

ORIGINAL ARTICLE

Epidemiology and Genetics

Hereditary angioedema with a mutation in the plasminogen gene

K. Bork¹  | K. Wulff² | L. Steinmüller-Magin³ | I. Brænne⁴ | P. Staubach-Renz¹ | G. Witzke¹ | J. Hardt⁵

¹Department of Dermatology, Johannes Gutenberg University, Mainz, Germany

²University Medicine, Ernst Moritz Arndt University, Greifswald, Germany

³Institute of Laboratory Medicine and Human Genetics, Singen, Germany

⁴Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA

⁵Department of Medical Psychology and Medical Sociology, Johannes Gutenberg University, Mainz, Germany

Correspondence

Konrad Bork, Department of Dermatology, Johannes Gutenberg University, Mainz, Germany.

Email: bork@hautklinik.klinik.uni-mainz.de

Edited by: Werner Aberer

Abstract

Background: Hereditary angioedema (HAE) with normal C1-INH (HAEnCI) may be linked to specific mutations in the coagulation *factor 12* (FXII) gene (HAE-FXII) or functional mutations in other genes that are still unknown. We sought to identify and characterize a hitherto unknown type of HAE with normal C1-INH and without mutation in the *F12* gene.

Methods: The study comprised analysis of whole-exome sequencing, Sanger sequencing, and clinical data of patients.

Results: We detected a mutation in the plasminogen (PLG) gene in patients with HAEnCI. The mutation c.9886A>G was located in exon 9 leading to the missense mutation p.Lys330Glu (K330E) in the kringle 3 domain of the PLG protein. The mutation was identified by next-generation sequencing in 14 patients with HAEnCI belonging to 4 of 7 families. Family studies revealed that this type of HAE was transmitted as an autosomal dominant trait. The PLG gene mutation was present in all studied symptomatic patients and was also found in 9 of 38 index patients from 38 further families with HAEnCI. Most patients had swelling of face/lips (78.3%) and tongue (78.3%). A total of 331 of all 3.795 tongue swellings (8.7%) were associated with dyspnea, voice changes, and imminent asphyxiation. Two women died by asphyxiation due to a tongue swelling.

Conclusions: Hereditary angioedema with a mutation in the PLG gene is a novel type of HAE. It is associated with a high risk of tongue swellings.

KEYWORDS

bradykinin, hereditary angioedema, mutation in the plasminogen gene, plasminogen, whole-exome sequencing

1 | INTRODUCTION

Hereditary angioedema (HAE) includes HAE due to C1 inhibitor (C1-INH) deficiency (HAE-C1-INH), HAE with a specific mutation in the factor XII gene (HAE-FXII), and HAE with an unknown genetic background.^{1,2} HAE-C1-INH is caused by mutations in the gene encoding C1-INH (*SERPINC1*) resulting in low plasma activity of C1-INH. The other types of HAE are associated with normal level and function of

C1-INH in plasma (HAEnCI). In some of the families with HAEnCI, a specific mutation in the *F12* gene was detected in symptomatic patients.³ In most families with HAEnCI, however, the genetic background is still unknown (HAE-unknown).³⁻⁶ In a previous study, we found that this was true for 73.9% of families with HAEnCI.⁵ Therefore, we investigated patients with HAE-unknown to identify novel gene aberrations linked with the expression of HAE. For identifying potential disease-causing gene variants, we used next-generation

sequencing of the whole exome combined with family studies. Hereby we found a mutation in the plasminogen (PLG) gene, which was previously unknown to be disease-causing and cosegregated with clinical symptoms in patients as an autosomal dominant trait. These findings suggest a novel type of HAE characterized by normal C1-INH function and level, absence of a specific *F12* gene mutation, and presence of the novel PLG gene mutation (HAE-PLG).

2 | METHODS

The study comprises analysis of whole-exome sequencing (WES), Sanger sequencing, and clinical and biochemical data of patients.

2.1 | Probands

Probands included patients, symptom-free family members, and healthy controls. Study patients with HAE were selected by the following criteria: (i) Patients had recurrent swellings of skin, abdominal pain attacks, tongue swellings, or laryngeal attacks in the absence of urticaria; (ii) patients had normal C1-INH function and concentration in plasma; (iii) patients had no HAE-specific *F12* gene mutations comprising the missense mutations p.Thr328Lys and p.Thr328Arg, the large deletion c.971_1018 + 24del 72, and the duplication c.892_909dup; (iv) patients came from families with 2 or more symptomatic patients with HAE; and (v) within the families, the patients came from two or more generations. All patients were surveyed in the Angioedema Outpatient Service at the Johannes Gutenberg University, Mainz, Germany (AOS). Twenty-two subjects (20 patients and two healthy persons) were investigated by WES and Sanger sequencing (sample A). Additionally, 13 relatives of 4 families and 38 index patients of 38 unrelated families (sample B) were examined by Sanger sequencing. One hundred and five healthy individuals (210 alleles), comprising 53 males and 52 females, were sequenced for the presence of the novel PLG gene mutation as controls.

