

Received Date : 07-Mar-2014

Revised Date : 22-Jul-2014

Accepted Date : 12-Aug-2014

Article type : Original Article: Epidemiology and Genetics

Editor : Thomas Bieber

Hereditary angioedema with *F12* mutation: factors modifying the clinical phenotype

Short title: Factors modifying hereditary angioedema phenotype

Delphine Charignon,^{1,2} Arije Ghannam,^{1,2} Federica Defendi,^{1,2} Denise Ponard,¹ Nicole Monnier,¹
Margarita López Trascasa,^{3,4} David Launay,^{1,5} Teresa Caballero,^{3,4} Kamel Djenouhat,⁶ Olivier Fain,^{1,7}
Sven Cichon,^{8,9} Ludovic Martin,^{1,10*} and Christian Drouet,^{1,2 **}

¹ Centre de Référence des Angioedèmes CREAK, France

² Université Joseph Fourier, GREPI/AGIM CNRS FRE 3405, Grenoble, France

³ Hospital Universitario La Paz, Institute for Health Research (IdiPaz), Madrid, Spain

⁴ Biomedical Research Network on Rare Diseases-U754 (CIBERER), Madrid, Spain

⁵ Université du Droit et de la Santé Lille 2, Département de Médecine Interne, Lille, France

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.12515

This article is protected by copyright. All rights reserved.

⁶ Institut Pasteur, Département d'Immunologie, Alger, Algeria

⁷ Université Paris XIII, Département de Médecine Interne, Bondy, France

⁸ Universität Bonn, Institute für Humangenetics, Bonn, Germany

⁹ Universität Basel, Departement Biomedizin, Basel, Switzerland

¹⁰ L'UNAM Université, Département de Dermatologie, Hôpital d'Angers, Angers, France

* These authors each contributed equally to this work.

[†]Correspondence to: Christian Drouet, Centre de Référence des Angioedèmes, CHU Grenoble & EFS Rhône-Alpes, BP 35, F-38701 La Tronche. E-mail: christian.drouet@ujf-grenoble.fr;

Abstract:

Background: Hereditary angioedema (HAE) with normal C1 inhibitor (C1Inh) associated with the c.983C>A and c.983C>G mutations of the *F12* gene (HAE-FXII) is a rare condition, and presents with highly variable clinical expression. Based on data gathered from a large carrier cohort, we assessed the modifiers affecting the clinical phenotype.

Methods: We analyzed clinical and biological data recorded from 118 mutation carriers (80 symptomatic and 38 asymptomatic), 58 non-carrier relatives from 40 families, and 200 healthy donors. Disease severity was scored in relation to frequency and location of edema, as well as age at disease onset. In order to predict HAE-FXII disease severity, we analyzed the biological phenotype

(C1Inh, C4, spontaneous amidase, angiotensin-I converting enzyme [ACE], aminopeptidase P [APP], and carboxypeptidase N/M [CPN]) by means of logistic regression (Akaike information criterion) and odds ratio (OR).

Results: Meaningful variables contributed to HAE-FXII, with the kinin catabolism enzymes ACE and CPN exhibiting a significant inverse relationship with disease severity (OR=0.36, 95% CI 0.23-0.59, $P<0.001$; OR=0.58, 95% CI 0.36-0.91, $P<0.05$, respectively). CPN activities was 37.5 [28.5–41.3] nmol•mL⁻¹•min⁻¹ and 38.5 [32.8–45.6] for HAE-FXII asymptomatic and symptomatic carriers respectively and 37.9 [30.5–43.7] nmol•mL⁻¹•min⁻¹ for non-carriers. ACE activities was 58 [44–76] and 49 [35–59] nmol•mL⁻¹•min⁻¹ for HAE-FXII asymptomatic and symptomatic carriers respectively, and 56 [49–66] nmol•mL⁻¹•min⁻¹ for non-carriers.

Conclusions: The HAE-FXII is associated with modifiers, *e.g.* kinin catabolism enzymes, ACE and CPN, different from those recognized in HAE with C1Inh deficiency.

KEY WORDS: angiotensin-I converting enzyme; carboxypeptidase N/M; factor XII mutation; hereditary angioedema with normal C1 inhibitor; bradykinin.

