

BRIEF COMMUNICATION

# ***F12-46C/T* polymorphism as modifier of the clinical phenotype of hereditary angioedema**

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## **Keywords**

C1-inhibitor deficiency; *F12-46C/T* polymorphism; hereditary angioedema.

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## **Abstract**

The factors influencing the heterogeneous clinical manifestation of hereditary angioedema due to C1-INH deficiency (C1-INH-HAE) represent one of the oldest unsolved problems of the disease. Considering that factor XII (FXII) levels may affect bradykinin production, we investigated the contribution of the functional promoter polymorphism *F12-46C/T* in disease phenotype. We studied 258 C1-INH-HAE patients from 113 European families, and we explored possible associations of *F12-46C/T* with clinical features and the *SERPING1* mutational status. Given that our cohort consisted of related subjects, we implemented generalized estimating equations (GEEs), an extension of the generalized linear model accounting for the within-subject correlation. *F12-46C/T* carriers exhibited a significantly delayed disease onset ( $P < 0.001$ ) and did not need long-term treatment ( $P = 0.02$ ). In a GEE linear regression model, the presence of *F12-46C/T* was significantly associated with a 7-year delay in disease onset ( $P < 0.0001$ ) regardless of *SERPING1* mutational status. It is concluded that *F12-46C/T* carriage acts as an independent modifier of C1-INH-HAE severity.

Hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) is a rare autosomal dominant disorder that manifests with recurrent incapacitating attacks of edema, spontaneously developing in any body location and possibly leading to life-threatening asphyxiation when upper airways are affected. The clinical expression of C1-INH-HAE is characterized by a large heterogeneity in regard to the age at disease onset, the number of attacks, triggers of acute episodes, the severity and localization of edema, and prodromal signs and symptoms. These characteristics may vary even among members of the same family, including cases where considerable C1-INH deficiency rarely or never results in clinical symptoms of C1-INH-HAE (1, 2). Therefore, to be able to better manage the disabling and potentially fatal manifestations of C1-INH-HAE, we need to be able to predict their development.

The genetics of C1-INH-HAE are complex; over 400 different alterations have already been identified in the *SERPING1* gene, encoding for C1-INH (HAEdb: hae.enzim.hu; Human Gene Mutation Database: <http://www.hgmd.cf.ac.uk/ac/>

index.php). These mutations entail reduced levels (type I) or impaired function (type II) of the C1-INH resulting in uncontrolled activation of various plasma enzyme cascades leading to increased production of bradykinin that is considered the major mediator of edema (3–5). Recent evidence indicates that patients carrying missense *SERPING1* mutations present with a less severe C1-INH-HAE clinical course (6, 7).

A third type of HAE with normal C1-INH levels has been recently described that is caused by mutations in the *F12* gene encoding for coagulation factor XII (*F12*) (8). Carriage of these mutations results in increased activity and expression of factor XII (FXII) (9), but the exact mechanisms by which they lead to the appearance of angioedema remain as yet controversial (10–12). Based on that, we hypothesized that the functional promoter polymorphism *F12-46C/T* (rs1801020) that results in decreased translation efficiency and low plasma levels of FXII activity and antigen (13, 14) might affect the disease phenotype of C1-INH-HAE patients, as it was recently indicated by Bors et al. (6).

## Patients and methods

One hundred and fifty-two (152) patients with C1-INH-HAE (125 with type I and 27 with type II) from 74 unrelated families (34 Greek, 18 German, 14 Romanian, and 8 Hungarian families) previously genotyped for *SERPING1*(6, 7, 15) were enrolled in the study. To further elevate the power of our study toward uncovering possible genotype–phenotype correlations, these patients were pooled with 106 C1-INH-HAE patients from 39 Hungarian families previously genotyped for the *F12-46C/T* polymorphism (6). Thus, a pooled analysis including 258 C1-INH-HAE patients from 113 unrelated European families was performed (Table 1). Medical records of the patients were reviewed, and data regarding the disease onset, treatment modalities, and the major clinical manifestations were recorded. The annual number of attacks was not considered as a variable of disease severity due to the inclusion of patients under long-term prophylactic treatment (LTP) and the resulted significant underestimation.