2.2 | Collection of clinical data

Clinical data were collected from the patients' medical history and from clinical examinations as well as follow-up records. The study was conducted in the AOS. All subjects analyzed in this study gave written informed consent prior to participation. The local ethics committee approved the study.

2.3 | Whole-exome sequencing

EDTA blood was collected from all study participants. All samples were stored at -80°C. DNA was extracted from whole blood according to standard protocols (Qiagen, Hilden, Germany). Exome sequencing was performed as previously described.⁷ Briefly, read alignment was performed with BWA (v. 0.5.8) and the human genome assembly hg19 (GRCh37) was used as reference. Single

nucleotide variants and small insertions and deletions (indels) were detected using SAMtools (v 0.1.7) using default parameters. Furthermore, we required putative single nucleotide variants to fulfill the following criteria: (i) median base quality of the variant bases of at least 15; (ii) a minimum of 15% of reads showing the variant base; and (iii) the variant base is indicated by at least 5% of reads coming from different strands. Variant calling was performed with ANNOVAR.⁸ Further annotation was based on several databases provided by ANNOVAR such as UCSC, dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>), Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), 1000 Genomes (<http://www.internationalgenome.org/>), and the Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>). In addition, we also annotated several functional prediction scores such as SIFT,⁹ CADD,¹⁰ PolyPhen-2,¹¹ and MutationTaster.¹²

2.4 | Haplotype analysis

After the PLG gene mutation was identified by WES analysis, a number of intragenic single nucleotide polymorphisms (SNPs) were used for haplotype analysis. We selected four SNPs flanking this mutation: rs14224 (c.771 T/C), in codon 257; rs1130656 (c.924 C/T), in codon 314; rs13231 (c.1083 A/G), in codon 361; and rs4252125 (c.1414 G/A), in codon 472. The four SNPs occur in frequencies of 0.30–0.50 in the European population (Single Nucleotide Polymorphism Database; <https://www.ncbi.nlm.nih.gov/snp>).

2.5 | Sanger sequencing

Variant validation of the WES results was performed using PCR and Sanger sequencing. Primers (Biomers, Ulm, Germany) that were used for validation are as follows:

PLG-9-1 (forward) CTT AGT TTT AGT TAC TGT AGG AAC GCA GG and PLG-9-2 (reverse) CAG GCT TTC TGA CCA CAA TAG C. PCR was carried out in 25 µL volume containing about 40 ng genomic DNA, 0.5 µL of each primer, and 12.5 µL AmpliTaq Gold Master 360 Mix (ThermoFisher Scientific, Darmstadt, Germany). Samples were proceeded in an Applied Biosystems Veriti thermal cycler (ThermoFisher Scientific) with a standard PCR program (62°C annealing temperature, 40 cycles). PCR products were purified with the Rapid PCR Cleanup Enzyme set (New England Biolabs, Frankfurt, Germany) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (ThermoFisher Scientific). Electrophoresis of sequenced products was performed on an Applied Biosystems 3500Dx Genetic Analyzer (ThermoFisher Scientific).

The presence of HAE-specific *F12* gene mutations and of mutations of the *SERPING1* gene was tested as described elsewhere.^{13,14}

2.6 | Biochemical analysis

Plasma from patients and unaffected family members was separated by means of centrifugation of freshly collected citrated blood at 1,500 g for 10 minutes at 4°C. All samples were placed in aliquots

and stored at -80°C . Samples were collected during symptom-free intervals between attacks. Function and protein levels of C1-INH and C4 were measured as described earlier.⁵ Plasminogen activity was determined using a chromogenic assay: PLG is activated through reaction with an excess of streptokinase in the presence of fibrinogen. The PLG-streptokinase complex is determined by the rate of hydrolysis of the chromogenic substrate pyroGlu-Phe-Lys-pNA (HemosIL Plasminogen, Instrumentation Laboratory (IL), Bedford, MA, USA).

3 | RESULTS

3.1 | Evidence of a disease-causing mutation in the plasminogen gene in 4 families with HAE with normal C1-INH and without specific mutations in the F12 gene (sample A)

By WES, the mutation hg19.chr6:161139762A>G; NM_000301:exon9:c.A988G:p.K330E in the plasminogen gene was identified in 14 patients belonging to 4 of the 7 (57%) families (families A to D) (Figure 1). The mutation was located in exon 9 leading to the missense mutation p.Lys330Glu (K330E) in the kringle 3 domain of the PLG protein (Figure 2). In two healthy persons from families A and B, this mutation was not found. Besides the families A to D (Figure 1), three further families with symptomatic patients were included in the WES study. The PLG gene mutation described here or any other rare mutation in the PLG gene was not present in the WES analysis in any of 6 examined patients.

3.2 | Pedigree analysis and Sanger sequencing in 4 families with the PLG gene mutation

The mutation found by WES was confirmed by Sanger sequencing. Furthermore, the presence of the PLG gene mutation was determined by Sanger sequencing in 17 additional family members (13 of them were relatives) solely out of families A to D. The mutation was found in 6/13 relatives, 4/6 were symptomatic, and 2/6 were asymptomatic (Figure 3). In total, 20 members of the 4 families carried the PLG gene mutation, 18/20 were symptomatic, and 2/20 were asymptomatic. In family A (Figure 1), there were two asymptomatic carriers of the PLG gene mutation (III:7 and IV:3). One of them (IV:3) was an obligate carrier. The existence of symptom-free mutation carriers documented an incomplete penetrance of the mutation. In families B, C, and D, all investigated carriers of the PLG gene mutation were symptomatic patients. In all PLG gene mutation carriers, the genotype was heterozygous.

