

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

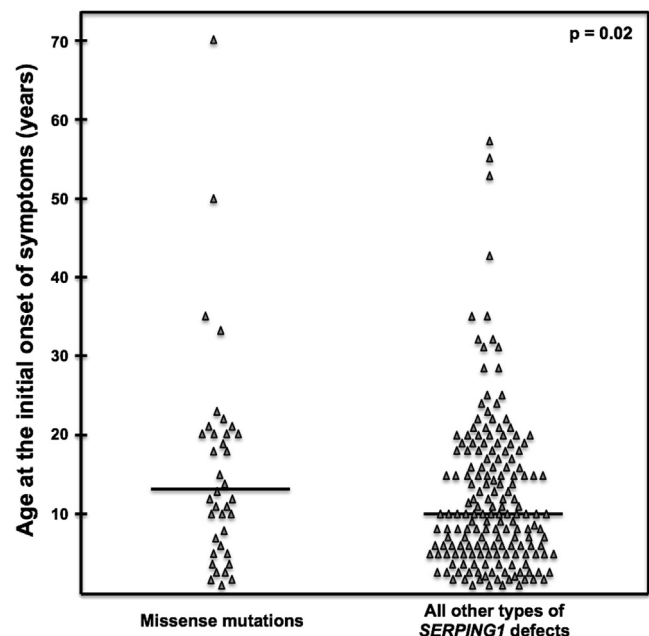


FIG 1. Age at the initial onset of symptoms in patients with HAE-C1-INH. Symptoms appeared at a statistically significantly later age in patients with missense mutations compared with patients with all other defects. Lines indicate median values. The significant P value refers to Mann-Whitney U test.

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

the expression of at least some of these gene defects. Since *SERPING1* is rich in Alu repeats, and therefore sensible in epigenetic alterations, different environmental factors (eg, dietary habits, radiation, hormones, etc) might induce different *SERPING1* defects. The variability of *SERPING1* defects might result in differential post-translational alterations affecting protein structure and/or function and consequently disease phenotype. This result indicates that environmental factors might affect the expression of at least some of these gene defects. This, however, does not justify the use of *SERPING1* molecular analysis for routine diagnostic purposes.

We thank the HAE families and all the clinicians for their collaboration.

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This work was partially supported by an unrestricted grant from Shire Hellas.

Disclosure of potential conflict of interest: M. Speletas is employed by the University of Thessaly School of Health Sciences; has received research support unrelated to this

manuscript from Greek Secretariat of Research in Greece; has received lecture fees from Amgen; and has received travel support from Shire, Behring, Bristol-Meyer, and Novartis. F. Psarros has received travel support from Shire. D. Moldovan has received travel support from Shire and CSL Behring; has received investigator fees from CSL Behring, Viropharma/Shire, and Pharming Technologies; and has received sponsorship of a local conference on HAE and PID from Pharming, Shire HGT, and CSL Behring. M. Magerl has received consultancy/honorarium fees from Shire, Viropharma, CSL Behring, and Sobi. E. Gramoustianou has received research support from Shire. A. E. Germenis has received research support from Shire and Novartis; has received lecture fees from Novartis and Amgen; and has received travel support from Shire. A. Avramouli has received research support from Shire. H. Varga has received travel support from CSL Behring, Pharming, and Shire. M. Maurer has received research support, consultancy and lecture fees, and travel support from Shire; has received consultancy and lecture fees from Biocryst and Viropharma; and is on the boards of Shire, Biocryst, and Viropharma. H. Farkas is on the boards of CSL Behring, Swedish Orphan Biovitrum, Shire, and Viropharma; and has received lecture fees from Swedish Orphan Biovitrum and Shire. The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2014.08.007>

METHODS

Subjects

One hundred forty-nine patients with HAE (121 with HAE type I and 28 with HAE type II) from 79 unrelated families (29 Greek, 18 German, 14 Romanian, and 18 Hungarian), completing the international consensus criteria,^{E1,E2} were subjected to molecular analysis of *SERPING1* gene. Medical records of all the patients were reviewed for disease onset, treatment modalities, and major clinical manifestations, ie, presence of laryngeal attacks, predominance of abdominal presentation during lifespan, and need of long-term prophylactic treatment.

Moreover, in order to investigate genotype-phenotype co-segregation, additional relatives from each family were also included when possible, for a total number of 207 individuals analyzed. Among these additional individuals, 9 showed no clinical symptoms, but complement abnormalities were consistent with HAE-C1-INH, and search for *SERPING1* mutations was conclusive; therefore, these 9 were included in the group of HAE-C1-INH patients. The demographic data of the individuals and the clinical data of the patients included in the study are presented in Table E1.

