

RESEARCH LETTER

The functional promoter *F12*-46C/T variant predicts the asymptomatic phenotype of C1-INH-HAE

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To the Editor,

Hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE, OMIM 106100) is a rare autosomal dominant disease with few cases of homozygous mutations reported in consanguineous patients, characterized by swelling of the face, lips, tongue, larynx, abdomen, genitalia or extremities.^{1,2} It is caused by mutations in the *SERPING1* gene, which codes for C1-INH, resulting in low levels of C1-INH (C1-INH-HAE type I, 85% of cases) or normal levels of non-functional C1-INH (C1-INH-HAE type II, 15% of cases).¹⁻³ In addition to C1-INH-HAE, other types of HAE include F12-HAE, PLG-HAE and ANGPT1-HAE, which are caused by specific mutations in the factor XII (*F12*) gene, plasminogen (*PLG*) gene, and angiopoietin-1 (*ANGPT1*) gene, respectively.⁴

C1-INH-HAE is characterized by highly variable clinical expression, as some patients suffer frequent, life-threatening attacks, others mild, while some remain asymptomatic throughout their entire life.³ Despite great efforts in clarifying the severity of the disease, variability in clinical expression remains an unsolved issue. Mutation type, specifically mutations with a clear effect on C1-INH function, accounts for a more severe disease phenotype, the need for long-term prophylaxis, and earlier disease onset.^{3,5-8} However, only a small proportion of the variance can be predicted with the *SERPING1* gene mutation type, and even in families with several affected members sharing the same mutation, differences in disease severity are well known, and by some estimations, up to 14% of carriers of the *SERPING1* gene mutation remain asymptomatic throughout their entire life.^{3,5,6} Therefore, research on genetic predisposition influencing the clinical expression of the disease and identifying patients who

are more likely to develop severe and frequent attacks in comparison to individuals who are more likely to be asymptomatic despite carrying the same disease-causing *SERPING1* gene mutation has been focused on other genes mainly involved in bradykinin metabolism. The most promising functional variant in the *F12* gene (*F12*-46C/T, rs1801020) was found (also by our group) to be strongly associated with disease onset.^{6,7,9} Since the association of this *F12* variant with disease onset was clearly demonstrated, we speculated that it might also predict the asymptomatic phenotype of C1-INH-HAE.

In the present study, we investigated whether the frequency of the *F12*-46C/T variant differs between symptomatic and asymptomatic C1-INH-HAE patients and whether it is a modifying genetic factor predisposing to the appearance of symptoms. To achieve that, a large cohort of 88 clinically well-characterized C1-INH-HAE south-eastern European patients, including nine, asymptomatic adults, from 42 unrelated families, were analysed for the *F12*-46C/T variant as described previously.⁶ For more details, see Data S1.

The genotype distributions of the *F12*-46C/T (rs1801020) variant were in Hardy-Weinberg equilibrium (chi-squared test *P* value = .741). When addressing the possible association of the *F12*-46C/T (rs1801020) variant with the appearance of clinical symptoms in 88 C1-INH-HAE patients and including only adult asymptomatic carriers of the *SERPING1* gene mutation (since the majority of patients, over 90%, are symptomatic by the age of 23 years^{2,10}), we found striking differences in frequencies of the *F12*-46C/T variant between symptomatic and asymptomatic C1-INH-HAE patients (Table 1, Figure 1). Genotype CC is highly over-represented in symptomatic C1-INH-HAE patients in comparison to asymptomatic

TABLE 1 Distribution of allele and genotype frequencies for variant F12-46C/T (rs1801020) in symptomatic and asymptomatic C1-INH-HAE patients

F12-46C/T (rs1801020)	Symptomatic	Asymptomatic		Fisher's exact test P value, OR (CI 95%)	GEE P value, β coefficient
Allele, N (%)					
C	127 (80)	9 (50)	C vs T	.007, 4.10 (1.56-10.68)	
T	31 (20)	9 (50)			
Genotype, N (%)					
CC	50 (63)	2 (22)	CC vs CT + TT	.029, 6.03 (1.16-29.66)	.044, .133
CT	27 (34)	5 (56)	CC vs TT	.022, 25.00 (2.23-280.1)	.030, .449
TT	2 (3)	2 (22)			

