Letter to the Editor

Hereditary angioedema: Molecular and clinical differences among European populations

To the Editor:

Hereditary angioedema due to C1 inhibitor deficiency (HAE-C1-INH) is a rare disorder caused by reduced level (type I) or impaired function (type II) of the C1 inhibitor (C1-INH). C1-INH, encoded by the *SERPING1* gene, is an inhibitor not only of the complement system, but also of various other cascades leading in bradykinin production. The phenotype of the disease is characterized by a large heterogeneity, with its clinical features varying even among members of the same family. More than 400 different *SERPING1* gene alterations associated with HAE-C1-INH have been described, but none of these have been proven to be closely associated with the phenotypic expression of the disease.[1] However, functional or segregation studies in kindreds have been carried out only on a small fraction of *SERPING1* alterations,[2,3] 25% of which represent *de novo* mutations.[4] On the other hand, attempts towards uncovering etiological associations between *SERPING1* alterations and clinical phenotypes in HAE-C1-INH patients are restricted to specific ethnic groups and include a rather low number of patients.[5-9]

Aiming to uncover correlations between genetic defects of *SERPING1* and the disease phenotype, as well as differences between different countries, a large cohort of 149 HAE-C1-INH patients from 79 unrelated families derived from 4 different countries of the European continent (Greece, Germany, Romania, and Hungary) were subjected to molecular analysis of the *SERPING1* gene. Medical records of all the patients were reviewed for disease onset, treatment modalities, and major clinical manifestations. This sample was pooled with patients previously genotyped by our groups,[5,6] and an additional pooled analysis of 265 HAE-C1-INH patients from 117 unrelated families was performed. The local institutional review boards approved the study, and written informed consent was obtained from each individual or an accompanying relative. For more details, see the Methods section and Tables E1 and E2 in this article's Online Repository at www.jacionline.org.

SERPING1 alterations, found in the patients analyzed in this study, are presented in Fig E1 in this article's Online Repository at www.jacionline.org and are summarized in Table I along with those of the pooled sample. Missense mutations were the most common alteration, followed by small deletions/insertions (including frameshift defects). Missense mutations and frameshift defects were more prevalent among Romanian and German patients, respectively ($P < .001$). In 30 patients (38%), there was no family history of HAE and, therefore, they were considered as sporadic cases. In 5 out of these 30 cases, it was confirmed by genetic analysis of the parents. Thirty-eight of the identified defects (48%) were novel and are presented in detail in Table E3 in this article's Online Repository at www.jacionline.org. Several patients and their healthy relatives carried the *SERPING1* polymorphism V480M (Table E4 in this article's Online Repository at www.jacionline.org).

Analysis of all missense mutations identified in this study by the use of 3 different bioinformatic methods (SIFT, PolyPhen2, and MutationTaster, for more details, see the Methods section in this article's Online Repository) uncovered that one of them, namely R366H located in exon 7, might be tolerant (benign). Subsequent molecular analysis of the entire *SERPING1* gene in a Hungarian patient whose disease was initially attributed to this mutation revealed an additional nonsense mutation located within exon 3, namely E85X, which results in a truncated protein and, obviously, represents the causative alteration (Table E4). Extending the bioinformatic analysis to all the 95 amino acid substitutions presented in HAEdb (C1 inhibiTor gene muTATion dATAbAse, available at hae.enzim.hu[7]), we found that several additional alterations previously described by other researchers were estimated to be tolerant for the protein function (Table E5 in this article's Online Repository at www.jacionline.org). Therefore, we suggest that bioinformatic analysis performed in cases of novel (missense) mutations could provide indications for a comprehensive analysis of *SERPING1*.

No defects of the *SERPING1* gene were identified in 5 patients (from 3 families) who displayed typical HAE-C1-INH symptoms and pathologic complement tests (Table E6 in this article's Online Repository at www.jacionline.org). Genomic defects located in intronic or untranslated regions and possibly modifying *SERPING1* expression that are not analyzed by current approaches might be responsible for this finding. Moreover, *SERPING1* alterations might not be the only causative damages of HAE-C1-INH, and factors resulting in increased post-translational consumption of C1-INH could be the factor leading to the disease.

