

Letter to the Editor

Less severe clinical manifestations in patients with hereditary angioedema with missense *C1INH* gene mutations

To the Editor:

Hereditary angioedema (HAE-C1INH) (OMIM#106100) caused by reduced level (type I) or impaired function (type II) of the C1 inhibitor (C1INH) is a rare disorder characterized by edematous attacks. The rate, location, and severity of recurring edemas are highly variable among patients.¹⁻³ In spite of repeated efforts aimed at clarifying the underlying causes of this heterogeneity, no definite explanation has been published yet. Thus, the aim of the current study was to analyze genotype-phenotype correlations in a well-characterized group of patients with HAE-C1INH.

The study population of 106 subjects of Caucasian origin, comprising 39 unrelated patients and their family members diagnosed with type I HAE-C1INH, included all patients followed up at the Hungarian HAE center from 2006 (see Table E1 in this article's Online Repository at www.jacionline.org). The annual numbers of attacks of different locations, as well as the number of ampoules of human plasma-derived C1INH concentrate (500 international units; Berinert; CSL Behring, Marburg, Germany) consumed over a year, were recorded for each patient for 5 years between 2006 and 2010. Besides the annual frequencies of these parameters, the age at the occurrence of the first attack was included in the analysis. The local institutional review board approved the study, and all subjects gave informed consent.

For more details, see the Methods section in this article's Online Repository at www.jacionline.org; in brief, screening for mutations of the *C1INH* gene (approved gene symbol *SERP-ING1*) was done by sequencing after PCR amplification while relative quantification of exons—where no mutation was detected by sequencing—was performed by using quantitative PCR.⁴ The genotype of factor XII (*F12*) 46C/T (rs1801020) polymorphism was determined by using PCR-RFLP.⁵

Mutations, found in the studied 39 families, are summarized in Table E2 in this article's Online Repository at www.jacionline.org. Based on a preliminary analysis comparing the characteristics of the clinical course among the carriers of different types of mutations, only missense mutations exhibited a significant difference from the other categories. Therefore, patients with missense mutations were then compared with those carrying other types of mutations of the *C1INH* gene. Two types of evaluations were carried out. In type 1, only 1 patient—who was first admitted to the center—from each family, while in type 2, all patients of the cohort were included in the analysis. In type 2 analysis, significance was estimated by applying the Mann-Whitney test or the Poisson generalized estimating equation that accounts for the clustering of patients within families.

In both types of evaluation, the median value of the annual frequency of the sum of all types of attacks as well as that of subcutaneous and abdominal attacks was significantly lower in the group with missense mutations (Table I). The evaluation of severe attacks (abdominal and upper airway attacks) also revealed a significant difference between the 2 groups. Patients with missense mutations consumed significantly less C1INH concentrate than did the other cohort of study subjects. According to the

type 2 evaluation, the first symptoms appeared at a significantly higher age in patients with than in those without missense mutations. Of note, similar differences were obtained when the median values of each variable calculated from data of all members of each family were considered.

Next, restricted to the unrelated patients, we performed a multiple logistic regression analysis adjusted to the sex and the age of the patients (1-year increment), as well as the use of the attack-preventing drug danazol (yes/no). Dependent variables (different markers of disease characteristics) were dichotomized by using receiver operating characteristic curve analysis, to achieve the best possible discrimination power between low and high values. Missense mutation carriers had a more than 10 times lower adjusted odds (odds ratio, 0.089; 95% CI, 0.009-0.895; $P = .040$) for high (>1 per year) attack frequency than did noncarriers. The odds ratio was even lower (odds ratio, 0.017; 95% CI, 0.001-0.423; $P = .013$) when only high number (>0.4 per year) of severe attacks were considered. Of note, when Poisson generalized linear model was applied, missense mutation carriers showed a lower relative risk for high number of all types of attacks ($\exp(B) = 0.326$; 95% CI, 0.129-0.819; $P = .017$) and severe attacks ($\exp(B) = 0.216$; 95% CI, 0.052-0.892; $P = .034$) as well.

Finally, we tested the 46T>C polymorphism of the *F12* gene and found an association between the single nucleotide polymorphism and the age of the onset of the first symptoms, in both types of evaluation (Fig 1). Symptoms appeared at a much younger age in homozygous carriers of the C allele than in the remainder of patients.