Adult patients with recurrent attacks were receiving LTP, involving anabolic steroids (danazol) in Greece, Romania, and Hungary, and anabolic steroids, tranexamic acid, or human C1-INH (Cinryze) in Germany. Moreover, human

plasma-derived C1-INH concentrate (Berinert PR; CSL Behring, Marburg, Germany) or icatibant (a specific antagonist of bradykinin B2 receptor; Firazyr, Shire, Lexington, USA) was given to adult patients, in the case of severe attacks involving airway obstruction, as well as severe attacks of abdominal pain. Pediatric patients were receiving only purified C1-INH concentrate during HAE attacks. The decision for the treatment option is based on clinicians' experience, according to international consensus algorithms for patients' management (16).

The local institutional review boards approved the study, and written informed consent was obtained from each individual or an accompanying relative.

The detection of the *F12-46C/T* polymorphism was performed by PCR-RFLP (17). Detailed methods are given in the online repository (Appendix S1).

## Results and discussion

Among the 258 C1-INH-HAE patients we examined, 74 were heterozygotes and nine were homozygotes for the *F12-46C/T* polymorphism (allele frequency 17.8%). Carriers of *F12-46C/T* (either homozygotes or heterozygotes) were found to be

**Table 1** Demographic, clinical, and molecular data of the patients included in the study

|                                                               | Total       | Greek       | Romanian     | German       | Hungarian   |
|---------------------------------------------------------------|-------------|-------------|--------------|--------------|-------------|
| No (families, patients)                                       | 113, 258    | 34, 85      | 14, 20       | 18, 28       | 47, 125     |
| Sex (male/female)                                             | 122/136     | 47/39       | 7/13         | 13/15        | 55/70       |
| Age at analysis (median, range)                               | 36.0 (1–82) | 35.0 (1–80) | 37.0 (10–72) | 36.5 (13–81) | 37.0 (7–82) |
| Age at onset (median, range)                                  | 10.0 (1–70) | 10.0 (1–57) | 22.0 (2–70)  | 13.0 (3–28)  | 10.0 (1–53) |
| Age at diagnosis (median, range)                              | 22.0 (1–78) | 28.2 (2–78) | 35.0 (10–70) | 18.0 (3–78)  | 21.0 (1–72) |
| Delay of diagnosis (years median, range)                      | 8.0 (0–72)  | 17.3 (0–58) | 2.0 (0–52)   | 2.5 (0–72)   | 7.5 (0–60)  |
| Patients without disease (n, %)                               | 16 (6.2)    | 8 (9.4)     | 1 (5.0)      |              | 7 (5.6)     |
| Age (mean, range)                                             | 10.0 (1–72) | 4.5 (1–16)  | 45.0         |              | 42.0 (3–72) |
| Families with type II HAE (n, %)                              | 11 (9.7)    | 4 (11.8)    | 1 (7.1)      | 2 (11.1)     | 4 (8.5)     |
| Patients with type II HAE (n, %)                              | 27 (10.5)   | 12 (14.1)   | 2 (10.0)     | 3 (10.7)     | 10 (8.0)    |
| Patients with laryngeal attacks (n, %)*                       | 139 (57.4)  | 44 (57.9)   | 12 (63.2)    | 13 (46.4)    | 70 (59.3)   |
| Patients with abdominal attacks*†                             |             |             |              |              |             |
| Rare (n, %)                                                   | 107 (44.2)  | 21 (27.3)   | 1 (5.3)      | 7 (25.0)     | 78 (66.1)   |
| Common (n, %)                                                 | 85 (35.1)   | 32 (41.5)   | 12 (63.1)    | 16 (57.1)    | 25 (21.2)   |
| Main (n, %)                                                   | 50 (20.7)   | 24 (31.2)   | 6 (31.6)     | 5 (17.9)     | 15 (12.7)   |
| Patients on LTP (n, %)*                                       | 96 (39.7)   | 37 (48.1)   | 3 (15.8)     | 9 (32.1)     | 47 (39.8)   |
| <i>SERPING1</i> defects (families)‡                           |             |             |              |              |             |
| Regulatory (n, %)                                             | 5 (4.4)     | 2 (5.9)     | 0 (0.0)      | 0 (0.0)      | 3 (6.4)     |
| Missense mutations (n, %)                                     | 37 (32.7)   | 12 (35.3)   | 6 (42.9)     | 6 (33.3)     | 13 (27.7)   |
| Nonsense mutations (n, %)                                     | 13 (11.5)   | 4 (11.8)    | 1 (7.1)      | 0 (0.0)      | 8 (17.0)    |
| Splice defects (n, %)                                         | 9 (8.0)     | 5 (14.7)    | 2 (14.3)     | 1 (5.6)      | 1 (2.1)     |
| Small deletions or insertions (frameshift alterations) (n, %) | 31 (27.4)   | 9 (26.5)    | 2 (14.3)     | 6 (33.3)     | 14 (29.8)   |
| Large deletions or insertions (n, %)                          | 12 (10.6)   | 1 (2.9)     | 2 (14.3)     | 3 (16.7)     | 6 (12.8)    |
| Unidentified defects (n, %)                                   | 6 (5.3)     | 1 (2.9)     | 1 (7.1)      | 2 (11.1)     | 2 (4.2)     |
| <i>F12-46C/T</i> , allele frequency (%)                       | 17.8        | 11.7        | 17.5         | 30.3         | 19.2        |