Furthermore, in order to further investigate arising assumptions about potential genotype-phenotype correlations, the present sample of patients was pooled with 118 HAE patients from 38 families previously genotyped by our groups.^{E3,E4} Thus, a pooled analysis from 265 HAE patients from 117 unrelated families, derived from 4 different regions of the European continent (32 Greek, 18 German, 14 Romanian, and 53 Hungarian families) was performed. Cumulatively, the demographic data of the individuals and the clinical data of the patients of the pooled sample are presented in Table E2.

Molecular techniques

Genomic DNA was isolated from peripheral blood, and *SERPING1* mutations were detected by sequencing after PCR amplification of all 8 exons and the exon-intron boundaries, as described.^{E3,E4} For the detection of large gene rearrangements (exon deletion[s] or duplication[s]), 2 different techniques, namely long-range PCR and multiplex ligation-dependent probe amplification (MLPA), were used for Greek, German, and Romanian samples, while the detection of large gene rearrangements of Hungarian patients was performed as described, including MLPA.^{E5} Examples of the detection of large gene rearrangements, resulting in exon(s) deletion or duplication, are presented in Fig E2. Particularly, in long-range PCR, segments of the *SERPING1* gene encompassing exons 3 to 6 (≈ 7 kb) and 3 to 8 (≈ 15 kb) were amplified using the forward primer 5'-CTTCCTGCTTTGAGTATTT TAGA-3' and the reverse primers 5'-AAAAGATAGGGTGGAATACA GAT-3', and 5'-AAAAACAAAGGCAAAGCAGAGA-3', respectively. Reactions were carried out in a volume of 25 μ L, using the LongAmpTaq DNA Polymerase Protocol (New England Biolabs, Ipswich, Mass) with 0.3 mM dNTPs, 0.3 μ M of each primer, 2% dimethyl sulfoxide (DMSO), 2.5 units of LongAmp Polymerase, and 250 to 500 ng of DNA. After 30 seconds at 94°C, cycling conditions were 5 cycles at 94°C for 15 seconds (denaturation), 56°C for 30 seconds (annealing), 65°C for 7 or 15 minutes (for the amplification of exons 3-6 and 3-8, respectively; elongation), followed by 25 cycles with the same conditions except for the elongation time, which is increased by 20 seconds at each cycle. Final extension was at 65°C for 10 minutes. All PCR procedures were carried out in the PCR-engine apparatus PTC-200 (MJ Research, Watertown, Mass), and the PCR products were analyzed in 0.7% Tris-borate-EDTA (TBE) buffer agarose gels, stained with ethidium bromide, and visualized under UV light.

MLPA was performed with the SALSA MLPA probe mix P243-A2 *SERPING1* kit (MRC-Holland, Amsterdam, The Netherlands). MLPA is a high throughput, sensitive technique for detecting copy number variations in genomic sequences.^{E6} The MLPA P243-A2 kit contains probes for each of the 8 exons of *SERPING1*, 1 flanking probe for APLNR (located

approximately 364 kb upstream of *SERPING1*), and several reference probes for other autosomal chromosomal locations. Data normalization and analysis were carried out according to the manufacturer's instructions.

Bioinformatic analysis

Bioinformatic analyses for all *SERPING1* missense amino acid substitutions identified in our patients was carried out by the use of 3 different software packages, namely SIFT (Sorting Intolerant From Tolerant; available at <http://sift.bii.a-star.edu.sg/>), PolyPhen2 (available at <http://coot.embl.de/PolyPhen/>), and MutationTaster (available at <http://www.mutationtaster.org>). Bioinformatic analyses were extended to *SERPING1* missense amino acid substitutions reported to HAEdb, aiming to predict which changes might be deleterious and, whenever possible, to determine which aspects of protein function are affected.^{E7-E9}

Statistical analysis

Categorical variables were analyzed with Fisher exact test. Normality of continuous variables was assessed with Kolmogorov-Smirnov test. Normally distributed data were analyzed with Student *t* test and 1-way ANOVA, as appropriate. Skewed data were analyzed with nonparametric methods (Mann-Whitney *U* test or Kruskal-Wallis test, as appropriate). Given the fact that our patient population consisted of correlated subjects (members of individual families), we implemented generalized estimating equations (GEE) in order to model the association of response variables (age at disease onset, need for long-term prophylactic treatment) with explanatory variables (patients' origin, gender, genotype, HAE type, and clinical manifestations regarding larynx and abdomen attacks). Age at disease onset was modeled as a continuous variable in linear GEE models, and need for long-term prophylactic treatment was entered as a binary variable in logistic GEE models. In all GEE models, an unstructured correlation structure was used, and the Quasi Likelihood Information Criterion was used for model selection. Data analysis was performed with SPSS ver. 17.0 (IBM Corporation, Armonk, NY). For all analyses, alpha was set at 0.05 (2-tailed).