The aim of the current study was to investigate one of the oldest unsolved problems of HAE-C1INH: the factors influencing its heterogeneous clinical manifestation.¹⁻³ Our novel findings indicate that disease manifestations are at least partially determined by genetic variations in the genes encoding proteins involved in the pathogenesis of angioedema. We established that the missense mutations of the *C1INH* gene are associated with a less severe form of the disease, as indicated by lower annual attack frequency and C1INH concentrate usage found in missense mutation carriers compared with the remainder of patients.

The pathological nature of amino acid substitutions in the C1INH protein was analyzed in only a few functional studies. As for the mutations found in our study population, experimental data are available on 3 missense mutations. One of these (Cys108Tyr) presumably lowers molecular stability and induces a latent conformation by removing a cysteine involved in an intramolecular disulphide bridge.⁶ Two mutations—Val451Met and Pro476Ser located at the same residues as 2 substitutions identified in our study (Val451Glu and Pro476Arg, respectively)—have been shown to result in a partially secreted protein that is dysfunctional in binding to target C1s, and therefore, phenotypes of their carriers were considered as an intermediate between the classically defined type I and type II.⁷ It is rational to hypothesize that if particular (or all) missense mutations result in a reduced amount of C1INH with a residual activity against target proteases, it may influence the symptoms, as consumption of the enzyme to the level where the unregulated activation of complement and contact cascades results in edema generation can occur less frequently. Another possible explanation for the observed, milder manifestation of HAE-C1INH with missense mutations is the differential regulation

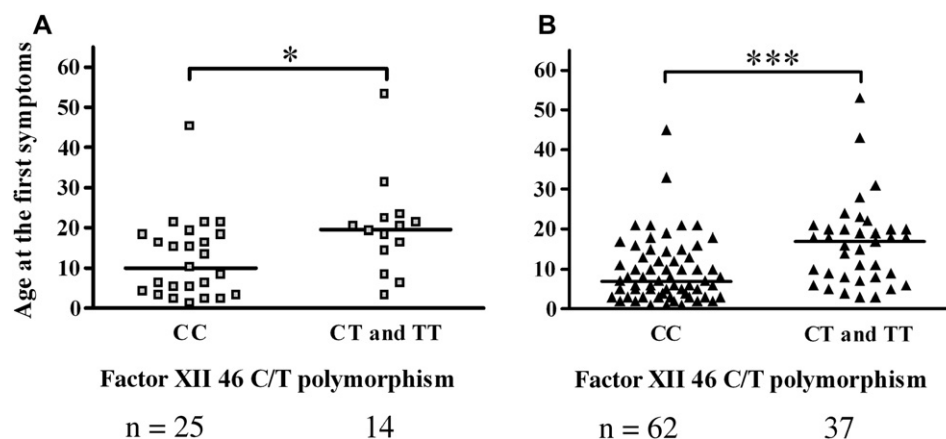
TABLE I. Differences in the clinical course of the disease between patients with HAE carrying missense mutations, or other mutations of the *C1INH* gene

Clinical property, median (interquartile range)	Genetically unrelated patients			All patients		
	Missense (n = 7)	Nonmissense (n = 32)	P value*	Missense (n = 11)	Nonmissense (n = 95)	P value* P value†
Age at the onset of first symptoms (y)	18.0 (10.0-23.0)	14.3 (5.0-19.8)	.174	18.0‡ (14.0-28.0)	8.5‡ (5.0-16.3)	.008 .001
Annual attack frequency	0.8 (0.2-5.2)	7.2 (2.5-10.4)	.008	0.5 (0.0-4.0)	4.6 (1.4-10.0)	.004 <.001
Subcutaneous attacks	0.6 (0.2-3.2)	4.1 (1.2-7.8)	.033	0.0 (0.5-3.2)	2.2 (0.6-5.8)	.027 .001
Abdominal attacks	0.0 (0.0-0.4)	0.8 (0.2-3.1)	.022	0.0 (0.0-0.4)	0.8 (0.0-2.8)	.009 .002
Severe attacks (abdominal and laryngeal)	0.0 (0.0-0.4)	1.5 (0.5-4.1)	.007	0.0 (0.0-0.4)	1.0 (0.2-3.4)	.003 .002
Annual frequency of C1INH concentrate usage	0.0 (0.0-0.2)	0.5 (0.1-2.0)	.022	0.0 (0.0-0.2)	0.4 (0.0-1.4)	.032 <.001

Significant values are shown in boldface.

*Significance was estimated by applying the Mann-Whitney test.

†Significance was estimated considering correlation among family members by applying Poisson generalized estimating equations with independent correlation matrix and robust variance.