\*Asymptomatic patients displaying *SERPING1* mutations were excluded from the statistical analysis.

†The abdominal attacks were categorized as rare when their frequency was <1–2 per year, as common when their frequency was >2 per year, and as main when these represent the most common manifestation of HAE patients throughout their life.

‡Four Greek families not included in our previous papers (7, 15) carried two novel (g.1769G>A, c.1A>C, p.M1L; g.18077-18083delAGACCTG, c.1503-1503+6delAGACCTG, p.X501fs) and two recurrent *SERPING1* mutations (g.9699T>C, c.878T>C, p.I293T; g.10045G>A, c.1029+1G>A).

significantly older at disease onset (mean  $\pm$  SD:  $17.1 \pm 11.9$  vs  $11.3 \pm 9.0$  years,  $P < 0.001$ ) and less often needed LTP as compared to noncarriers ( $P = 0.021$ ) (Fig. 1A). In a generalized estimating equation (GEE) linear regression model with age at disease onset as dependent variable, the presence of *F12*-46C/T polymorphism was significantly associated with a 7-year delay in disease onset ( $P = <0.0001$ ) adjusting for sex,

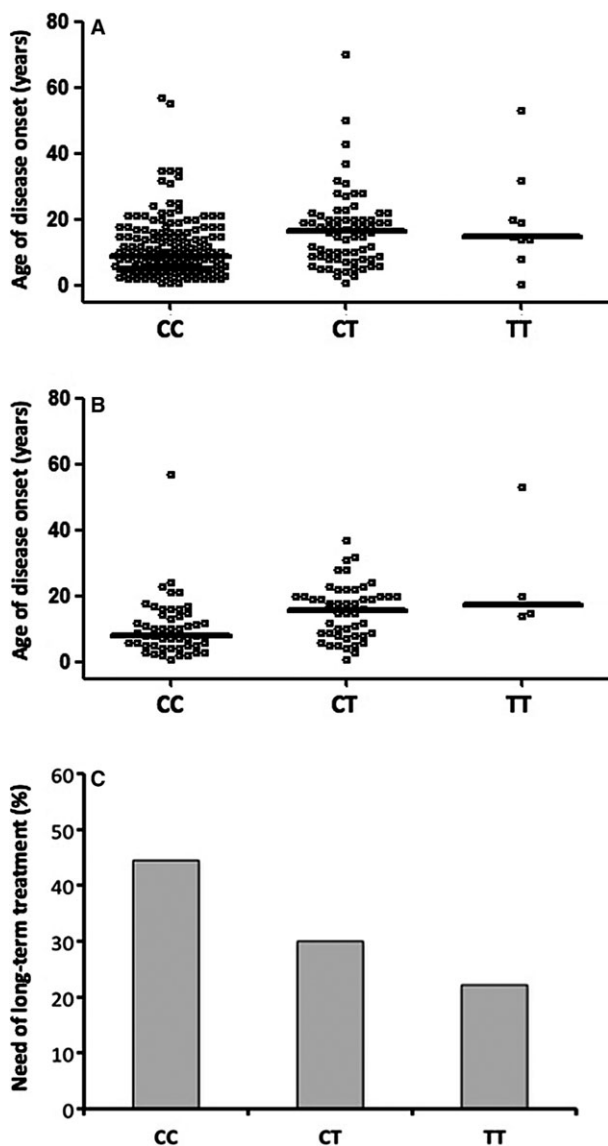


**Figure 1** (A) Differences in the age at disease onset between wild-type (CC), heterozygote (CT), and homozygote (TT) HAE patients for the *F12*-46C/T polymorphism ( $P < 0.001$ ). (B) Differences in the age at disease onset in families with at least one affected member who was carrier of the *F12*-46C/T polymorphism (CT or TT) and at least one affected wild-type (CC) member ( $P < 0.001$ ). (C) Percentage of CC, CT, and TT HAE patients who received long-term treatment ( $P = 0.021$ ). A line across the plots indicates the median value for each group;  $P$ -values were calculated by the Kruskal–Wallis  $H$ -test.

ethnicity, and the presence of *SERPING1* missense mutations. Similarly, the need for LTP as dependent variable was significantly and negatively associated with the carriage of *F12*-46C/T polymorphism (odds ratio = 2.8, 95% CI = 1.1–7.0,  $P = 0.029$ ) after adjustment for the same variables (Fig. 1C). The same results were obtained after the exclusion of C1-INH-HAE type II patients from the analysis.