Introduction

Hereditary angioedema (HAE) is a rare disease exhibiting autosomal-dominant inheritance, characterized by sudden, episodic, pale and non-itching swellings of the subcutaneous or submucosal tissues. Swellings are deforming and disabling phenomena, with gastrointestinal edemas being painful, and upper respiratory tract disorders potentially life-threatening (1-3).

Accepted Article

In 1986, HAE associated with normal C1Inh function (nC1Inh-HAE-) was identified (4) and then described (5-7). In 2006, two causative mutations of nC1Inh-HAE were described within exon 9 of the *F12* gene encoding the coagulation factor XII (FXII), namely c.983C>A and c.983C>G (FXII-HAE [MIM 610618]) (8-9). Other pathogenic mutations also discovered in the *F12* gene consist of a 72-bp deletion (c.971_1018+24del72) (10) and a duplication (c.892_909dup) (11). Typically, only a few individuals presenting with nC1Inh-HAE carry an *F12* mutation (12). Vasoactive BK has been recognized as potentially the primary mediator responsible for increased vascular permeability in HAE pathophysiology (13-16). Once released, BK is rapidly metabolized, principally by angiotensin I-converting enzyme (ACE) yet also by aminopeptidase P (APP), whereas carboxypeptidase N (CPN) transforms BK into its active metabolite: *desArg*⁹-BK. The relevance of the BK degradation pathway has been demonstrated by reports of angioedema in CPN deficiency or cases involving ACE inhibitor treatment (17-18). The *desArg*⁹-BK action onto the endothelium has been shown as being short-lived by APP and ACE (17). Decreased plasma APP activity was associated with a significantly slower degradation of *desArg*⁹-BK *in vitro* (19), and low APP activity has proven a severity risk factor for C1Inh-HAE (20).

Diagnosis of FXII-HAE is rendered problematic by its variable and unpredictable expressivity (22-23). Biological testing of HAE patients typically includes the complement parameters C1Inh and C4. HAE-associated kinin accumulation is a consequence of a kinin production-degradation imbalance. For this reason, we chose to investigate the enzymes involved in kinin metabolism (amidase, APP, CPN, and ACE activities). Recent reports have argued for modifiers of strategic importance for the phenotype (11, 22-23). This prompted us to investigate biochemical markers of individuals from 40 mutation-carrier families in order to predict disease severity. Based on clinical and biological phenotypes, we found that disease severity was associated with a decrease in kinin catabolism.

Materials and Methods

Ethics and consent

All procedures were performed in accordance with the Helsinki declaration and French ethical policy, and data was obtained from the biological sample collection (with Ministry of Health authorization). Informed consent was obtained from all patients and healthy blood donors.

Study design

Our analysis was based on exhaustive case reports on members of carrier families of the c.983C>A missense mutation on the *F12* gene.

Sampling procedures and laboratory methods

The mutation was established from DNA extracted through direct sequencing of exon 9 of the *F12* gene (8). Citrated plasma samples were analyzed for enzymatic parameters, and serum samples were used for measuring C1Inh and C4 antigenic levels quantified by nephelometry (BN2 Siemens, Munich, Germany). Samples were harvested out of ACE inhibitor intake, and whenever possible, during attacks. Spontaneous amidase activity was measured in line with Defendi *et al.* (24) study methods. APP and CPN activities were assessed by means of fluorometric assays, as previously described (17, 25). ACE activity was measured with the commercial ACE kinetic[®] kit (Bühlmann Laboratories AG, Allschwil, Switzerland). C1Inh function was quantified by means of a chromogenic assay (26).

Severity score

Clinical data were collected by physicians. Angioedema phenotypes were classified into four groups (asymptomatic, mild, intermediate, and severe) according to the frequency and location of attacks and the patient's age at symptom onset, as per the methods of Cumming *et al.* (27) and Freiburger *et al.* (28). Classification was determined following adaptation to the specificity of nC1Inh-HAE clinical phenotype (attack frequency and age at symptom onset).

Statistical analysis

Qualitative data was reported as numbers and frequencies. Quantitative variables were expressed as a median and interquartile range (IQ25- IQ75), distributions tested by means of the Shapiro-Wilk test and compared using the Mann-Whitney test (two group comparisons), or the Kruskal-Wallis test (three or more groups). P-values < 0.05 were considered statistically significant.