In the pooled cohort of HAE-C1-INH patients, a significantly lower delay in diagnosis was observed in countries with a higher awareness for the disease (especially Hungary) (Fig E3 in this article's Online Repository at www.jacionline.org). The presence of at least 1 episode of larynx edema was found to significantly influence the decision for the initiation of long-term prophylactic treatment (unadjusted OR: 2.77; 95% CI: 1.34-5.72: $P = .007$ for the newly diagnosed patients, and OR: 2.72; 95% CI: 1.58-4.67; $P < .001$ in the pooled sample), with male patients receiving long-term prophylactic treatment (mainly attenuated androgens) more frequently than female patients (OR: 2.38; 95% CI: 1.17-4.84; $P = .017$ for the newly diagnosed patients, and OR: 1.86; 95% CI: 1.11- 3.13; $P = .018$ for the entire cohort of patients). We also observed geographical differences in some clinical manifestations of the disease: Romanian patients displayed a significantly later disease onset compared to other geographical groups (Mann-Whitney *U* test; $P = .009$ and $P = .006$, for the newly diagnosed and the entire cohort of patients, respectively; Fig E3). Hungarian patients less often exhibited abdominal attacks (Mann-Whitney *U* test; $P < .001$), and long-term prophylactic treatment was more often administered in Greek patients compared with other geographical groups (Mann-Whitney *U* test; $P = .024$), a difference which persisted in the pooled sample.

We further investigated the above associations, modeling the long-term treatment as a binary dependent variable in logistic generalized estimating equations (GEE) models with gender, ethnicity, and laryngeal manifestations as categorical explanatory variables. The simplest and most informative model, yielding the lowest Quasi Likelihood Information Criterion, was the one including main effects of these 3 variables. Although the effect of

TABLE I. *SERPING1* alterations of the patients analyzed in this study (current) and those of the pooled sample

	Total		Greek		Romanian		German		Hungarian	
	Current	Pooled	Current	Pooled	Current	Pooled	Current	Pooled	Current	Pooled
No. (families, patients studied)	79, 149	117, 265	29, 61	32, 77	14, 20	14, 20	18, 28	18, 28	18, 40	53, 139
Missense mutations (n, %)	34, 43.0*	40, 34.2*	11, 37.9	11, 34.4	7, 50.0	7, 50.0	6, 33.3	6, 33.3	10, 55.6*	16, 30.2*
Nonsense mutations (n, %)	7, 8.9	14, 12.0	2, 6.9	4, 12.5	1, 7.1	1, 7.1	0, 0	0, 0	4, 22.2	9, 17.0
Splice defects (n, %)	8, 10.1	9, 7.7	3, 10.3	3, 9.4	2, 14.3	2, 14.3	1, 5.6	1, 5.6	2, 11.1	3, 5.7
Small deletions or insertions (including frameshift alterations) (n, %)	21, 26.6	33, 28.2	10, 34.5	10, 31.3	2, 14.3	2, 14.3	6, 33.3	6, 33.3	3, 16.7	15, 28.3
Regulatory mutations (n, %)	0, 0	4, 3.4	0, 0	1, 3.1	0, 0	0, 0	0, 0	0, 0	0, 0	3, 5.7
Large deletions or insertions (n, %)	7, 8.9	13, 11.1	2, 6.9	2, 6.2	2, 14.3	2, 14.3	3, 16.7	3, 16.7	0, 0	6, 11.3
Unidentified defects (n, %)	3, 3.8	5, 4.3	1, 3.4	1, 3.1	0, 0	0, 0	2, 11.1	2, 11.1	0, 0	2, 3.8
Novel defects (n, %)	38, 48.1*	38, 32.5*	13, 44.8	13, 40.6	6, 42.9	6, 42.9	10, 55.6	10, 55.6	9, 50.0*	9, 17.0*

*A Hungarian patient displayed 2 novel defects: a missense mutation proved tolerant by bioinformatic analysis, and a causative nonsense mutation.

ethnicity did not reach statistical significance ($P = .113$), it was kept in the final model for adjusting effects of the other variables. This model showed that male gender (OR: 1.93; 95% CI: 1.14-3.27; $P = .015$) and history of at least 1 episode of laryngeal edema (OR: 3.11; 95% CI: 1.81-5.36; $P < .001$) were independently associated with the use of long-term prophylactic therapy.