The pedigrees (Figure 1) reveal that the clinical HAE symptoms occurred in 2, 3, or 4 generations, respectively, of the 4 families and that they were transmitted as an autosomal dominant trait. The PLG gene mutation was found in all 18 analyzed symptomatic patients in families A to D and segregated in agreement with the clinical symptoms. There was no symptomatic patient who did not carry the mutation.

According to the pedigrees in Figure 1, there were a total of 32 patients with clinical symptoms of HAE-PLG. Twenty-five (78%) of them were females and 7 (22%) males. The gender-specific rate of females to males is 3.6–1 in those 4 families. In family D, all identified patients were females.

3.3 | Haplotype analysis: common founder effect

In families A to D, haplotypes linked to the mutant c.9886G allele and wild-type c.9886A allele were determined (Figure 1). The same haplotype was present in all mutant c.9886G alleles in all 14 symptomatic patients of the 4 unrelated families. Hence, a founder effect, that is, an identical origin of the c.9886A>G mutation in PLG gene, is likely.

3.4 | PLG gene mutation in further families (sample B)

To cross-validate the results found in the 7 families analyzed by WES and Sanger sequencing, we determined the presence of the described PLG gene mutation in 38 index patients of 38 different families with HAE_{EnCI} without HAE-specific F12 gene mutations using Sanger sequence analysis. The PLG gene mutation was found in 9/38 index patients (37.5%). Summing up over samples A and B, the mutation was found in 27 symptomatic patients and 2 asymptomatic individuals coming from 13/45 (28.9%) families. The PLG gene mutation K330E was not present in the 105 control subjects (210 independent chromosomes).

3.5 | Prediction scores

The variant is predicted to be benign and tolerated based on SIFT,⁹ CADD,¹⁰ PolyPhen-2,¹¹ and MutationTaster.¹²

3.6 | Clinical symptoms

Clinical data could be obtained from 60 symptomatic patients coming from 13 families with the reported PLG gene mutation and are summarized in Table 1. Forty-seven of 60 patients had recurrent skin and 47/60 tongue swellings (Figure 4). Most skin swellings affected face and lips. Compared to a series of 61 patients with HAE-FXII,⁵ significantly more patients with HAE-PLG had tongue swellings and significantly less patients had facial swellings, swellings of the extremities, and abdominal attacks (Table 2). Forty-seven of 60 patients had a total of 3.795 tongue swelling, on average 80.7 tongue swellings per patient (SD 121; range: 1 to 626). Eleven of 60 patients had exclusively recurrent tongue swellings (range: 1 to 150) and no other clinical signs. Three hundred and thirty-one of all tongue swellings (8.7%) in 23 patients were associated with dyspnea, voice changes, and imminent asphyxiation. Two women died by asphyxiation due to a tongue swelling with upper airway obstruction, one woman at age 36 (III:3 of family A) and another woman at age 47 (IV:3 of family C). The latter woman had 160 tongue swellings