patients (63% vs 22%, CC vs CT + TT, Fisher's exact test: $P = .029$, OR = 6.03, 95% CI 1.16-29.66, GEE: $P = .044$, β coefficient = .133, adjusted for gender and age; GEE: $P = .048$, β coefficient = .127, adjusted for gender, age and mutation group). Similarly, the C allele was significantly more often in symptomatic than in asymptomatic (80% vs 50%, Fisher's exact test: $P = .007$, OR = 4.10, 95% CI 1.56-10.68). Using the additive homozygous model, we found that carriers of the CC genotype have a 25-fold greater risk of developing symptoms in comparison to carriers of the TT genotype (CC vs TT, Fisher's exact test: $P = .022$, OR = 25.00, 95% CI 2.23-280.1, GEE: $P = .030$, β coefficient = .449, adjusted for gender and age; GEE: $P = .045$, β coefficient = .433, adjusted for gender, age and mutation group). Additionally, there were significant differences in serum levels of FXII between F12-46C/T genotypes [median (IQR) CC: 31.0 (15.2) $\mu\text{g/mL}$, CT: 24.5 (11.8) $\mu\text{g/mL}$, TT: 17.6 (5.4) $\mu\text{g/mL}$; Kruskal-Wallis test, P value = .005; Figure S1]. However, we observed more modest and statistically non-significant differences between symptomatic and asymptomatic C1-INH-HAE patients [median (IQR) serum levels of FXII: 27.9 (14.9) $\mu\text{g/mL}$ vs 23.5 (16.6) $\mu\text{g/mL}$].

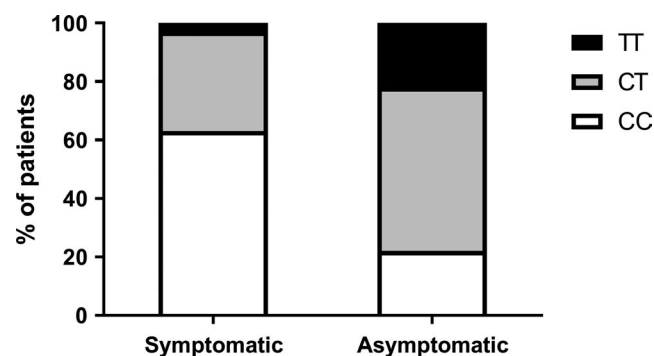
High variability in the clinical expression and incomplete penetrance of C1-INH-HAE remains an unsolved issue. Our results confirm that the F12-46C/T (rs1801020) variant is a modifying factor in C1-INH-HAE disease expression. Furthermore, we have demonstrated for the first time that a genetic factor, the F12-46C/T variant, can influence the penetrance of C1-INH-HAE. Our results

indicate that the T allele in F12-46C/T might be protective, as the C allele and CC genotype were highly over-represented among symptomatic C1-INH-HAE patients. The F12-46C/T variant is located in the promoter region, and the presence of the T allele results in a decreased transcription of F12 and lower FXII plasma concentration and activity, which was also confirmed in our study.¹¹ Similarly, F12 mutations responsible for F12-HAE were shown to enhance susceptibility of FXII activation by plasmin and activation of the kallikrein-kinin system, resulting in increased production of bradykinin.⁴ Higher activity of FXII, which is not properly controlled due to deficient C1-INH, could ultimately result in even higher bradykinin production (cause uncontrolled bradykinin production). This in turn could explain not only the reason for the association of the F12-46C/T variant with earlier disease onset, as reported previously^{3,6,7,9} but also the differences in genotypes between clinically affected and symptom-free individuals (carriers of the SERPING1 gene mutation). Furthermore, the frequencies of F12-46C/T genotypes differ significantly between various ethnic groups (GnomAD), and the highest frequency of the protective TT genotype reported in East Asians correlates with lower reported prevalence of C1-INH-HAE, and more asymptomatic and later disease onset in comparison to Caucasians, who have a lower reported frequency of TT genotype.^{2,5,6,8,10,12} If our results are confirmed in larger sample sizes and in different ethnic groups, the information on the F12-46C/T variant could contribute to improvements in the management of this potentially life-threatening disease. The detection of the F12-46C/T (rs1801020) variant in infants carrying the SERPING1 mutation before the appearance of clinical symptoms could be useful to predict the age of disease onset and disease expression.

In conclusion, our novel findings indicate that the F12-46C/T variant is an important factor modifying the clinical expression of C1-INH-HAE. More importantly, the F12-46C/T variant may differentiate between clinically affected and symptom-free individuals and add new insights for our understanding of the clinical variability in C1-INH-HAE expression.

