P \leq 0.048$).

There were no relevant differences between patients with HAE-FXII and patients with HAE-unknown in tests for C1-INH protein, C1q, A2M, AT III, and ACE.

Among the patients with HAE-FXII, two patients had a slightly decreased C1-INH activity and C4 protein, two others a low PAI-1 activity and a slightly shortened aPTT, and one patient a slightly decreased C4 protein and a decreased

PAI-1 activity. Among the patients with HAE-unknown, three patients had an increased FXII activity, a decreased PAI-1 activity, and a shortened aPTT, two patients had an increased C4 protein and an increased FXI activity, one patient an increased FXI and FXII activity, and one patient a decreased PAI-1 activity and a shortened aPTT. Otherwise, there were no clusters of pathological results.

Discussion

The exact incidence and prevalence of HAE-unknown and of HAE-FXII are still unknown. The present two large data sets suggest a higher prevalence of HAE-unknown than HAE-FXII, with HAE-unknown patients representing a larger proportion of all HAE patients. Based on the distribution of all HAE types in our center and an assumed prevalence of HAE-C1-INH of approximately 1 : 50 000 (15), an estimate for the prevalence of HAE-unknown could be placed at 1 : 100 000, and at 1 : 400 000 for HAE-FXII and 1 : 150 000 for HAE-unknown. The pedigree analysis confirmed the previously described autosomal-dominant inheritance of HAE-FXII with incomplete penetrance (9) and revealed the same for HAE-unknown.

As an autosomal-dominant trait, the genotype would be expected to occur in 50% of the offspring and to affect males and females equally. Our data indicate, however, that more women than men manifested clinical symptoms of HAE-FXII and HAE-unknown. In addition, a pedigree analysis of the phenotype in both HAE-FXII and HAE-unknown showed that in a significantly higher number of cases, the disease was passed on by the mothers than by the fathers, indicating a predominance of female carriers. This was confirmed by the mutation analysis which revealed significantly more female than male carriers in HAE-FXII families. In addition, genotype correlation of parents and their offspring showed a

Table 5 Clinical impact of oral contraceptives, hormonal replacement therapy, and pregnancies in 58 women with HAE-FXII and 144 women with HAE-unknown

Subgroups	HAE-FXII Number of women (%)	HAE-unknown Number of women (%)	Difference between groups <i>P</i> -value*
Women with high-estrogen periods			
Total	58 (100)	144 (100)	–
No influence†	10 (17.2)(–)	92 (63.9)(+)	–
Moderate impact‡	24 (41.4)	42 (29.2)	–
Severe impact‡,†	24 (41.4)(+)	10 (6.9)(–)	≤0.001
Women with oral contraceptives			
Total	48 (100)	110 (100)	–
No influence	5 (10.4)(–)	76 (69.1)(+)	–
Onset or exacerbation of symptoms	43 (89.6)(+)	34 (30.9)(–)	≤0.001
Improvement	0	0	–
Women with hormonal replacement therapy			
Total	10 (100)	19 (100)	–
No influence	4 (40)	10 (52.6)	–
Onset or exacerbation of symptoms	6 (60)	9 (47.4)	≤0.700
Improvement	0	0	–
Number of pregnancies			
Total	92 (100)	239 (100)	–
No influence	42 (45.7)(–)	202 (84.5)	–
Onset or exacerbation of symptoms†	43 (46.7)(+)	15 (6.3)(–)	–
Improvement	7 (7.6)	22 (9.2)	≤0.001

HAE-FXII, hereditary angioedema (HAE) with normal C1 inhibitor (C1-INH) and HAE-specific mutation in the *F12* gene; HAE-unknown, HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene.

*When tests were performed over multiple lines, the *P*-value was displayed in the last line involved. Entries of the previous lines were left empty. Single cells were indicated by (–) and (+) if the observed value was significantly lower or higher than expected.

†Differences between HAE-FXII and HAE-unknown were significant ($P \leq 0.05$) in these lines.

‡Moderate impact: recurrent angioedema during and outside of high-estrogen periods; severe impact: recurrent angioedema exclusively limited to high-estrogen periods.

higher proportion of daughters and a significantly lower proportion of sons with the *F12* gene mutation than expected for an autosomal-dominant trait for both maternal and paternal transmission. This low number of sons who were born with the *F12* mutation might indicate a negative influence of the *F12* gene mutation on the development of the male fetus.

Likewise, it could be speculated that the predominant maternal disease transmission reported for HAE-unknown is due to a higher number of female than male carriers of the hitherto unknown mutation.