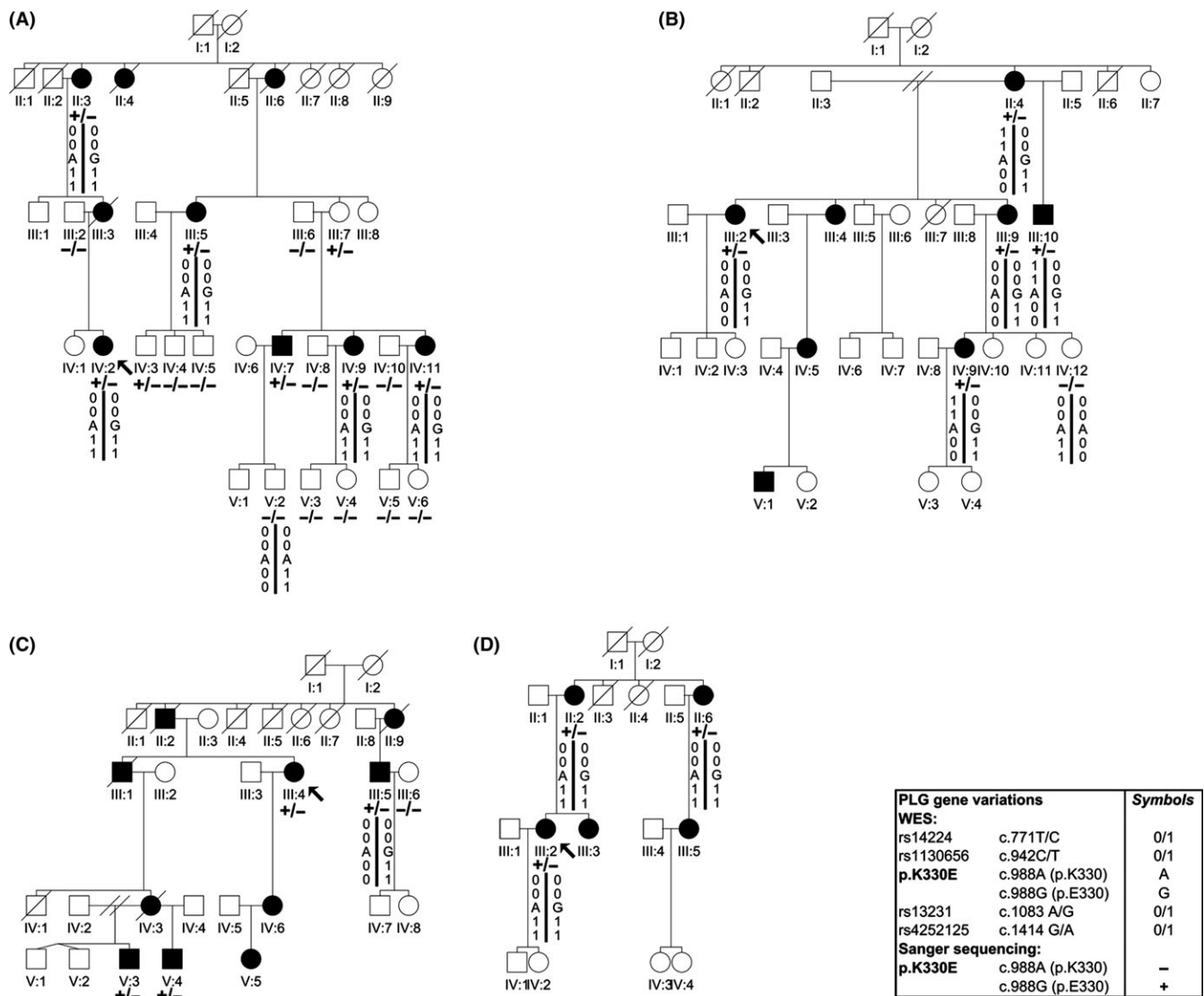


FIGURE 1 Pedigrees of 4 families A-D, with HAE-PLG. Results of whole-exome sequencing and Sanger sequence analysis and haplotypes linked with the mutation c.988A>G are presented. Circle = female. Square = male. Symbols with a slash indicate deceased family members. Filled symbols = individuals affected by recurrent angioedema. Empty symbols = symptom-free subjects

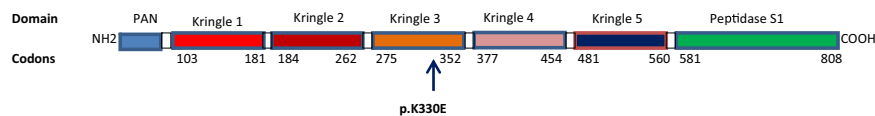


FIGURE 2 Schematic presentation of the plasminogen protein domains with numbering of the amino acids and localization of the here described plasminogen gene mutation in the kringle 3 domain (UniProtKB: P00747)

before the fatal upper airway obstruction. One woman with a severe tongue swelling needing intubation was published in 2012.¹⁵ This patient now turned out to carry the PLG gene mutation. An erythema marginatum was not reported by any of the patients.

3.7 | Estrogen impact

In 6/43 (14%) women, the first clinical symptoms started after beginning with oral contraceptives. Thirty-one women with HAE-PLG had

a total of 73 pregnancies. An onset or worsening of disease during pregnancy was not reported.

3.8 | Treatment

Nine patients were treated 25 times in an intensive care unit for attacks of upper airway obstruction. Intubation was required in four patients and cricothyrotomy in two patients. One woman received 30 mg Icatibant (bradykinin-2 receptor antagonist) subcutaneously