Additionally, an effect of HAE-C1-INH type on the disease onset was observed: Greek patients with HAE-C1-INH type I had an earlier disease onset compared with Greek patients with type II ($P = .048$). We pursued this finding of interaction between ethnicity and HAE type in our pooled analysis further, fitting linear GEE models with age at disease onset as the dependent variable and ethnicity and HAE type as explanatory variables. The strongest interaction effect was recorded in Romanian patients (β coefficient for interaction was 22.14; $P < .001$), which translates to a 16-year delay of disease onset in Romanian patients with HAE-C1-INH type I compared with Hungarian patients with type II.

Furthermore, we examined whether the type of *SERPING1* defect is correlated with the clinical phenotype of the disease and especially with disease onset, ie, the age at the occurrence of the first HAE attack. Interestingly, no significant association was found in the group of 149 newly diagnosed patients (in total and among family members, and regardless of HAE-C1-INH type). Thereafter, we performed a similar analysis in the whole group of patients, which was restricted to HAE-C1-INH type I, considering that HAE-C1-INH type II represents a disease entity with distinct laboratory findings. In particular, we investigated the effect of missense mutations, as they lead only to the change of a single amino acid, in contrast to all other defects, because the latter alterations result usually in a truncated protein. We found that patients with missense mutations displayed a significantly later disease onset compared with patients with all other defects (Fig 1). Moreover, in a logistic GEE model with age at disease onset modeled as a binary response variable, patients carrying missense mutations displayed a significantly lower probability of manifesting HAE attacks before the 10th year of age (unadjusted OR: 0.51; 95% CI: 0.31-0.82; $P \leq .006$; OR adjusted for ethnicity: 0.52; 95% CI 0.28-0.99; $P = .047$). Bearing in mind that early onset of HAE symptoms is predictive of high severity of the disease course,[8,9] these findings are of obvious clinical significance.

As mentioned above (Table E1), 9 members of patients' families out of 58 examined (15.5%) for reasons of confirmation of our results exhibited *SERPING1* alterations without any

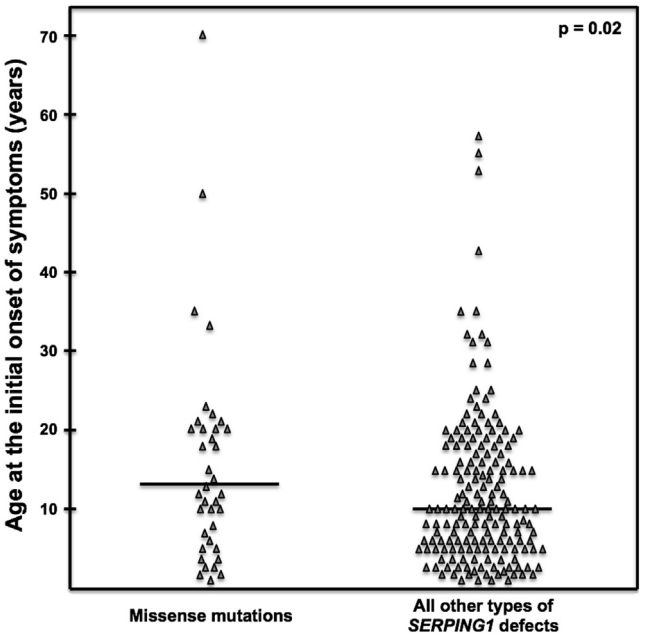

FIG 1. Age at the initial onset of symptoms in patients with HAE-C1-INH. Symptoms appeared at a statistically significantly later age in patients with missense mutations compared with patients with all other defects. *Lines* indicate median values. The significant *P* value refers to Mann-Whitney *U* test.

symptom or sign of the disease during their lifespan. Two of these individuals were older than 45 years. Since we did not examine all healthy members of analyzed families, it is obviously not a representative percentage. Given that some HAE-C1-INH patients develop their first symptoms in late adulthood, this finding underlines the need for screening all of their family members, at least by serum C4 and C1-INH measurements, independently of their age.

In conclusion, our study establishes the notion that carriage of *SERPING1* missense mutations represents an index of a less severe HAE-C1-INH clinical course, and provides additional evidence that *SERPING1* alterations are not the only responsible factor determining the clinical phenotype of the disease. The different distribution of *SERPING1* defects detected among the 4 countries was unexpected given that all populations were of Caucasian origin. This result indicates that environmental factors might affect the variety of *SERPING1* alterations and/or