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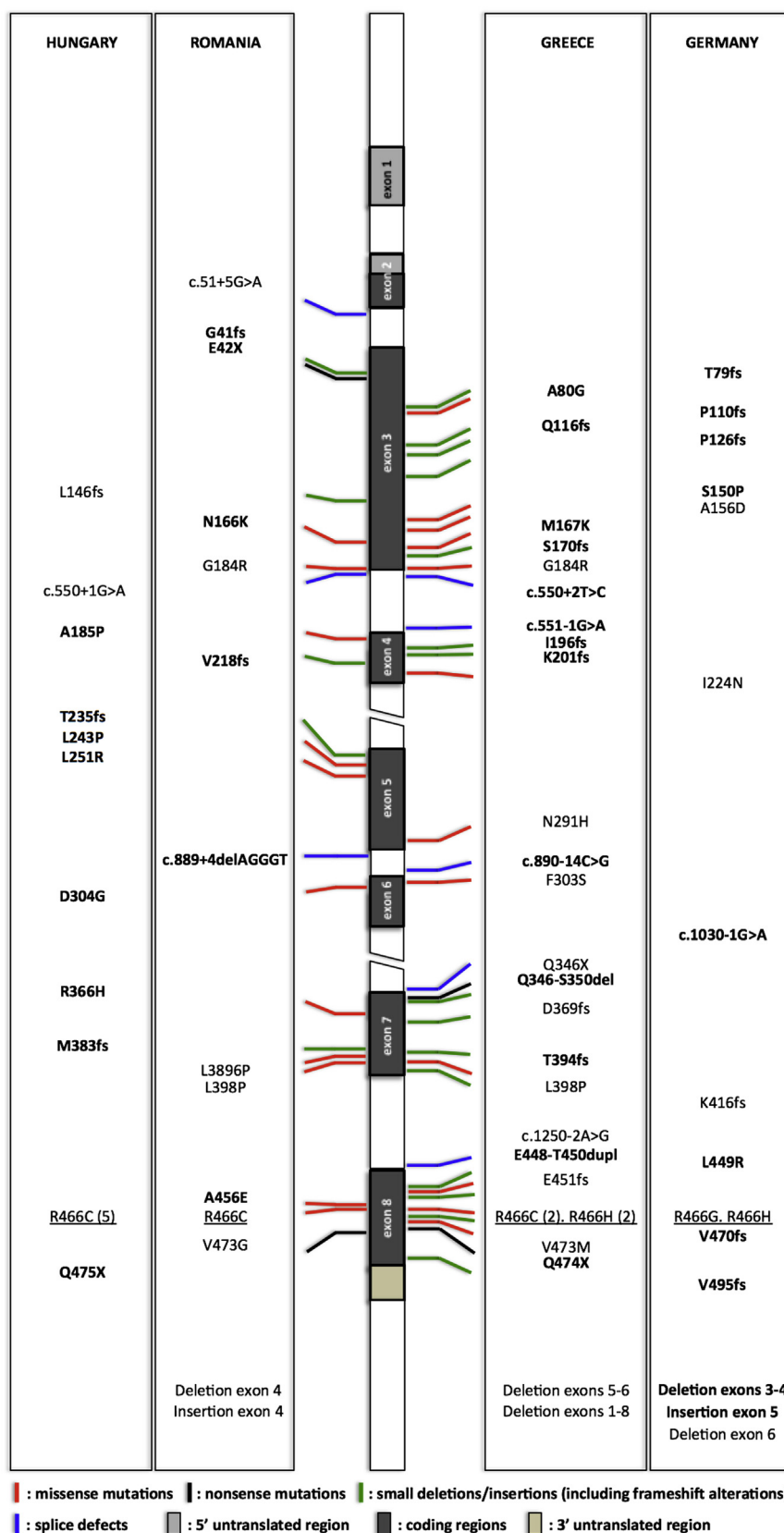


FIG E1. *SERPING1* alterations identified in the patients of the study. The 8 exons of the *SERPING1* are shown as colored boxes (including 5'- and 3'-untranslated regions), and the introns are shown as white boxes. The locations of the mutations are shown with colored arrows, according to the type of alteration (missense, nonsense, small deletions/insertions, and splice defects). The novel mutations/alterations are shown in boldface.