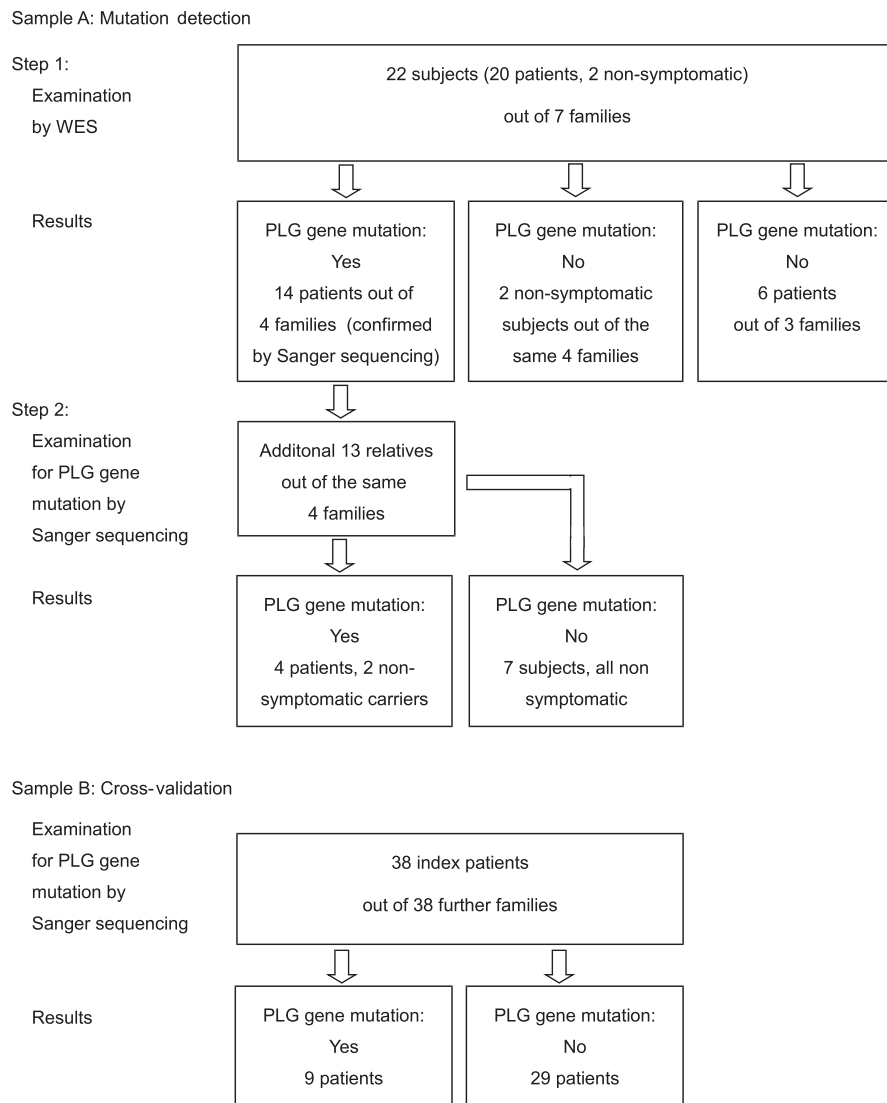


FIGURE 3 Flowchart of plasminogen gene mutation analysis in 20 patients and 2 healthy persons (sample A) by whole-exome sequencing and in 13 relatives and 38 index patients of further families by Sanger sequencing (samples A and B)

TABLE 1 Clinical data of patients with HAE-PLG

Description	Data
Number of families with HAE-PLG patients	13
Number of patients with HAE-PLG	60
Male/female patients	13/47
Mean age at onset of clinical symptoms [y]	30.5 (SD 15.5; range: 5 to 72)
Mean duration of period of angioedema attacks [y]	20.6 (SD 16.8; range: 1 to 72)

for 33 abdominal attacks and 9 skin swellings. She reported a shortening of attacks to 20% compared to untreated attacks. Another woman received Icatibant for 32 tongue swellings. On average, symptom relief started after 10 minutes and the duration of treated tongue swellings was shortened to 50% in the mean compared to

untreated tongue swellings. Three patients were successfully treated for 17 abdominal attacks with plasma-derived C1-INH concentrate. One patient received C1-INH concentrate for 3 tongue swelling with lacking efficacy. Seven patients received low-dose short-time antihistamines combined with corticosteroids for a total of 151 tongue swellings and 137 facial swellings. This treatment was not effective. Two patients received 3 g tranexamic acid (TXA) per day orally for long-term prophylaxis, one man for 12 years and one woman for 4 years. Both were completely symptom-free under this treatment.

3.9 | Biochemical results

In 29 patients, mean C1-INH activity was 91% (reference range: 70%-130%) (SD 16.9%; range: 63% to 125%), C1-INH protein 26.8 mg/dL (reference range: 17-44 mg/dL) (SD 6; range: 15.4 to 43.4 mg/dL), and C4 25.7 mg/dL (reference range: 10-40 mg/dL)

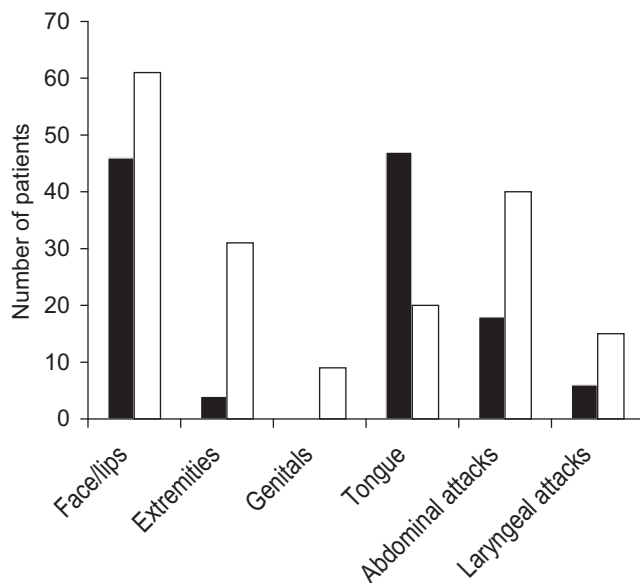


FIGURE 4 Clinical symptoms in 60 patients with HAE-PLG (filled bars) compared to a historical series of 61 patients with HAE-FXII⁵ (empty bars)

(SD 8.4; range: 12.2 to 43.4 mg/dL). Plasminogen activities in three women were 131%, 96%, and 70% (reference range: 70%-130%).