‡The number of symptom-free patients with *C1INH* deficiency in the group of subjects carrying missense and nonmissense mutations was 2 and 5, respectively.**FIG 1.** Age at the initial onset of symptoms in patients carrying different genotypes of the *F12* gene. Symptoms appeared at a younger age in patients with homozygous CC genotype than in carriers of the T allele considering either unrelated (**A**, Mann-Whitney test, $P = .013$) or all symptomatic patients (**B**, Mann-Whitney test, $P = .0002$; Poisson generalized estimating equation, $P = .001$). Lines indicate median values. * $P < .05$ and *** $P < .001$.

of transcription in its carriers. A detailed evaluation of total *C1INH* messenger RNA expression did not find a difference between controls and patients with missense mutations, but revealed a highly significant decrease in comparison to nonsense and splicing mutation carriers.⁸ This suggests that the transinhibition of wild-type *C1INH* translation by mutant messenger RNA and/or protein may be less pronounced in missense mutants, resulting in a higher expression of normal protein, which could result in a milder phenotype.

Besides *C1INH* mutations, the potential disease-modifying role of a variation in *F12* gene was evaluated. The studied 46C>T single nucleotide polymorphism was reported as the only common variation accounting for the diversity in levels of factor XII⁹ that has an essential role in bradykinin release. Our results indicate that in carriers of the C/C genotype, associated with high factor XII level,⁵ symptoms develop at an earlier age than in patients carrying the T allele.

The main strength of our study is the inclusion of a well-characterized and closely monitored patient population, followed up by the same clinician for the last 5 years or longer. However, we are aware of the limitations of our study, namely, low sample size and the inclusion of a relatively small number of mutations. As only 6 missense mutations occurred in the studied group, we cannot rule

out that our findings are true only for some of the missense mutations in the *C1INH* gene while others cause more severe phenotype. Hence, detailed evaluation of different missense mutations, particularly interaction kinetics with target proteases, should be undertaken to clarify their role as phenotype-modifying factors.

Our findings may prove clinically relevant as—provided they will be confirmed in additional populations—the assessment of these variations may be considered an essential component of the initial workup of new cases and potentially useful also for patient counseling.

We thank Dr Zoltán Prohászka for his valuable comments and suggestions on this work.

Andras Bors, PhD^a
Dorottya Csuka, MSc^b
Lilian Varga, PhD^b
Henriette Farkas, MD, DSc^b
Attila Tordai, MD, DSc^a
George Füst, MD, DSc^{b,†}
Agnes Szilagyi PhD^b

From ^athe Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, and ^bthe 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary. E-mail: szilagi@kut.sote.hu.

†Deceased.

The National Scientific Research Fund (grant nos. OTKA-NKTH 80842 and OTKA K 100886) supported this work.

Disclosure of potential conflict of interest: D. Csuka has received one or more payments for travel/accommodations/meeting expenses from CSL Behring, Viropharma, and Shire. H. Farkas has consultancy arrangements with Shire; has received one or more payments for lecturing from or is on the speakers' bureau for Shire Human Genetic Therapies, Inc, Pharming, CSL Behring, and Viropharma; and has received one or more payments for travel/accommodations/meeting expenses from CSL Behring, Viropharma, and Shire. G. Füst has been supported by one or more grants from or has one or more grants pending with and has received one or more payments for travel/accommodations/meeting expenses from the National Research Fund. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Agostoni A, Aygören-Pürsün E, Binkley KE, Blanch A, Bork K, Bouillet L, et al. Hereditary and acquired angioedema: problems and progress: Proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. *J Allergy Clin Immunol* 2004;114:S51-131.
2. Bork K, Meng G, Staubach P, Hardt J. Hereditary angioedema: new findings concerning symptoms, affected organs, and course. *Am J Med* 2006;119:267-74.
3. Cicardi M, Zanichelli A. Angioedema due to C1 inhibitor deficiency in 2010. *Intern Emerg Med* 2010;5:481-6.
4. Kalmar L, Bors A, Farkas H, Vas S, Fandl B, Varga L, et al. Mutation screening of the C1 inhibitor gene among Hungarian patients with hereditary angioedema. *Hum Mutat* 2003;22:498.
5. 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;91:2010-4.
6. Simonovic I, Patston PA. The native metastable fold of C1-inhibitor is stabilized by disulfide bonds. *Biochim Biophys Acta* 2000;1481:97-102.
7. Verpy E, Couture-Tosi E, Eldering E, Lopez-Trascasa M, Spath P, Meo T, et al. Crucial residues in the carboxy-terminal end of C1 inhibitor revealed by pathogenic mutants impaired in secretion or function. *J Clin Invest* 1995;95:350-9.
8. de la Cruz RM, Lopez-Lera A, Lopez-Trascasa M. Analysis of SERPING1 expression on hereditary angioedema patients: quantitative analysis of full-length and exon 3 splicing variants. *Immunol Lett* 2012;141:158-64.
9. Calafell F, Almasy L, Sabater-Lleal M, Buil A, Mordillo C, Ramirez-Soriano A, et al. Sequence variation and genetic evolution at the human F12 locus: mapping quantitative trait nucleotides that influence FXII plasma levels. *Hum Mol Genet* 2010;19:517-25.