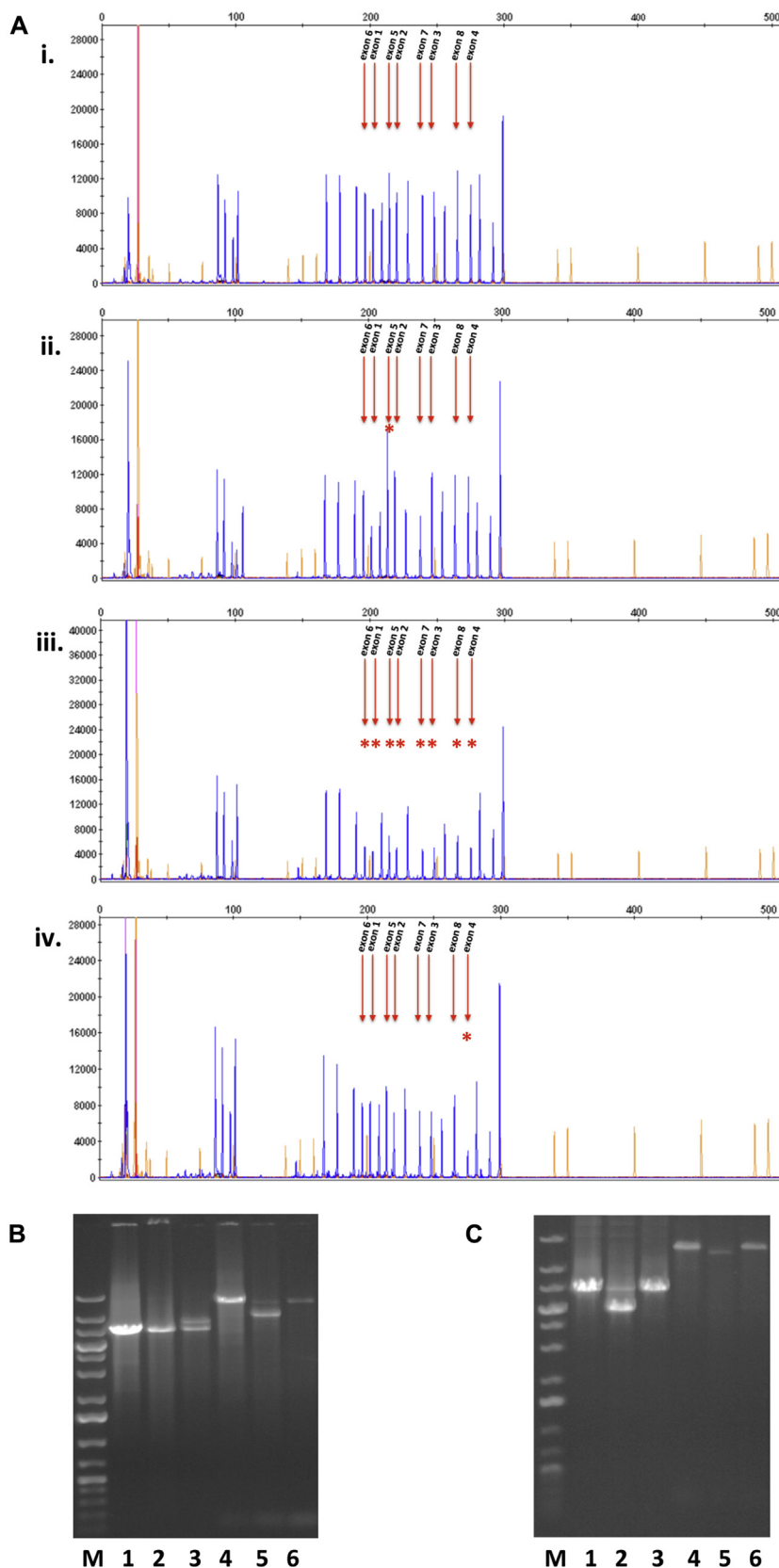


FIG E2. Detection of exon deletions and duplications in the patients in the study. Electrophoretogram images represent fluorescent profiles of multiplex PCR products of MLPA (**A**). Fluorescent intensities of each exon (indicated by arrows) were normalized by comparing the peaks of several reference genes from other autosomal chromosomal locations. The asterisk(s) show(s) the deleted or duplicated exon(s). A healthy individual (wild-type) (**i**), a patient with duplication of exon 5 (**ii**), a patient with an apparently complete gene deletion (**iii**), and a patient with deletion of exon 4 (**iv**). Long-range PCR for exons 3 to 6 (lines 1-3) and for exons 3 to 8 (lines 4-6) (**B**). M: GeneRuler 1 kb Plus DNA Ladder (Fermentas, Pittsburgh, Pa); Lines 1, 2, 4, 6: samples without defect; Line 3: sample with a duplication of exon 5; Line 5: sample with a deletion of exons 5 and 6. A deletion of exon 4, as shown by our long-range PCR protocol: Line 2 (amplification of exons 3-6) and Line 4 (amplification of exons 3-8) (**C**). M: GeneRuler 1 kb Plus DNA Ladder, Lines 1, 3, 4, 6: samples without defect.