Twenty-nine of the 113 examined families had at least one affected member who was carrier of the *F12*-46C/T polymorphism (heterozygotes or homozygotes) and at least one affected wild-type (CC) member. The comparison of phenotypic features between carriers (49 heterozygotes and four homozygotes) and wild-type members ( $n = 51$ ) of these families revealed that the need for LTP was not different ( $P > 0.05$ ) but the age at disease onset was significantly affected by the carriage of *F12*-46C/T polymorphism (wild-type:  $10.5 \pm 8.9$  years, heterozygotes:  $15.6 \pm 8.2$  years, homozygotes:  $25.5 \pm 18.5$  years, Kruskal–Wallis test  $P = 0.0005$ ) (Fig. 1B).

The factors influencing the heterogeneous clinical manifestation of C1-INH-HAE represent one of the oldest unsolved problems of the disease. Our results confirm preliminary data by Bors et al. (6), and provide strong evidence that the carriage of the *F12*-46C/T polymorphism is independently associated with a less severe clinical phenotype of C1-INH-HAE. This fact may be attributed to the effect that the polymorphism has on the synthesis of FXII. The *F12*-46C/T polymorphism is located in the promoter region of the *F12* gene at nt46 creating a new initiation codon (ATG) for transcription of the mRNA and a frameshift that produces a truncated protein. The T allele destroys the Kozak consensus sequence (GCCAGCCATGG) for translation initiation signaling and prevents proper recognition of the translation initiation site (13, 14). However, despite the fact that the *F12*-46C/T polymorphism leads to a decreased *F12* transcription, and plasma FXII antigenic level and activity (13), its possible consequences on the production of bradykinin remain to be elucidated.

Our novel finding indicates that genetic alterations in genes, other than *SERPING1*, encoding proteins other than C1-INH involved in the pathogenesis of angioedema, may modify C1-INH-HAE manifestation. From the practical point of view, the detection of *F12*-46C/T polymorphism might be useful for the prediction of the age of disease onset in a healthy offspring carrying the *SERPING1* mutation of their C1-INH-HAE suffering parent. Moreover, given that an earlier onset of symptoms appears to be inversely correlated with the subsequent course of the disease and, eventually, the need of LTP (18, 19), carriage of this polymorphism might be a predictor of the severity of the disease.

#### Author contributions

MS, HF, MM, and AEG designed the study. AS performed the genetic analysis. MK and MS analyzed the data. DC, NK, FP, DM, MM, and LV collected the patients' clinical data. MS and AS supervised the analysis. MS and AEG wrote the manuscript. All authors reviewed the manuscript.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Methods and statistical analysis.