<http://dx.doi.org/10.1016/j.jaci.2012.11.015>

METHODS

Patients

The study was performed in 106 patients of Caucasian origin with type I HAE-C1INH, comprising 39 unrelated patients and their available family members diagnosed with HAE-C1INH (Table E1). All patients followed up at the Hungarian HAE center between 2006 and 2010 were included in the study. HAE-C1INH was diagnosed according to international consensus criteria^{E1,E2}: if the patients had recurrent edematous attacks, their levels of functional and antigenic C1INH were repeatedly below normal, with a low C4 concentration. We also included 7 asymptomatic patients with low levels of functional and antigenic C1INH as well as a family history of HAE-C1INH. In 33 families, the diagnosis was confirmed by identifying a mutation of the *SERPINC1* (*C1INH*) gene. The annual follow-up of the patients included recording the details of the therapy administered along with the number of attacks of different location, as excerpted from the patients' diaries. In addition, the serum concentrations of C4 and C1INH and the functional activity of the latter were measured at each visit. The local institutional review board approved the study, and all subjects gave informed consent.

Sample collection and analysis

EDTA-anticoagulated blood samples were obtained for genomic DNA preparation by using the salting-out method of Miller et al.^{E3} Screening for mutations was done by DNA sequencing after PCR amplification as reported previously,^{E4} with the following modifications. Amplification of genomic DNA was carried out in 35 cycles (95°C, 30 seconds; 55°C, 40 seconds; 72°C, 60 seconds), and PCR products were purified with the ExoSAP-IT cleaning method. To confirm the presence of a particular mutation, alternative amplification primers were used or another affected family member was sequenced. In those patients in which no mutation was detected by sequencing, dosage analysis was performed by applying the sequencing primers for exons 3 to 8 to detect whole exon deletions (as according to Stoppa-Lyonnet et al,^{E5} the deletion of only exons 1 and 2 is very unlikely). Relative quantification by quantitative PCR was performed as described previously,^{E4} except for amplification (55 cycles of 95°C, 10 seconds; 55°C, 10 seconds; 72°C, 20 seconds from 20 ng whole genomic DNA).

Genotyping of *F12* 46C/T (rs1801020) polymorphism was carried out by using PCR-RFLP as reported previously.^{E6}

Complement measurements

To quantify C4 and antigenic C1INH levels, radial immunodiffusion was performed by using antibodies from DiaSorin (DiaSorin Inc, Stillwater, Minn) and a human calibrator serum (DAKO, Glostrup, Denmark) for C4, as well as

an in-house calibrator for C1INH. The functional concentration of C1INH was measured with an enzyme immunoassay kit (Quidel, San Diego, Calif).

Statistical analysis

Statistical analysis was performed by using the GraphPad Prism 3.0 and IBM SPSS Statistics 20 software. Between-group differences were evaluated by using the Mann-Whitney test or Poisson generalized estimating equations (with robust covariance matrix and independent correlation matrix), a method introduced by Zeger and Liang^{E7} that accounts for correlation among family members. Cut points with optimum sensitivity and specificity for the selected indicators of the disease course were obtained with receiver operating characteristic analysis. The strength of association between genetic variations and the indicators was calculated by using multiple logistic regression or Poisson generalized linear models (with robust covariance matrix), adjusted to the age and sex of the patients, as well as treatment with danazol. Possible confounding variables for adjustment were selected on the basis of significance in univariate tests (sex, age) or prior knowledge (treatment with danazol was included in the model of attack frequency as this drug is frequently administered to prevent attacks). In the case of generalized models, the number of attacks during the tested 5-year period was included instead of annual attack frequency (to avoid working with nonintegers).