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**FIGURE 1** Distribution of genotype frequencies for variant F12-46C/T (rs1801020) in symptomatic and asymptomatic C1-INH-HAE patients

diagnosis and management of patients with hereditary angioedema and to T. Krumpstar for his skilful assistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

MR and PK were involved in conception and design of the study; MK, SA, LKL and VGP recruited the subjects, collected relevant clinical data and were involved in interpretation of the data; MR performed the laboratory experiments and, data analysis, interpreted the data and wrote the main manuscript. All authors read, revised, and approved the final version of the manuscript for publication.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

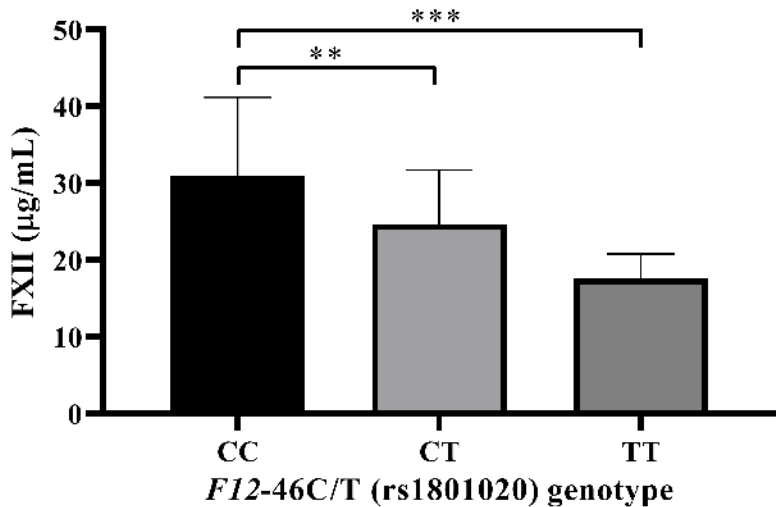
1. Zuraw B. Hereditary angioedema. *N Engl J Med*. 2008;359:1027-1036.
2. Maurer M, Magerl M, Ansotegui I, et al. The international WAO/EAACI guideline for the management of hereditary angioedema—The 2017 revision and update. *Allergy*. 2018;73:1575-1596.
3. Garmen AE, Speletas M. Genetics of hereditary angioedema revisited. *Clin Rev Allergy Immunol*. 2016;51:170-182.

4. Zuraw BL. Hereditary angioedema with normal C1 inhibitor: four types and counting. *J Allergy Clin Immunol*. 2018;141:884-885.
5. Andrejević S, Korošec P, Šilar M, et al. Hereditary angioedema due to C1 inhibitor deficiency in Serbia: two novel mutations and evidence of genotype-phenotype association. *PLoS ONE*. 2015;10:e0142174.
6. Grivčeva-Panovska V, Košnik M, Korošec P, Andrejević S, Karadža-Lapić L, Rijavec M. Hereditary angioedema due to C1-inhibitor deficiency in Macedonia: clinical characteristics, novel SERPING1 mutations and genetic factors modifying the clinical phenotype. *Ann Med*. 2018;50:269-276.
7. Bors A, Csuka D, Varga L, et al. Less severe clinical manifestations in patients with hereditary angioedema with missense C1INH gene mutations. *J Allergy Clin Immunol*. 2013;131:1708-1711.
8. Speletas M, Szilagyi A, Psarros F, et al. Hereditary angioedema: Molecular and clinical differences among European populations. *J Allergy Clin Immunol*. 2015;135:570-573.
9. Speletas M, Szilagyi A, Csuka D, et al. F12-46C/T polymorphism as modifier of the clinical phenotype of hereditary angioedema. *Allergy*. 2015;70:1661-1664.
10. 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.
11. Kanaji T, Okamura T, Osaki K, 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.
12. Jung J-W, Suh D, Park H, et al. Clinical features of hereditary angioedema in Korean patients: a Nationwide Multicenter Study. *Int Arch Allergy Immunol*. 2018;176:272-279.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supporting information

Data S1.

Patients

Eighty-eight C1-INH patients, including nine asymptomatic adults, from 42 unrelated families were included (Table E1) (Croatia, 13 symptomatic and 3 asymptomatic ¹; Macedonia, 13 symptomatic and 1 asymptomatic ²; Serbia, 40 symptomatic and 4 asymptomatic ³; and Slovenia, 13 symptomatic and 1 asymptomatic ⁴). In all patients, the diagnosis was confirmed by laboratory results (C1 inhibitor antigenic levels and C1 inhibitor function), and the disease-causing *SERPING1* mutation was identified, as previously reported ¹⁻⁴. For the association analysis, we only included adult asymptomatic patients aged 23 years or more, since the majority of patients, over 90%, are symptomatic by the age of 23 years^{5,6}. The study was conducted in accordance with the amended Declaration of Helsinki and all participants or the relatives of minor participants provided written informed consent.