Prior to conducting logistic regression, we verified the independence of paired variables by means of the Pearson's chi-squared test, the variables then being standardized and reduced to remove the impact of variable weight. Then, the data was submitted to logistic regression with backward, forward, and both developments. The best model was selected by applying the lowest Akaike Information Criterion (AIC) (29) and lowest Bayesian Information Criterion (BIC) (30).

Statistical analyses were performed with R 2.15.1 software programs (R Foundation for Statistical Computing, Vienna, Austria).

Results

Population description

This study involved 40 unrelated families and 176 individuals (Supp. Table S1), 118 of whom were carriers of the c.983C>A mutation on the *F12* gene. Non-carriers (n=58) were included as controls within the families. These subjects were compared with 200 healthy blood donors.

A total of 80 carriers were symptomatic, and the disease was more commonly found in women (n=72) than men (n=8; Fig. 1). At the time of examination, 38 carriers (32%) were still asymptomatic. Family history was not reported in 12 families, and the proportion of symptomatic individuals varied from 0.17 to 1.00 (mean 0.67 ± 0.25 ; Supp. Table S1). Disease severity was determined according to edema location, age at onset, and attack frequency (see Supp. Tables S1). Patient disease severity was then classified as mild for 22 (19%), intermediate for 42 (35%), and severe for 14 (12%). The disease severity of two carriers could not be determined (2%; Fig. 1).

Biological phenotype of the families

Kinin production-degradation is imbalance in HAE-associated kinin accumulation. For this reason, we chose to investigate the enzymes involved in kinin metabolism (amidase, APP, CPN, and ACE activities) in order to compare patients with healthy donors or between FXII-HAE patient groups. C1Inh and C4 antigenic levels and C1Inh function were within normal range in all groups (data not shown). Nevertheless, C1Inh function was lower in symptomatic patients ($20.7 [18.1-23.3] \text{ U} \cdot \text{mL}^{-1}$; n=58) compared to the asymptomatic carriers ($25.2 [22.3-27.6] \text{ U} \cdot \text{mL}^{-1}$; n=21 $P < 0.001$). Increased spontaneous amidase activity was observed in all groups in contrast to that of blood donors ($2.4 [1.9-3.0] \text{ nmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$; $P < 0.001$, Fig. 2A). Spontaneous amidase activity was $8.6 [4.1-21.7]$

nmol•mL⁻¹•min⁻¹ in carriers (n=100) and was 6.6 [4.0–11.2] nmol•mL⁻¹•min⁻¹ in non-carriers (n=45), with significantly higher levels reported in symptomatic carriers (10.1 [4.3–28.8] nmol•mL⁻¹•min⁻¹ n=72) compared to the asymptomatic non carriers (6.1 [4.0–12.0] nmol•mL⁻¹•min⁻¹ n=28; *P* < 0.01; Fig. 2A). APP activity was higher in the carrier group (1.24 [0.63–2.02] nmol•mL⁻¹•min⁻¹ n=101) compared to the control (0.83 [0.40–1.33] nmol•mL⁻¹•min⁻¹; *P* < 0.001), with the same increase also observed in symptomatic carriers (1.25 [0.63–2.02] nmol•mL⁻¹•min⁻¹ n=73; *P* < 0.001) and asymptomatic carriers (1.18 [0.55–1.96] nmol•mL⁻¹•min⁻¹ n=28; *P* < 0.05) when compared to the control (Fig. 2B). CPN activities proved significantly lower in both carriers (37.8 [31.6–45.0] nmol•mL⁻¹•min⁻¹ n=102) and non-carriers (37.9 [30.5–43.7] nmol•mL⁻¹•min⁻¹ n=45), as well as in both symptomatic (38.5 [32.8–45.6] nmol•mL⁻¹•min⁻¹ n=74) and asymptomatic (37.5 [28.5–41.3] nmol•mL⁻¹•min⁻¹ n=28) carriers, compared to healthy donors (46.6 [41.7–50.4] nmol•mL⁻¹•min⁻¹ *P* < 0.001; Fig. 2C). ACE activities proved also significantly lower in both carriers (50 [39–65] nmol•mL⁻¹•min⁻¹ n=101) and non-carriers (56 [49–66] nmol•mL⁻¹•min⁻¹ n=44), as well as in both symptomatic (49 [35–59] nmol•mL⁻¹•min⁻¹ n=73) and asymptomatic (58 [44–76] nmol•mL⁻¹•min⁻¹ n=28) carriers, compared to healthy donors (*P* < 0.001). In addition, ACE activity was decreased in symptomatic individuals with regard to that of asymptomatic subjects (*P* < 0.01; Fig. 2D).