REFERENCES

- E1. 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.
- E2. Cicardi M, Bork K, Caballero T, Craig T, Li HH, Longhurst H, 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;67:147-57.
- E3. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- E4. Kalmar L, Bors A, Farkas H, Vas S, Fandl B, Varga L, et al. Mutation screening of the C1 inhibitor gene among Hungarian patients with hereditary angioedema. *Hum Mutat* 2003;22:498.
- E5. Stoppa-Lyonnet D, Duponchel C, Meo T, Laurent J, Carter PE, Arala-Chaves M, et al. Recombinational biases in the rearranged C1-inhibitor genes of hereditary angioedema patients. *Am J Hum Genet* 1991;49:1055-62.
- E6. 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;91:2010-4.
- E7. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986;42:121-30.

TABLE E1. The demographic and clinical characteristics of the 106 patients with HAE-C1INH who were studied

Property	Value
Man-to-woman ratio	47/59
Age at inclusion (y), mean $\pm$ SD	37.9 $\pm$ 17.2
Age at the onset of the first symptoms (y), mean $\pm$ SD	12.0 $\pm$ 9.6
Type I/type II HAE-C1INH	106/0
Frequency of all types of attacks per year, median (IQR)	4.0 (1.0-9.6)
Frequency of subcutaneous attacks per year, median (IQR)	2.2 (0.5-5.6)
Frequency of abdominal attacks per year, median (IQR)	0.6 (0.0-2.5)
Frequency of severe (abdominal and laryngeal) attacks per year, median (IQR)	0.8 (0.0-2.8)
Use of C1INH concentrate, ever/never*	61/45
Number of C1INH concentrate administrations, ampoules per year, median (IQR)	0.3 (0.0-1.2)
Danazol use, ever/never	48/58
Mean concentration of C4 (g/L), median (IQR)	0.06 (0.03-0.10)†
Mean concentration of C1INH (g/L), median (IQR)	0.04 (0.03-0.05)†
Mean activity of C1INH (%), median (IQR)	27.6 (16.6-37.5)†

IQR, Interquartile range.

With the exception of demographic data, all values represent the period between 2006 and 2010.

*The administration of C1INH concentrate for prophylaxis (eg, before surgery) was disregarded.

†The normal ranges of C4 and C1INH concentrations are 0.15-0.55 and 0.15-0.3 g/L, respectively. The activity of C1INH was defined as the percentage of pooled normal human plasma with a normal range of 70% to 100%, as determined in our laboratory.

TABLE E2. Summary of the mutations identified in 39 families of HAE-C1INH type I

No. of affected families	Traditional nomenclature	Location	Affected exon	Mutation type
1	Deletion of exons (1-)-3-8*		(1-)-3-8*	Large deletion
1	Met-22Val	g.1769A>G	2	Initiation codon mutation
2	IVS2SD+1G>A	g.1820G>A	Intron 2	Splice site mutation
1	Ser-1X	g.3391C>G	3	Nonsense
1	Gln10X	g.3420C>T	3	Nonsense
2	Cys108Tyr	g.3715G>A	3	Missense
1	2534_2535delCT	g.3716_3717del	3	Small deletion
3	2579-2620del42	g.3761_3802del	3	Small deletion
1	IVS2SD+1G>A	g.3877G>A	Intron 3	Splice site mutation
1	2696-2697insT	g.3878_3879insT	Intron 3	Splice site mutation
1	Gln201X	g.5649C>T	4	Nonsense
3	Deletion of exon 4		4	Large deletion
1	14107delA	g.15289delA	7	Small deletion
2	14126_14127insC†	g.15308_15309insC	7	Small insertion
1	Thr372Pro	g.15363A>T	7	Missense
1	Deletion of exon 7		7	Large deletion
1	Deletion of exons 7 and 8		7-8	Large deletion
1	Asp386Val	g.16106A>T	8	Missense
1	16748_16749delTG†	g.17930_17931delTG	8	Small deletion
1	16749_16774dup	g.17931_17956dup	8	Small insertion
1	16783_16784delTG†	g.17965_17966delTG	8	Small deletion
1	Val451Glu	g.17992T>A	8	Missense
1	16858delC†	g.18040delC	8	Small deletion
1	Gly471Glu	g.18052G>A	8	Missense
1	Arg472X	g.18054C>T	8	Nonsense
1	Pro476Arg	g.18067C>G	8	Missense
6	Mutation not identified			Not identified

Missense mutations are shown in boldface.

*Experimentally proved for exons 3 to 8.

†Previously not reported mutations.