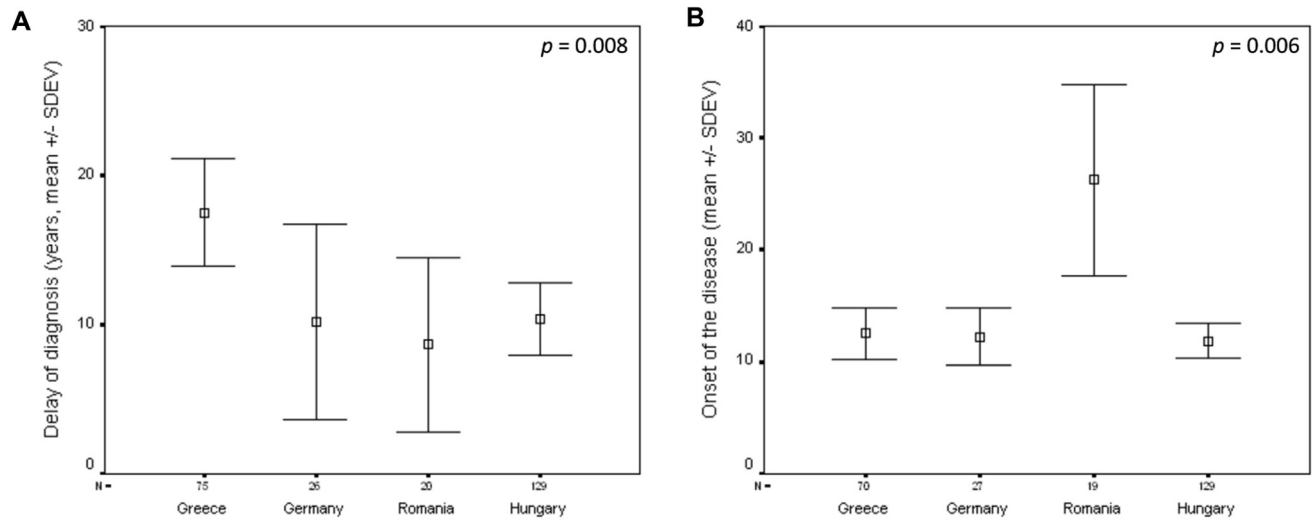


FIG E3. The delay of diagnosis (**A**) and the disease onset (**B**) in different European countries. The charts describe the algorithms for error bar computation of the mean $\pm$ 2 standard errors (age, years). The significant *P* value refers to Kruskal-Wallis H test.

TABLE E1. Demographic and clinical characteristics of the patients of the study

	Total	Greek	Romanian	German	Hungarian
No. (families, individuals studied)	79, 207	29, 88	14, 30	18, 39	18, 40
Sex (male/female)	85/122	43/45	14/26	16/23	12/28
Age analysis (mean, range)	37.2, 1-82	35.5, 1-80	35.8, 8-72	46.3, 13-81	33.7, 3-82
No. (patients)	149	61	20	28	40
Sex (male/female)	63/86	31/30	7/13	13/15	12/28
Age analysis (mean, range)	37.2, 2-81	37.2, 2-73	37.5, 10-72	41.9, 13-81	33.7, 3-68
Age onset (mean, range)	14.8, 0.5-70	12.3, 1-57	26.3, 2-70	12.3, 3-28	14.7, 0.5-53
Age diagnosis (mean, range)	26.6, 1-78	27.7, 2-63	36.2, 10-70	22.8, 3-78	22.6, 1-66
Years delay (mean, range)	12.1, 0-72	16.8, 0-56	8.7, 0-52	10.2, 0-72	8.0, 0-46
Patients without disease (n, %)	9, 6.0	5, 8.2	1, 5.0	0, 0	3, 7.5
Age (mean, range)	14.6, 2-45	7.0, 2-16	45		29.0, 3-45
Families without HAE history (n, %)	30, 37.9	10, 34.5	1, 7.1	10, 55.5	9, 50.0
Patients without HAE history (n, %)	30, 20.1	10, 16.4	1, 5.0	10, 35.7	9, 22.5
Families with type II HAE (n, %)	12, 15.2	4, 13.8	1, 7.1	2, 11.1	5, 27.8
Patients with type II HAE (n, %)	28, 18.8	12, 19.7	2, 10.0	3, 10.7	11, 27.5
Patients with laryngeal attacks (n, %)*	68, 48.6	28, 50.9	12, 63.2	13, 46.4	15, 40.5
Patients with abdominal attacks* (rare, common, main; n, %) [†]	53 (38.4), 57 (41.3), 28 (20.3)	18 (33.3), 22 (40.8), 14 (25.9)	1 (5.3), 12 (63.1), 6 (31.6)	7 (25.0), 16 (57.1), 5 (17.9)	27 (73.0), 7 (18.9), 3 (8.1)
Patients on long-term treatment (n, %)*	49, 35.5	27, 38.9	3, 15.8	9, 32.1	10, 27.0