Materials and Methods

The detection of the *F12*-46C/T (rs1801020) variant was performed by Sanger sequencing, using primers *F12*-46C/T-F 5'-GCTTTCCACAAACAGCCTGT-3' and *F12*-46C/T-R 5'-CCACAGCACTCACCGAAAG-3', as described previously^{2,4,7}.

The serum concentration of Factor XII was measured in 69 symptomatic and 9 asymptomatic C1-INH-HAE patients using an Abcam ELISA kit (Cambridge, UK) according to the manufacturer's instructions.

Statistical analysis

Data distribution was evaluated using the D'Agostino–Pearson test. Parametric statistics (unpaired t-tests) were used on normally distributed data, whereas non-parametric statistics (the Mann–Whitney) were used if the distribution deviated from normal. The two-sided Fisher's exact test was used to calculate the significance of the differences in frequencies of the *F12-46C/T* variant between symptomatic and asymptomatic C1-INH-HAE patients. A generalized estimating equation (GEE) with a robust covariate matrix and exchangeable correlation structure was implemented to model the association of response variables (symptoms) with explanatory variables (variant, age, and gender) because our patient population consisted of related subjects. Patient symptom status (symptomatic/asymptomatic) was entered as binary variables in the logistic GEE model. GraphPad Prism 5.0 software (San Diego, CA, USA), and SPSS 21.0 software (Chicago, IL, USA) was used for statistical analysis. A P-value of less than 0.05 was considered statistically significant.

References

1. Karadža-Lapić L, Korošec P, Šilar M, et al. Frequent life-threatening laryngeal attacks in two Croatian families with hereditary angioedema due to C1 inhibitor deficiency harbouring a novel frameshift mutation in SERPING1. *Ann Med*. 2016;48:485-491.
2. Grivčeva-Panovska V, Košnik M, Korošec P, Andrejević S, Karadža-Lapić L, Rijavec M. Hereditary angioedema due to C1-inhibitor deficiency in Macedonia: clinical characteristics, novel SERPING1 mutations and genetic factors modifying the clinical phenotype. *Ann Med*. 2018;50:269-276.
3. Andrejević S, Korošec P, Šilar M, et al. Hereditary Angioedema Due to C1 Inhibitor Deficiency in Serbia: Two Novel Mutations and Evidence of Genotype-Phenotype Association. *PLoS One*. 2015;10:e0142174.
4. Rijavec M, Korošec P, Šilar M, et al. Hereditary Angioedema Nationwide Study in Slovenia Reveals Four Novel Mutations in SERPING1 Gene. *PLoS One*. 2013;8:e56712.
5. Maurer M, Magerl M, Ansotegui I, et al. The international WAO/EAACI guideline for the management of hereditary angioedema—The 2017 revision and update. *Allergy*. 2018;73:1575-1596.
6. 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.
7. Bors A, Csuka D, Varga L, et al. Less severe clinical manifestations in patients with hereditary angioedema with missense C1INH gene mutations. *J Allergy Clin Immunol*. 2013;131:1708-1711.

Table E1. Clinical characteristic of C1-INH-HAE patients.

	Symptomatic	Asymptomatic†	Fisher's exact test, P
N individuals, families	79, 42	9, 7	
Age, years, mean (SD)	42 (16)	51 (20)	NS
Male sex, N (%)	38 (48)	5 (56)	NS
C1-INH-HAE type, type I, N (%)	70 (89)	8 (89)	NS
Age at onset of symptoms, years, mean (SD)	12 (8)	ND	
Clinical severity score, mean (SD)	6.6 (2.2)	0 (0)	
Skin oedema, N (%)	78 (99)	0 (0)	
Facial oedema, N (%)	65 (82)	0 (0)	
Abdominal oedema, N (%)	59 (75)	0 (0)	
Laryngeal oedema, N (%)	58 (73)	0 (0)	
Prophylactic treatment, N (%)	36 (46)	0 (0)	

†Only adult (≥ 23 years old) asymptomatic.

Figure E1. Correlation between *F12*-46C/T (rs1801020) genotypes and concentration of Factor XII in C1-INH-HAE patients' serum samples.
Mann-Whitney test; ** *P* value < 0.01; *** *P* value < 0.001.