Age at initial symptom onset was lower for the majority of patients with HAE-FXII compared to patients with HAE-unknown. Clinically affected children below 10 years of age were rare in both groups of patients.

While both, patients with HAE-FXII and with HAE-unknown, experienced skin swelling that was mainly located in the face and less frequently in the extremities, tongue swelling was less frequent and abdominal attacks were more frequent in HAE-FXII than HAE-unknown. Tongue swelling only was observed in HAE-unknown, but not in HAE-FXII. The distribution of edema differed from the pattern observed for HAE-C1-INH, where extremity swelling is far more frequent than facial swelling (16). The origin of these differences between HAE-FXII, HAE-unknown, and HAE-C1-INH has still to be determined. Laryngeal edema, however, affected a similar proportion of HAE-FXII and HAE-unknown patients and the incidence of asphyxiation was similar in both, thereby demonstrating that both conditions are potentially life-threatening, just as is the case for HAE-C1-INH (17).

The *F12* gene mutation in HAE-FXII, and most probably also the not yet identified gene mutation in HAE-unknown, represents the underlying genetic defect that is responsible for the development of angioedema. All of the four different *F12* gene mutations that were detected in patients with HAE-FXII are inframe mutations and might lead to configurational changes in the proline-rich region and thus a structural alteration of the FXII protein which could influence the activation of the kallikrein–kinin system (3, 18). This mechanism has been discussed to be the main, but not only, reason for the development of angioedema attacks in HAE-FXII (9). In addition, specific triggering events like changes in estrogen levels may elicit swelling attacks. Because of the estrogen-responsive element in the 5' flanking region of the *F12* gene, FXII expression, and consequently the activation of the kallikrein–kinin system, may be influenced by high estrogen levels (19). Although it has been observed that various other targets in the kallikrein–kinin system may be influenced by estrogen (20–22), the role and function of estrogen in the activation of the kallikrein–kinin system remains unclear.

In HAE-FXII, the impact of estrogen on the severity of the disease has been reported before (9) and is also well documented for HAE-C1-INH and idiopathic angioedema (13, 23, 24). In addition, our present data indicate that a certain impact of estrogen on HAE-unknown is likely, although less pronounced than for HAE-FXII. This phenomenon remains to be investigated. We speculate that the difference between the high estrogen impact on HAE-FXII and the relatively lower impact on HAE-unknown might be related to differences in the genetic background and, in addition, exogenous influences. Similar to data on HAE-FXII and idiopathic angioedema presented in other studies, in some women with HAE-FXII and HAE-unknown, clinical symptoms were limited to periods of OC intake and during HRT ('estrogen-dependent angioedema') (9, 13, 25–27).

Laboratory results revealed that the vast majority of patients had normal but a few patients had slightly

Table 6 Laboratory results of 24 patients with HAE-FXII and 33 patients with HAE-unknown during attack-free intervals

Biochemical markers	HAE-FXII			HAE-unknown			<i>P</i> -value
	Number of patients (%)	Mean (SD)	Range	Number of patients (%)	Mean (SD)	Range	
↔ C1-INH activity, %	19 (79.2)	93.3 (13.1)	72–119	31 (93.9)	94.6 (17.2)	72–127	–
↓ C1-INH activity, %	5 (20.8)	65 (4.7)	57–69	2 (6.1)	65.5 (0.7)	65–66	≤0.119
↔ C1-INH protein, mg/dl	24 (100)	23.1 (5.1)	16.4–33.5	33 (100)	21.8 (4.3)	15.4–33.2	≤0.302
↔ C4, mg/dl	17 (70.8)	25.3 (3.7)	16.4–28.8	27 (81.8)	23.1 (3.5)	16.4–26.3	–
↓ C4, mg/dl	4 (16.6)	12.7 (2.3)	9.8–15.4	0	0	0	–
↑ C4, mg/dl	3 (12.5)	40.7 (3.2)	37.5–43.9	6 (18.2)	37.4 (3.8)	33.8–43.4	≤0.048
↔ C1q, mg/dl	24 (100)	19 (4.8)	12–25	33 (100)	19.4 (3.5)	12–25	≤0.717
↔ A2M, g/l	24 (100)	1.8 (0.5)	1.3–2.6	33 (100)	1.9 (0.4)	1.3–2.7	≤0.405
↔ AT III, %	24 (100)	103.3 (9.9)	82–120	33 (100)	104.4 (9.8)	83–120	≤0.677
↔ ACE, U/l	24 (100)	27.1 (9.5)	14–44	33 (100)	30.3 (10.0)	14–60	≤0.229
↔ Factor XI, %	17 (70.8)	101 (9.5)	82–119	25 (75.8)	107 (11.0)	81–120	–
↑ Factor XI, %	7 (29.2)	125.9 (3.6)	121–131	8 (24.2)	136.5 (9.8)	121–144	≤0.677
↔ Factor XII, %	23 (95.8)	117.3 (17.6)	84.8–146	25 (75.8)	101.4 (29.7)	75.7–139	–
↑ Factor XII, %	1 (4.2)	152 (n.a.)	n.a.	8 (24.2)	154.3 (2.6)	151–157	≤0.064
↔ PAI-1, U/ml	17 (70.8)	3.3 (1.9)	1.1–76.	24 (72.7)	2.4 (1.3)	1–5.8	–
↓ PAI-1, U/ml	7 (29.2)	0.3 (0.1)	0.01–0.4	9 (27.3)	0.4 (0.1)	0.3–0.6	≤0.634
↔ aPTT, s	22 (91.7)	29.8 (1.9)	26.2–33.6	28 (84.8)	30.4 (2.6)	26–34.8	–
↓ aPTT, s	2 (8.3)	24.6 (0.8)	24–25.1	5 (15.2)	25 (0.7)	24.2–25.9	≤0.687