Biology and disease severity groups

Our study then proceeded to decipher the biological phenotype among different FXII-HAE severity phenotypes. Complement parameters and kinin metabolism activities were then investigated for a possible association between biological phenotype and disease severity. C1Inh and C4 antigenic levels and C1Inh function were similar in all four disease severity groups (data not shown; Kruskal-Wallis test, *P* > 0.05). Spontaneous amidase activity was significantly increased in the symptomatic patients, irrespective of disease severity, with 2.6 (2.0–4.3) nmol•mL⁻¹•min⁻¹ recorded for asymptomatic individuals, 8.3 (5.2–46.2) nmol•mL⁻¹•min⁻¹ for the mild severity group, 9.2 (3.4–28.1)

nmol•mL⁻¹•min⁻¹ for the intermediate severity group, and 12.5 (6.9–23.8) nmol•mL⁻¹•min⁻¹ for the severe phenotype group ($P < 0.001$, Kruskal-Wallis test; $P < 0.001$, Mann-Whitney test for asymptomatic vs. mild, asymptomatic vs. intermediate, and asymptomatic vs. severe). APP activity was significantly increased in the mild (1.43 [0.70–2.19] nmol•mL⁻¹•min⁻¹), intermediate (1.23 [0.70–1.62] nmol•mL⁻¹•min⁻¹), and severe disease (1.35 [0.69–2.20] nmol•mL⁻¹•min⁻¹) groups, compared to the controls (0.87 [0.41–1.44] nmol•mL⁻¹•min⁻¹; $P < 0.01$, Kruskal-Wallis test; $P < 0.05$, Mann-Whitney test for asymptomatic vs. mild, asymptomatic vs. intermediate, and asymptomatic vs. severe). CPN activity was significantly decreased in patients presenting with intermediate (36.3 [32.9–44.5] nmol•mL⁻¹•min⁻¹) and severe disease (37.9 [31.8–42.6] nmol•mL⁻¹•min⁻¹), in comparison with asymptomatic subjects (44.7 [38.5–49.4] nmol•mL⁻¹•min⁻¹; $P < 0.001$, Kruskal-Wallis test; $P < 0.001$, Mann-Whitney test for asymptomatic vs. intermediate, and $P < 0.01$ for asymptomatic vs. severe). No differences, however, were observed between the mild severity and asymptomatic groups (data not shown). ACE activity was decreased in all three disease severity groups, with values of 54 [46–62] nmol•mL⁻¹•min⁻¹ for the mild severity group, 47 [32–58] nmol•mL⁻¹•min⁻¹ for intermediate severity, and 44 [29–61] nmol•mL⁻¹•min⁻¹ for the severe clinical phenotype, in comparison with the controls : 64 [53–76] nmol•mL⁻¹•min⁻¹ ($P < 0.001$, Kruskal-Wallis test; $P < 0.01$, Mann-Whitney for asymptomatic vs. mild and asymptomatic vs. severe and $P < 0.001$ for asymptomatic vs. intermediate). These results suggest that distinct biological phenotypes of FXII-HAE correlated with disease severity.

Identification of risk factors for the severe FXII-HAE phenotype

We tested the recorded differences in biological phenotypes for their relevance to severity groups. Risk factors for disease severity were analyzed using logistic regression, initially designed based on the observations described in Fig. 2. The most apt model was subsequently selected using the AIC and BIC. Model D, which associated C1Inh antigenic level with spontaneous amidase, CPN, and ACE