4 | DISCUSSION

Hereditary angioedema with normal C1-INH was first described in 2000.^{16,17} In approximately 30% of patients, it is associated with mutations in the *F12* gene (HAE-FXII).^{3,5,6} Here, we report that HAE-PLG can be associated also with a mutation in the *PLG* gene. We identified a genetic variant in the *PLG* gene in patients with HAE-PLG. By whole-exome analysis the mutation was found in four families and by Sanger sequencing in further members of those families as well as in 9 index patients of further families. The mutation was found in a heterozygous genotype, was transmitted as an autosomal dominant trait, and cosegregated with clinical symptoms in patients. K330E is extremely rare (not found in dbSNP, ExAC, or 1000 Genomes; in gnomAD (<http://gnomad.broadinstitute.org/>) variant frequency is 1:31 591 in the European population). Comparing the frequency of K330E in HAE-PLG patients with frequency in public databases and 105 unrelated controls, we assume that the variant K330E is disease-caus-

Affected organs	HAE-PLG		HAE-FXII		Difference between groups* Fisher's exact test
	Number of patients** (%)	Number of attacks (%)	Number of patients** (%)	Number of attacks (%)	
Total	60 (100)	11 552 (100)	61 (100)	9969 (100)	No test
Skin					
Skin (total)	47 (78.3)	5373 (46.5)	61 (100)	5846 (58.6)	Pat: $P = .426$ Att: $P < .001$
Face	46 (76.7)	5032 (19.3)	61 (100)	2231 (22.4)	Pat: $P = .353$ Att: $P < .001$
Hands/arms	4 (6.7)	172 (1.5)	30 (49.2)	2258 (22.7)	Pat: $P = <.001$ Att: $P < .001$
Feet/legs	2 (3.3)	169 (1.5)	31 (50.8)	1325 (13.3)	Pat: $P = <.001$ Att: $P < .001$
Genitals	0 (0)	0 (0)	9 (14.8)	122 (1.2)	Pat: $P = .004$ Att: $P < .001$
Other organs					
Tongue (total)	47 (78.3)	3795 (32.8)	20 (32.8)	86 (0.9)	Pat: $P = .009$ Att: $P < .001$
Abdomen (total)	18 (30)	2343 (20.3)	40 (65.6)	3947 (39.6)	Pat: $P = .024$ Att: $P < .001$
Larynx (total)	6 (10)	41 (0.3)	15 (24.6)	90 (0.9)	Pat: $P = .098$ Att: $P = .027$

HAE-PLG, hereditary angioedema (HAE) with normal C1 inhibitor and mutation in the plasminogen gene; HAE-FXII, HAE with normal C1-INH with HAE-specific mutation in the *F12* gene.

*All tests on the per-patient and per-attack basis.

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

TABLE 2 Sites and numbers of attacks during the course of disease for 60 patients with HAE-PLG compared to a historical series of 61 patients with HAE-FXII⁵

ing for HAE-PLG despite benign *in silico* prediction. Our findings indicate a novel type of HAE-PLG which is linked to the described mutation in the PLG gene. The HAE type is termed HAE with normal C1-INH and a mutation in the PLG gene (HAE-PLG).

The mutation reported here leads to an amino acid substitution of lysin by glutamic acid in position 330 located in the kringle 3 domain in PLG. The kringle domains facilitate PLG binding to large surfaces, for example, fibrin, bacterial proteins, cell surfaces, and small-molecule ligands, and have a high influence on the arrangement and function of PLG protein.^{18,19}

Functional mutations in the PLG gene may lead to the phenotype of PLG deficiency (PLGD). PLG deficiency has been classified as hypoplasminogenemia or "true" PLGD type I and as dysplasminogenemia or PLGD type II.²⁰ Hypoplasminogenemia is autosomal recessive inherited and is associated with ligneous lesions on mucous membranes, most commonly ligneous conjunctivitis. Dysplasminogenemia has a normal or slightly reduced antigen and a strongly reduced PLG activity. In dysplasminogenemia, no specific clinical manifestation was reported.²¹ Angioedema was not mentioned in patients with PLGD type I or II. Here, we describe a mutation in the PLG gene, which is linked to a novel phenotype. The normal PLG activity reported here in 3 symptomatic patients with HAE-PLG seems to indicate that the PLG gene mutation has no influence on the PLG activity in blood plasma.