*Patients displaying *SERPING1* mutations but without disease were excluded from the statistical analysis.

[†]The abdominal attacks were categorized as rare when their frequency was less than 1 to 2 per year, as common when their frequency was >2 per year, and as main when these represented the most common manifestation of HAE patients throughout the patient's life.

TABLE E2. Demographic and clinical characteristics of all patients (including those previously genotyped by Bors et al^{E4} and Speletas et al^{E3})

	Total	Greek	Romanian	German	Hungarian
No. (families, individuals studied)	117, 320	32, 110	14, 30	18, 39	53, 139
Sex (male/female)	145/175	53/57	14/16	16/23	61/78
Age analysis (mean, range)	37.5, 1-82	35.5, 1-80	35.8, 8-72	46.3, 13-81	36.9, 3-82
No. (patients)	265	78	20	28	139
Sex (male/female)	122/143	40/37	7/13	13/15	61/78
Age analysis (mean, range)	37.6, 2-82	37.2, 2-80	37.5, 10-72	41.9, 13-81	37.0, 3-82
Age onset (mean, range)	13.1, 1-70	12.2, 1-57	26.3, 2-70	12.3, 3-28	11.9, 1-53
Age diagnosis (mean, range)	25.2, 1-78	27.9, 2-78	36.2, 10-70	22.8, 3-78	22.7, 1-72
Years delay (mean, range)	11.7, 0-72	16.8, 0-58	8.7, 0-52	10.2, 0-72	9.6, 0-60
Patients without disease (n, %)	17, 6.4	6, 7.7	1, 5.0	0, 0	10, 7.2
Age (mean, range)	25.1, 2-82	6.2, 2-16	45		34.4, 3-82
Families without HAE history (n, %)	32, 27.3	10, 31.3	1, 7.1	10, 55.5	21, 39.6
Patients without HAE history (n, %)	32, 12.1	10, 12.8	1, 5.0	10, 35.7	21, 15.1
Families with type II HAE (n, %)	12, 10.3	4, 12.5	1, 7.1	2, 11.1	5, 9.4
Patients with type II HAE (n, %)	28, 10.1	12, 15.4	2, 10.0	3, 10.7	11, 7.9
Patients with laryngeal attacks (n, %)*	137, 55.2	38, 55.1	12, 63.2	13, 46.4	74, 57.4
Patients with abdominal attacks* (rare, common, main; n, %) [†]	112 (45.7), 83 (33.9), 50 (20.4)	19 (27.5), 27 (39.1), 23 (33.3)	1 (5.3), 12 (63.1), 6 (31.6)	7 (25.0), 16 (57.1), 5 (17.9)	85 (65.9), 28 (21.7), 16 (12.4)
Patients on long-term treatment (n, %)*	97, 39.1	36, 52.2	3, 15.8	9, 32.1	49, 37.9

*Patients displaying *SERPING1* mutations but without disease were excluded from the statistical analysis.

[†]The abdominal attacks were categorized as rare when their frequency was less than 1 to 2 per year, as common when their frequency was >2 per year, and as main when these represented the most common manifestation of HAE patients throughout the patient's life.