activities, was selected for its capacity to best explain disease severity (likelihood ratio < 0.001). From this model, we calculated the odds ratio for the mild, intermediate, and severe phenotypes (Fig. 3). Increased spontaneous amidase activity significantly correlated with all three phenotypes, with odds ratio values of 1.95 ([95% CI, 1.33–2.86], $P < 0.001$), 2.15 ([95% CI, 1.48–3.11], $P < 0.001$), and 1.92 ([95% CI, 1.15–3.21], $P < 0.05$), respectively. Decreased CPN activity was shown to constitute a risk factor for disease severity (odds ratio of 0.58 [95% CI, 0.36–0.91]) as regards the intermediate phenotype ($P < 0.05$; Fig. 3). Furthermore, decreased ACE activity was also found to be a risk factor for the intermediate and severe phenotypes (odds ratio of 0.36 [95% CI, 0.23–0.59]; $P < 0.001$ and 0.34 [95% CI, 0.17–0.69]; $P < 0.001$, respectively; Fig. 3), with the increased severity index in correlation with decreased ACE activity, indicating that ACE activity strongly impacted the disease severity. C1Inh antigenic levels did not correlate to any severity index (Fig. 3). As expected from the AIC- and BIC-based analysis, APP activity did not correlate with severity (data not shown). In conclusion, low kinin catabolism with decreased ACE and CPN was revealed to correlate with a severe phenotype of FXII-HAE, a condition favorable to BK accumulation.

Discussion

This report describes an extended study of FXII-HAE families displaying high inter-individual variability of clinical and biological phenotypes. The remarkable variability in carriers observable in this group includes both the severity (location and frequency) of attacks and age at symptom onset, suggesting the influence of modifying factors. Although FXII-HAE exhibits an autosomal dominant transmission, family history is not currently recognized in a third of the cases (12/40 families), and there thus remains the possibility that this factor has been overlooked or has gone unrecognized. This is consistent with recent reports that advise not defining absence of family history as an exclusion criterion for FXII-HAE diagnosis (22, 23).

The clinical manifestations were quite similar for both FXII-HAE and C1Inh-HAE (31) . A large proportion of FXII-HAE patients (66%) developed severe edemas (laryngeal or abdominal). Nevertheless, the frequency of attacks was lower, with 13% and 58% of patients developing more than 12 attacks per year for FXII-HAE and C1Inh-HAE, respectively. In addition, the first attacks occurred later in the FXII-HAE group than the C1Inh-HAE, with symptoms developing after aged 12 in 82.5% of FXII-HAE patients (Table. S1) and 62% of those with C1Inh-HAE (28). Consequently, these considerations required the severity scaling of Freiburger *et al.* (28) to be modified for this study . nC1Inh-HAE was initially described as preferentially affecting women (22) several affected males were included. The family data was consistent with monogenic inheritance of FXII-HAE, with only slightly reduced penetrance observed in females and very small penetrance in males.

Complement parameters (C1Inh and C4) were within the normal range, reinforcing the disease's designation as being HAE with normal C1Inh (1). Early observations of nC1Inh-HAE reported that C1Inh function transiently decreased during the disease's active period and remained normal during asymptomatic periods (32), associated with the emerging cleaved C1Inh 95-kDa species (33). We hypothesized that this C1Inh 95-kDa species could have resulted from the high proteolytic activity during active periods. This underlines the relevance of the exact time samples are collected in terms of allowing us the opportunity to detect these transient modifications.

Spontaneous amidase activity was increased while ACE and CPN activities were decreased for all four severity groups, including both non-carriers and asymptomatic individuals, in comparison with healthy donors. This would indicate that BK accumulation in families carrying an *F12* mutation is emphasized by enzymes influencing kinin formation and degradation. This is consistent with a monogenic disease associated with modifying gene effects that is shared within the family. The

influence of BK metabolism parameters in clinical phenotype severity provides further support for the concurrent argument of kinin metabolism with genetic identification.

A higher APP activity in the intermediate and severe phenotype groups, which was expected to reduce the kinin half-life (mainly *desArg*⁹-BK), was not identified by logistic regression as a protective parameter for FXII-HAE. This directly contrasted to a prior observation where low APP activity was retained as a risk factor for C1Inh-HAE severity (20). A significant observation made was that ACE activity inversely correlated with severity scores and a decrease in CPN in the intermediate and severe groups. These parameters, implemented into the logistic regression model (Fig. 3), confer a possible decision for clinical phenotype prediction. Even if two first decisional parameters have been identified, the high deviation of values in all groups as seen in Figure 2 suggests that other factors could protect from or amplify the bradykinin accumulation process.