Plasminogen is a circulating zymogen that is converted to the active enzyme plasmin by cleavage of a peptide bond. Plasmin is the main enzyme of fibrinolysis. Fibrinolysis including plasmin seems to be involved in the pathogenesis of HAE-C1-INH and of HAE-FXII. This can be concluded from the effectiveness of the antifibrinolytic agent TXA. Tranexamic acid is a competitive inhibitor of the lysine-binding sites in the kringle domains of PLG and plasmin. It is effective in various degrees in the long-term prophylaxis in HAE-C1-INH,²²⁻²⁴ in HAE-FXII,²⁵ and in HAE-PLG as mentioned in this article. The exact mode of action of antifibrinolytics in HAE, however, is not fully understood. It is known that an activation of the fibrinolytic system and the generation of plasmin, for example, by recombinant tissue PLG activator can lead to the formation of angioedema of the tongue.^{26,27} In HAE-PLG with and without *F12* gene mutations, low levels of PLG activator inhibitors 1 and 2 have been found, indicating a consumption by PLG activators (possibly tissue PLG activator (tPA) and urokinase PLG activator (uPA)) and underscoring the role of fibrinolysis.²⁸

In 2006, we identified two missense mutations, T328K, T328R, and later the deletion c.971_1018 + 24del72 in the *F12* gene, all coding for the proline-rich region of FXII protein.^{3,29} Recently, it was reported that the three mutant FXII proteins had new sites sensitive to enzymatic cleavage by plasmin.³⁰ The atypical cleavage of the mutant FXII by plasmin could lead to an increased rate of FXIIa formation, kallikrein-kinin system (KKS) activation, and bradykinin production that is not properly controlled by C1-INH.

In contrast to HAE-FXII, in HAE-PLG the sensitivity of FXII for plasmin can be expected to be normal. In HAE-PLG, the PLG is

changed because of one mutation in the kringle 3 domain. The gene mutation might lead to a structural change of the molecule that potentially modifies the function of the mutant PLG. It may show a higher affinity for surfaces and/or may be more accessible for activators such as tPA and uPA. The consequence might be an increased activation of the fibrinolytic system with subsequent formation of plasmin, activation of KKS, and increased production of bradykinin. Furthermore, we could speculate that the amino acid substitution (K330E) could lead to introduction of a new site in FXII protein leading to enhanced FXII activation, with consequences as described for HAE-FXII.³⁰

As reported here, the bradykinin-2 receptor antagonist was effective in a high number of attacks in two patients with HAE-PLG. This provides evidence that bradykinin is produced in acute attacks and may be the main mediator of edema in HAE-PLG.

The high percentage of tongue swellings in HAE-PLG patients has not been reported for HAE-C1-INH and HAE-FXII.^{5,31} The severity and outcome of the tongue swellings including ICU treatments as well as emergency measures and the two asphyxiation cases emphasize the clinical importance of HAE-PLG.

The number of women affected with HAE-PLG was more than three times higher than males. The percentage of affected men, however, was higher in HAE-PLG than in HAE-FXII.⁵ Onset or worsening of clinical symptoms after intake of oral contraceptives or during pregnancies is lower than in HAE-FXII.⁵ Thus, estrogens seem to play a less important role in HAE-PLG than in HAE-FXII.

Limitations of this study are as follows: (i) Until now, we have no clear information on the pathomechanism of HAE-PLG. To assess the effect of the PLG gene mutation K330E on gene function or organism phenotype, appropriately designed functional studies or mutagenesis experiments will be required. (ii) The exact level of penetrance could not be determined because the carrier status could not be investigated in all relatives of patients.

The identification of HAE-PLG shows that HAE-PLG comprises at least three different types of HAE: HAE-FXII, HAE-PLG, and HAE with a genetic background still unknown. Up to now, there is no specific plasma test available to identify the different types of HAE-PLG. HAE-FXII and now also HAE-PLG can be diagnosed by genotyping. Diagnosing HAE-PLG is important because patients have a high risk of tongue swellings including tongue swellings with partial or, rarely, even complete upper airway obstruction. Furthermore, it is important to determine the risk of developing HAE-PLG also in asymptomatic members of families with HAE-PLG because the symptoms of HAE-PLG may start nearly at any age. The fact that patients with HAE-PLG can now be identified by determining the basic genetic variant and thus be differentiated from other types of HAE enables us to study the biochemical aspects of the pathophysiology of HAE-PLG and also to develop the urgently needed treatments for this group of patients including studies with drugs effective in other form of bradykinin-mediated angioedema.

ADDENDUM

After submission of our manuscript, an abstract was published reporting a series of 29 unrelated patients with idiopathic angioedema or HAE-unknown. Two patients showed the PLG mutation reported here.³² Furthermore, an Italian family with HAE and a mutation in the angiotensinogen gene was reported.³³

ACKNOWLEDGMENTS

We are extremely grateful to all the families who took part in this study.

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. LS and IB contributed to conception and design of the study and data acquisition and interpretation. PS, GW, and JH were involved in data collection and analysis and drafted the manuscript. All authors contributed to drafting the article, revised the manuscript critically for important intellectual content, and approved the final version.

CONFLICTS OF INTEREST

Dr. Bork reports speaker fees from CSL Behring and Shire, outside the submitted work. The other authors have no conflict of interests to disclose.