## References

1. Bork K, Davis-Lorton M. Overview of hereditary angioedema caused by C1-inhibitor deficiency: assessment and clinical management. *Eur Ann Allergy Clin Immunol* 2013;**45**:7–16.
2. Zuraw BL. Hereditary angioedema. *N Engl J Med* 2008;**359**:1027–1036.
3. Zuraw BL. The pathophysiology of hereditary angioedema. *World Allergy Organ J* 2010;**3**(9 Suppl.):S25–S28.
4. Kaplan AP. Bradykinin and the pathogenesis of hereditary angioedema. *World Allergy Organ J* 2011;**4**:73–75.
5. Walford HH, Zuraw BL. Current update on cellular and molecular mechanisms of hereditary angioedema. *Ann Allergy Asthma Immunol* 2014;**112**:413–418.
6. Bors A, Csuka D, Varga L, Farkas H, Tordai A, Füst G et al. Less severe clinical manifestations in patients with hereditary angioedema with missense C1INH gene mutations. *J Allergy Clin Immunol* 2013;**131**:1708–1711.
7. Speletas M, Szilagyi A, Psarros F, Moldovan D, Magerl M, Kompoti M et al. Hereditary angioedema: molecular and clinical differences among European populations. *J Allergy Clin Immunol* 2015;**135**:570–573.
8. Charignon D, Ghannam A, Defendi F, Ponard D, Monnier N, López Trascasa M et al. Hereditary angioedema with F12 mutation: factors modifying the clinical phenotype. *Allergy* 2014;**69**:1659–1665.
9. 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.
10. Duan QL, Binkley K, Rouleau GA. Genetic analysis of Factor XII and bradykinin catalytic enzymes in a family with estrogen-dependent inherited angioedema. *J Allergy Clin Immunol* 2009;**123**:906–910.
11. Björkqvist J, de Maat S, Lewandrowski U, Di Gennaro A, Oschatz C, Schöning K et al. Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III. *J Clin Invest* 2015;**125**:3132–3146.
12. 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.
13. Kanaji T, Okamura T, Osaki K, Kuroiwa M, Shimoda K, Hamasaki N et al. A common genetic polymorphism (46 C to T substitution) in the 5'-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. *Blood* 1998;**91**:2010–2014.
14. Lammle B, Willemin W, Huber I, Krauskopf M, Zurcher C et al. Thromboembolism and bleeding tendency in congenital factor XII deficiency: a study of 74 subjects from 14 Swiss families. *Thromb Haemost* 1991;**65**:117–121.
15. Speletas M, Boukas K, Papadopoulou-Alataki E, Tsitsami E, Germentis AE. Hereditary angioedema in Greek families caused by novel and recurrent mutations. *Hum Immunol* 2009;**70**:925–929.
16. Bowen T, Cicardi M, Farkas H, Bork K, Longhurst HJ, Zuraw B et al. 2010 International consensus algorithm for the diagnosis, therapy and management of hereditary angioedema. *Allergy Asthma Clin Immunol* 2010;**6**:24.
17. Zito F, Lowe GDO, Rumley A, McMahon AD, Humphries SE. Association of the factor XII 46C>T polymorphism with risk of coronary heart disease in the WOSCOPS study. *Atherosclerosis* 2002;**165**:153–158.
18. Farkas H, Varga L, Szeplaki G, Visy B, Harmat G, Bowen T. Management of hereditary angioedema in pediatric patients. *Pediatrics* 2007;**120**:713–722.
19. 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.

## Appendix S1. Methods and statistical analysis

The detection of the *F12-46C/T* polymorphism was performed by PCR-RFLP. Briefly, a total of 100-200 ng of DNA was amplified by PCR in a 30  $\mu$  l reaction mixture using 62.5  $\mu$ M of each deoxynucleoside triphosphate, 20 pmol of each primer, 1.5 mM MgCl<sub>2</sub>, 10% DMSO and 1.0 U Taq polymerase (Bioline, London, UK) in a buffer supplied by the manufacturer. The PCR primers were forward primer 5' - GATAGGCAGCTGGACCAACG - 3' and reverse primer 5'- TGATAGCGACCCCCAGAAC -3' and the PCR conditions were 2 min at 94 °C, followed by 34 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and 5 min at 72 °C after the last cycle. The protocol was designed on the basis of the fact that the wild-type allele results in the creation of a DNA sequence recognized by the restriction enzyme *BsaHI* (New England BioLabs, Hitchin, UK). The presence of undigested PCR products (142 bp) was indicative of homozygous samples, whereas the wild-type samples resulted in the digestion of the PCR products to 116-bp and 26-bp fragments. All PCR and digestion procedures were carried out in the PCR engine apparatus PTC-200 (MJ Research, Watertown, MA), and the PCR and digestion products were analyzed in 3% Tris-borate-EDTA agarose gels. To confirm the PCR-RFLP analysis results, randomly chosen PCR products positive and negative for the *F12-46C/T* polymorphism were purified by use of a PCR purification system (Invitrogen, Life Technologies, Thessaloniki, Greece) and were directly sequenced using an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA) and a BigDye Terminator DNA sequencing kit (Applied Biosystems).

Appropriate statistical tests were used for the analysis of the results. Given, however, that our patient population consisted of related subjects (members of the same families), we implemented generalized estimating equations (GEE), an extension of the generalized linear model that accounts for the within-subject correlation. GEE was used to model the association of response variables (age at disease onset, need for long-term prophylactic treatment) with explanatory variables (race, gender, genotype, C1-INH-HAE type, clinical manifestations such as attacks affecting the larynx or the abdomen as well as *SERPING1* mutational status). In all GEE models an unstructured correlation structure was used and the Quasi Likelihood Information Criterion (QIC) was used for model selection. Data analysis was performed with SPSS 17.0 (IBM Corporation, NY, 2008).