CPN and ACE are the main catabolic proteases involved in degrading BK, the B₂ receptor agonist, while *desArg*⁹-BK, the B₁ receptor agonist, primarily involves APP for its catabolism. Based on the present results with FXII-HAE, along with those found with C1Inh-HAE (20), the two following hypotheses for the edema etiopathogenesis can be distinguished: FXII-HAE is potentially more closely associated with BK and B₂ receptors, and C1Inh-HAE with *desArg*⁹-BK and B₁ receptors, with a putative impact observed for the treatment option using an appropriate kinin receptor antagonist (34). In conclusion, an *F12* mutation is the principal FXII-HAE predictor, with the disease expression influenced by individual variations in kinin degradation enzyme activities.

Acknowledgements

We would like to express our gratitude to the physicians of the French National Centre for Angioedema, and others for their helpful participation in this collaborative study, these include: Michel Bouvier, Brigitte Coppéré, Pauline Pralong Lyon; Bernard Goichot, Joelle Goetz, Strasbourg; Anne Gompel, Louis Affo, Paris; Stéphane Gayet, Bruno Graffin, Marseille; Stéphane Guez, Anne Sarrat, Bordeaux; Isabelle Pruvost, Lille; Hervé Maillard, Le Mans; Olivier Michel, Said Tas, Brussels; Denise Anne Moneret-Vautrin, Etienne Beaudouin, Epinal; Yann Ollivier, Caen; Hervé Watier, Tours; Nadia Raison-Peyron, Aurélie Du Thanh, Montpellier; Géraldine Jeudy, Fabien Pelletier, Besançon; Antoine Guégnard, Le Breuil. The authors are indebted to Françoise Csopaki, Marion Allegret-Cadet, Grenoble, and Hélène Humeau, Angers, for their skillful work, and are grateful to Sébastien Bailly for his support in the statistical analysis.

This work was supported by an ERA-Net E-RARE-1 research program conducted within European framework 7 (acronym HAEIII; S. Cichon, coordinator) and by the National Rare Disease Plan (French Ministry of Health, 2011-2015).

Author contributions:

DC performed the data collection, data analyses and written the article. AG, FD, DP and NM contributed to data collection and review the article. MLT, DL, TC, KD, OF contributed to sample collection and revision of the article. SC, LM and CD contributed to the study management and review the article.

Conflicts of interest

Disclosure of potential conflict of interest: TC, OF and LM received funds from Shire HGT, CSL Behring, and ViroPharma. TC received funds from SOBI, Dyax, Pharming. The other authors declare no competing financial interests.

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 Suppl 1**:S145–156.
2. Bork K, Staubach P, Eckardt AJ, Hardt J. Symptoms, course, and complications of abdominal attacks in hereditary angioedema due to C1 inhibitor deficiency. *Am J Gastroenterol* 2006;**101**:619–627.
3. Cugno M, Zanichelli A, Foiени F, Caccia S, Cicardi M. C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. *Trends Mol Med* 2009;**15**:69–78.
4. Warin RP, Cunliffe WJ, Greaves MW, Wallington TB. Recurrent angioedema: familial and oestrogen-induced. *Br J Dermatol* 1986;**115**:731–734.
5. Bork K, Barnstedt SE, Koch P, Traupe H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* 2000;**356**:213–217.
6. Binkley KE, Davis A 3rd. Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema. *J Allergy Clin Immunol* 2000;**106**:546–550.
7. Martin L, Degenne D, Toutain A, Ponard D, Watier H. Hereditary angioedema type III: an additional French pedigree with autosomal dominant transmission. *J Allergy Clin Immunol* 2001;**107**:747–748.
8. Cichon S, Martin L, Hennies HC, Müller F, Van Driessche K, Karpushova A et al. Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. *Am J Hum Genet* 2006;**79**:1098–1104.
9. 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.