A2M, alpha2-macroglobulin; ACE, angiotensin-converting enzyme; aPTT, activated partial thromboplastin time; AT III, antithrombin III; C1-INH, C1 inhibitor; HAE-FXII, hereditary angioedema (HAE) with normal C1-INH and HAE-specific mutation in the *F12* gene; HAE-unknown, HAE with normal C1-INH and without HAE-specific mutation in the *F12* gene; PAI-1, plasminogen activator inhibitor-1.

Reference (normal) ranges: C1-INH activity, 70–130%; C1-INH protein, 15.4–33.8 mg/dl; C4, 16.4–31.3 mg/dl; C1q, 10–25 mg/dl; A2M, 1.3–3.0 g/l; AT III, 80–120%; ACE, 12–68 U/l; factor XI activity, 70–120%; factor XII activity, 70–150%; PAI-1, 1–7 U/ml; and aPTT, 26–36 s. When tests were performed over multiple lines, the *P*-value was displayed in the last line involved. Entries of the previous lines were left empty.

decreased C1-INH activity. This has been observed before in HAE-FXII (9, 25, 27), but not in HAE-unknown. The increased activity of coagulation factors XI and XII in some of the patients could indicate an increased activation of coagulation. The decrease of PAI-1 in some patients with HAE-FXII and HAE-unknown, on the other hand, which has also been reported in HAE-C1-INH patients and is therefore not specific for HAE-C1-INH (28), could be an indication for the activation of the fibrinolytic system (29). Overall, HAE-FXII and HAE-unknown did not significantly differ in laboratory parameters. Also the clustering of pathological results did not reveal a clearly different pattern between patients with HAE-FXII and HAE-unknown. The above-discussed HAE-FXII-specific mutation of the *F12* gene, which is not present in HAE-unknown, however, suggests differences in the pathomechanisms of HAE-FXII and HAE-unknown.

Limitations of the study

The present study has the following limitations: First, and most importantly, the basic genetic defect of HAE-unknown is still unknown. Therefore, it cannot be excluded that HAE-unknown is not one entity but represents a number of different genotypes with different mutations and differences in

pathomechanisms. Second, whereas in sporadic patients with HAE-FXII inheritance can be confirmed by *F12* gene analysis (11, 13, 27), this is at present not possible for sporadic patients with HAE-unknown because the basic genetic defect is not known and asymptomatic mutation carriers in these families cannot be identified. Third, the partly retrospective nature of the analysis may introduce bias, resulting from the review of family history and medical records but also the accuracy of the physicians' and patients' memories when they were asked to recall frequency, intensity, and distribution of their angioedema.

Conclusions

Given the mentioned limitations, our results show that, besides the presence or absence of a *F12* gene mutation, HAE-FXII and HAE-unknown have similar as well as different features. Both HAE-FXII and HAE-unknown are inherited as autosomal-dominant traits with incomplete penetrance, affecting mainly women and significantly less men. However, HAE-FXII and HAE-unknown differ in various important features including prevalence, gender distribution, transmission of disease, age at onset of clinical symptoms, affected organs, and the impact of estrogen on disease severity.

Author contributions

KB designed the study, performed data acquisition and analysis, and wrote the manuscript. KW designed the study, interpreted and analyzed the data, and drafted the manuscript. GW performed data analysis and interpretation and drafted the manuscript. JH contributed to conception and design of the study and performed data analysis and interpretation. All authors contributed to drafting the article and revising the manuscript and approved the final version.

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Conflicts of interest

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