TABLE E3. Novel *SERPING1* gene alterations identified in the patients analyzed in this study

No.	Origin	Location	cDNA numbering*	DNA numbering*	Predicted effect on protein function
1	Ro	exon 3	c.122delG	g.3448delG	G41fs
2	Ro	exon 3	c.124G>T	g.3450G>T	E42X
3	Ge	exon 3	c.235delA	g.3561delA	T79fs
4	Gr	exon 3	c.239C>G	g.3565C>G	A80G
5	Hu†	exon 3	c.253G>T	g.3579G>T	E85X
6	Ge	exon 3	c.350_362del13bp	g.3655_3667del13bp	P110fs
7	Gr	exon 3	c.347delA	g.3673delA	Q116fs
8	Ge	exon 3	c.378_379ins20bp	g.3704_3705ins20bp	P126fs
9	Ge	exon 3	c.448T>C	g.3774T>C	S150P
10	Ro	exon 3	c.498C>A	g.3824C>A	N166K
11	Gr	exon 3	c.500T>A	g.3826T>A	M167K
12	Gr	exon 3	c.508delT	g.3834delT	S170fs
13	Gr	intron 3	c.550+2T>C	g.3878T>C	splice defect
14	Gr	intron 3	c.551-1G>A	g.5532G>A	splice defect
15	Hu	exon 4	c.553G>C	g.5535G>C	A185P
16	Gr	exon 4	c.586_589delATCC	g.5568_5571delATCC	I196fs
17	Gr	exon 4	c.601insC	g.5583insC	K201fs
18	Ro	exon 4	c.652_653delGT	g.5634_5635delGT	V218fs
19	Hu	exon 5	c.705delC	g.9526delC	T235fs
20	Hu	exon 5	c.728T>C	g.9549T>C	L243P
21	Hu	exon 5	c.751C>T	g.9572C>T	L251R
22	Ro	intron 5	c.889+4delAGGGT	g.9714_9718delAGGGT	splice defect
23	Gr	intron 5	c.890-14C>G	g.9891C>G	splice defect
24	Hu	exon 6	c.911A>G	g.9926A>G	D304G
25	Ge	intron 6	c.1030-1G>A	g.15212G>A	splice defect
26	Gr	exon 7	c.1036_1050del15bp	g.15219_15233del15bp	Q346_S350del
27	Hu†	exon 7	c.1097G>A	g.10112G>A	R366H
28	Hu	exon 7	c.1147_1148insA	g.15330_g.15331insA	M383fs
29	Gr	exon 7	c.1180delA	g.15363delA	T394fs
30	Ge	exon 8	c.1346T>G	g.18020T>G	L449R
31	Gr	exon 8	c.1350ins9bp	g.17925ins9bp	E448-T450dupl
32	Ro	exon 8	c.1367C>A	g.17941C>A	A456E
33	Ge	exon 8	c.1409_c.1410insG	g.18982_g.18983insG	V470fs
34	Gr	exon 8	c.1420C>T	g.17994C>T	Q474X
35	Hu	exon 8	c.1423C>T	g.19797C>T	Q475X
36	Ge	exon 8	c.1483_7delGTATA	g.18057_61delGTATA	V495fs
37	Ge	deletion of exons 3 & 4			no functional protein
38	Ge	duplication of exon 5			no functional protein

Ge, German; Gr, Greek; Hu, Hungarian; Ro, Romanian.

*The cDNA numbering is according to GenBank accession number NM_000062.2, and the g. DNA numbering is according to GenBank accession number X54486.

†The same Hungarian patient displayed 2 novel alterations.

TABLE E4. Summary of the results from 3 bioinformatic methods applied to missense amino acid substitutions observed in *SERPING1* gene in this study*