- Accepted Article
10. 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.
 11. Kiss N, Barabás E, Várnai K, Halász A, Varga LÁ, Prohászka Z et al. Novel duplication in the F12 gene in a patient with recurrent angioedema. *Clin Immunol* 2013;**149**:142–145.
 12. Bork K, Gül D, Hardt J, Dewald G. Hereditary angioedema with normal C1 inhibitor: clinical symptoms and course. *Am J Med* 2007;**120**:987–992.
 13. Ghannam A, Defendi F, Charignon D, Csopaki F, Favier B, Habib M et al. Contact system activation in patients with HAE and normal C1 Inhibitor function. *Immunol Allergy Clin North Am* 2013;**33**:513–533.
 14. Kaplan AP, Joseph K. The bradykinin-forming cascade and its role in hereditary angioedema. *Ann Allergy Asthma Immunol* 2010;**104**:193–204.
 15. Seip KF, Bjerknes KC, Johansen HT, Nielsen EW, Landrø L, Reubsæet L. Bradykinin analysis revived - A validated method for determination of its stable metabolite in whole blood by LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2014;**947-948**:139–144.
 16. Davis AE 3rd. The pathophysiology of hereditary angioedema. *Clin Immunol* 2005;**114**:3–9.
 17. Cyr M, Lepage Y, Blais C, Gervais N, Cugno M, Rouleau JL et al. Bradykinin and des-Arg(9)-bradykinin metabolic pathways and kinetics of activation of human plasma. *Am J Physiol Heart Circ Physiol* 2001;**281**:H275–83.
 18. Matthews KW, Mueller-Ortiz SL, Wetsel RA. Carboxypeptidase N: a pleiotropic regulator of inflammation. *Mol Immunol* 2004;**40**:785–793.
 19. Molinaro G, Cugno M, Perez M, Lepage Y, Gervais N, Agostoni A et al. Angiotensin-converting enzyme inhibitor-associated angioedema is characterized by a slower degradation of des-arginine9-bradykinin. *J Pharmacol Exp Ther* 2002;**303**:232 –237.
 20. Drouet C, Désormeaux A, Robillard J, Ponard D, Bouillet L, Martin L et al. Metallopeptidase activities in hereditary angioedema: Effect of androgen prophylaxis on plasma aminopeptidase P. *J Allergy Clin Immunol* 2008;**121**:429–433.
 21. Cilia La Corte AL, Carter AM, Rice GI, Duan QL, Rouleau GA, Adam A et al. A functional XPNPEP2 promoter haplotype leads to reduced plasma aminopeptidase P and increased risk of ACE inhibitor-induced angioedema. *Hum Mutat* 2011;**32**:1326–1331.
 22. 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.

- Accepted Article
23. Gómez-Traseira C, López-Lera A, Drouet C, López-Trascasa M, Pérez-Fernández E, Favier B et al. Hereditary angioedema caused by the p.Thr309Lys mutation in the F12 gene: A multifactorial disease. *J Allergy Clin Immunol* 2013;**132**:986–989.e5.
 24. Defendi F, Charignon D, Ghannam A, Baroso R, Csopaki F, Allegret-Cadet M et al. Enzymatic assays for the diagnosis of bradykinin-dependent angioedema. *PLoS One* 2013;**8**:e70140.
 25. Molinaro G, Carmona AK, Juliano MA, Juliano L, Malitskaya E, Yessine M-A et al. Human recombinant membrane-bound aminopeptidase P: production of a soluble form and characterization using novel, internally quenched fluorescent substrates. *Biochem J* 2005;**385**:389–397.
 26. Drouet C, Alibeu C, Ponard D, Arlaud GJ, Colomb MG. A sensitive method to assay blood complement C1- inhibitor activity. *Clin Chim Acta* 1988;**174**:121–130.
 27. Cumming S, Halsall D, Ewan P, Lomas D. The effect of sequence variations within the coding region of the C1 inhibitor gene on disease expression and protein function in families with hereditary angioedema. *J Med Genet* 2003;**40**:e114.
 28. Freiburger T, Grombířiková H, Ravčuková B, Jarkovský J, Kuklínek P, Kryštůřková O et al. No evidence for linkage between the hereditary angioedema clinical phenotype and the BDKR1, BDKR2, ACE or MBL2 gene. *Scand J Immunol* 2011;**74**:100–106.
 29. Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control* 1974;**19**:716–723.
 30. Schwarz G. Estimating the Dimension of a Model. *Ann Statist* 1978;**6**:461–464.
 31. Bork K. Hereditary angioedema with normal C1 inhibitor. *Immunol Allergy Clin North Am* 2013;**33**:457–470.
 32. Bouillet L, Ponard D, Rousset H, Cichon S, Drouet C. A case of hereditary angio-oedema type III presenting with C1-inhibitor cleavage and a missense mutation in the F12 gene. *Br J Dermatol* 2007;**156**:1063–1065.
 33. 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**:975–977.
 34. Charignon D, Späth P, Martin L, Drouet C. Icatibant, the bradykinin B2 receptor antagonist with target to the interconnected kinin systems. *Expert Opin Pharmacother* 2012;**13**:2233–2247.