ORCID

K. Bork  <http://orcid.org/0000-0002-6084-4577>

REFERENCES

1. Cicardi M, Bork K, Caballero T, et al. Evidence-based recommendations for the therapeutic management of angioedema owing to hereditary C1 inhibitor deficiency: consensus report of an International Working Group. *Allergy*. 2012;67:147-157.
2. Zuraw BL, Christiansen SC. HAE pathophysiology and underlying mechanisms. *Clin Rev Allergy Immunol*. 2016;51:216-229.
3. 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;343:1286-1289.
4. Vitrat-Hinckley V, Gompel A, Dumestre-Perard C, et al. Type III hereditary angio-oedema: clinical and biological features in a French cohort. *Allergy*. 2010;65:1331-1336.
5. Bork K, Wulff K, Witzke G, Hardt J. Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations. *Allergy*. 2015;70:1004-1012.
6. Bouillet L, Boccon-Gibod I, Gompel A, et al. Hereditary angioedema with normal C1 inhibitor: clinical characteristics and treatment response with plasma-derived human C1 inhibitor concentrate (Berinert®) in a French cohort. *Eur J Dermatol*. 2017;27:155-159.
7. Braenne I, Kleiwecke M, Reiz B, et al. Systematic analysis of variants related to familial hypercholesterolemia in families with premature myocardial infarction. *Eur J Hum Genet*. 2016;24:191-197.
8. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38:e164.
9. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4:1073-1081.
10. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;46:310-315.
11. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248-249.
12. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7:575-576.
13. 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. *Haemophilia*. 2014;20:e372-e375.
14. Gösswein T, Kocot A, Emmert G, et al. Mutational spectrum of the C1INH (SERPING1) gene in patients with hereditary angioedema. *Cytogenet Genome Res*. 2008;121:181-188.
15. Dehne MG, Zimmer M, Deisz R, Bork K. Angioedema caused by C1-esterase inhibitor deficiency or ACE inhibitors? *Anaesthesist*. 2007;56:335-336. 338.
16. Bork K, Barnstedt SE, Koch P, Traupe H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet*. 2000;356:213-217.
17. Binkley KE, Davis 3rd A. Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema. *J Allergy Clin Immunol*. 2000;106:546-550.
18. Petersen TE, Martzen MR, Ichinose A, Davie EW. Characterization of the gene for human plasminogen, a key proenzyme in the fibrinolytic system. *J Biol Chem*. 1990;265:6104-6111.
19. Castellino FJ, Ploplis VA. Structure and function of the plasminogen/plasmin system. *Thromb Haemost*. 2005;93:647-654.
20. Tefs K, Gueorgieva M, Klammt J, et al. Molecular and clinical spectrum of type I plasminogen deficiency: a series of 50 patients. *Blood*. 2006;108:3021-3026.
21. Schuster V, Hügler B, Tefs K. Plasminogen deficiency. *J Thromb Haemost*. 2007;5:2315-2322.
22. Sheffer AL, Austen KF, Rosen FS. Tranexamic acid therapy in hereditary angioneurotic edema. *N Engl J Med*. 1972;287:452-454.
23. Zanichelli A, Vacchini R, Badini M, Penna V, Cicardi M. Standard care impact on angioedema because of hereditary C1 inhibitor deficiency: a 21-month prospective study in a cohort of 103 patients. *Allergy*. 2011;66:192-196.
24. Wintemberger C, Boccon-Gibod I, Launay D, et al. Tranexamic acid as maintenance treatment for non-histaminergic angioedema: analysis of efficacy and safety in 37 patients. *Clin Exp Immunol*. 2014;78:112-117.
25. 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;72:320-324.
26. Bozkurt S, Arslan ED, Köse A, Ayrik C, Yılmaz A, Dündar GA. Lingual angioedema after alteplase treatment in a patient with acute ischemic stroke. *World J Emerg Med*. 2015;6:74-76.
27. Hill MD, Barber PA, Takahashi J, Demchuk AM, Feasby TE, Buchan AM. Anaphylactoid reactions and angioedema during alteplase treatment of acute ischemic stroke. *CMAJ*. 2000;162:1281-1284.
28. 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;137:1822-1829.
29. 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;141:31-35.
30. de Maat S, Björkqvist J, Suffritti C, et al. Plasmin is a natural trigger for bradykinin production in patients with hereditary angioedema with factor XII mutations. *J Allergy Clin Immunol*. 2016;138:1414-1423.
31. Bork K, Meng G, Staubach P, Hardt J. Hereditary angioedema: new findings concerning symptoms, affected organs, and course. *Am J Med*. 2006;119:267-274.
32. Zamanakou M, Loules G, Csuka D, et al. Genotyping unknown hereditary angioedema (U-HAE) and idiopathic non-histaminergic acquired angioedema (InH-AAE): preliminary evidence of pathogenic variants. *Allergy Asthma Clin Immunol*. 2017;13(Suppl 2):29.
33. Bafunno V, Firinu D, D'Apolito M, et al. Mutation of angiopoietin-1 gene associates with a new type of hereditary angioedema. *J Allergy Clin Immunol*. 2017; pii: S0091-6749(17)30921-1. doi: 10.1016/j.jaci.2017.05.020. [Epub ahead of print]

How to cite this article: Bork K, Wulff K, Steinmüller-Magin L, et al. Hereditary angioedema with a mutation in the plasminogen gene. *Allergy*. 2017;00:1–9. <https://doi.org/10.1111/all.13270>