Figure Legends

Figure 1. Symptoms and severity distribution by gender. Male and female carriers (n=118) of the c.983C>A mutation were distributed for their asymptomatic phenotype or severity grades. Number (percentage).

Figure 2. Biological phenotype. **A:** spontaneous amidase activity, **B:** aminopeptidase P, **C:** carboxypeptidase N, **D:** angiotensin-I converting enzyme. Presentation of data from healthy donors, non-carriers (F12 C/C) and carriers (F12 A/C), and asymptomatic and symptomatic carriers. —: median;: normal range; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 3. Risk estimate for disease severity with biological phenotype. Odds ratios were calculated from the logistic regression model explaining the severity using C1 inhibitor (C1Inh) antigenic level and carboxypeptidase N (CPN), angiotensin-I converting enzyme (ACE) and spontaneous amidase activity data. The vertical bar represents the odds-ratio.: 95% CIs. The Wald test was used to calculate P value.

Figure 1

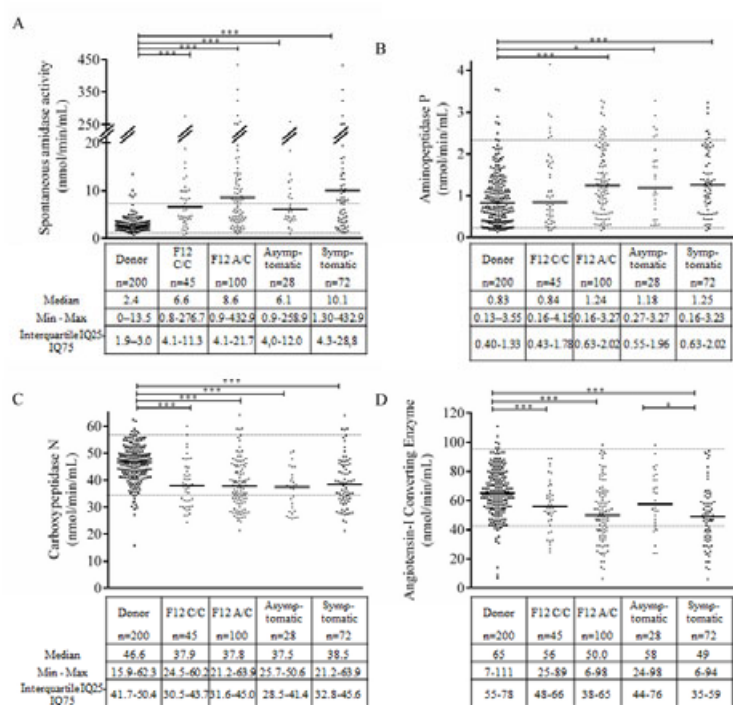
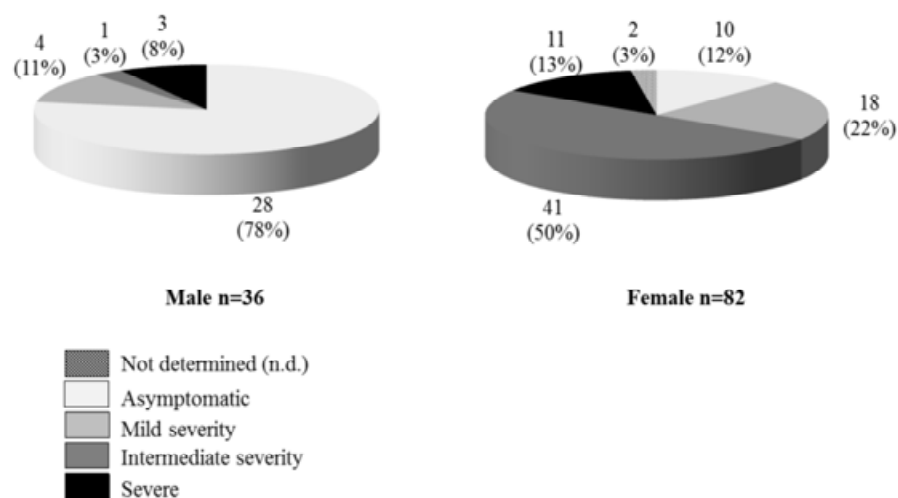


Figure 2

Figure 3