No.	Amino acid substitution	Origin	Effect on protein function			Total
			PolyPhen-2 (score, sensitivity, specificity)	SIFT (probability score)	MutationTaster (probability)	
1	A80G	Gr	Possibly damaging (0.601, 0.87, 0.91)	Tolerant (0.25)	Polymorphism (0.999)	X
2	S150P	Ge	Probably damaging (0.999, 0.14, 0.99)	Intolerant (0.03)	Disease causing (0.999)	XXX
3	A156D	Ge	Benign (0.350, 0.90, 0.89)	Tolerant (0.55)	Disease causing (0.897)	X
4	N166K	Ro	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Disease causing (0.995)	XXX
5	M167K	Gr	Probably damaging (0.996, 0.55, 0.98)	Intolerant (0.01)	Disease causing (0.982)	XXX
6	G184R	Gr, Ro	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Disease causing (1.000)	XXX
7	A185P	Hu	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.02)	Disease causing (0.999)	XXX
8	I224N	Ge	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Disease causing (0.999)	XXX
9	L243P	Hu	Probably damaging (0.987, 0.73, 0.96)	Intolerant (0.04)	Disease causing (0.999)	XXX
10	L251R	Hu	Probably damaging (0.987, 0.73, 0.96)	Intolerant (0.01)	Disease causing (0.768)	XXX
11	N291H	Gr	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Disease causing (0.999)	XXX
12	F303S	Gr	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Polymorphism (0.999)	XX
13	D304G	Hu	Possibly damaging (0.858, 0.83, 0.93)	Intolerant (0.01)	Polymorphism (0.999)	XX
14	R366H	Hu	Benign (0.038, 0.94, 0.82)	Tolerant (0.20)	Polymorphism (0.999)	
15	L396P	Ro	Probably damaging (0.997, 0.35, 0.98)	Intolerant (0.00)	Polymorphism (0.959)	XX
16	L398P	Gr, Ro	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.01)	Disease causing (0.999)	XXX
17	D408V	Hu	Probably damaging (0.983, 0.74, 0.96)	Intolerant (0.00)	Disease causing (0.999)	XXX
18	L449R	Ge	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Disease causing (0.999)	XXX
19	A456E	Ro	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.00)	Disease causing (0.999)	XXX
20	R466C	Gr, Ro, Hu	Probably damaging (0.921, 0.81, 0.94)	Tolerant (0.12)	Disease causing (0.999)	XX
18	R466G	Ge	Benign (0.290, 0.91, 0.89)	Tolerant (0.46)	Disease causing (0.999)	X
19	R466H	Gr, Ge	Possibly damaging (0.666, 0.86, 0.91)	Tolerant (0.13)	Disease causing (0.999)	XX
20	V473M	Gr	Probably damaging (0.997, 0.35, 0.98)	Intolerant (0.05)	Disease causing (0.658)	XXX
21	V473G	Ro, Hu	Probably damaging (1.000, 0.00, 1.00)	Intolerant (0.02)	Disease causing (0.999)	XXX
22	V480M	Gr, Ge, Ro	Benign (0.086, 0.92, 0.81)	Tolerant (0.06)	Polymorphism (0.999)	

We distinguish between XXX (consensus of the 3 methods predicts that this change is deleterious), XX (2 out of the 3 methods predict that the change is deleterious), X (only 1 of the methods predicts that the change is deleterious), and no mark (both methods predict that the change [in boldface] is benign).

Ge, German; Gr, Greek; Hu, Hungarian; Ro, Romanian.

*The V480M variant was found, even in homozygous state, in healthy relatives of the analyzed patients without any history of attacks or complement abnormality, confirming that it represents a polymorphism of the *SERPING1* gene.^{E1}

TABLE E5. Summary of the results from 3 bioinformatic methods applied to benign missense amino acid substitutions observed in C1-inhibitor gene mutation database (HAEdb)

No.	Amino acid substitution	Origin	Effect on protein function		
			PolyPhen-2 (score, sensitivity, specificity)	SIFT (probability score)	MutationTaster (probability)
1	H155R	USA (Ref. 20)	Benign (0.005, 0.97, 0.74)	Tolerant (1.00)	Polymorphism (0.726)
2	S255T	Germany (Ref. 24)	Benign (0.009, 0.96, 0.77)	Tolerant (0.51)	Polymorphism (0.999)
3	T301K	USA (Ref. 20)	Benign (0.056, 0.94, 0.84)	Tolerant (1.00)	Polymorphism (0.999)
4	T301I	USA (Ref. 20)	Benign (0.298, 0.91, 0.89)	Tolerant (0.14)	Polymorphism (0.999)
5	T467P	Spain (Ref. 21)	Benign (0.000, 1.00, 0.00)	Tolerant (0.27)	Polymorphism (0.999)
6	Q474E	France (Ref. 22)	Benign (0.164, 0.92, 0.87)	Tolerant (0.26)	Polymorphism (0.988)

TABLE E6. Clinical and laboratory findings of 5 HAE patients in the study without *SERPING1* alterations, derived from 3 different families

No.	Origin	Family history of HAE	Affected individuals	Laryngeal attacks*	Abdominal attacks†	Long-term treatment	C4 (mg/dL)‡	C1-INH antigenic levels (mg/dL)‡	C1-INH functional levels‡
1	Gr	Yes	3	Yes	Common	Yes (Danazol)	10.0**	8.0**	56.0%**
2	Ge	No	1	Yes	Rare	No	2.0	25.0	18.0%
3	Ge	No	1	No	Common	No	9.0	8.0	19.0%

Ge, German; Gr, Greek.

*At least one.

†The abdominal attacks were categorized as rare when their frequency was less than 1 to 2 per year, as common when their frequency was >2 per year, and main when these represented the most common manifestation of HAE-C1-INH.

‡Normal range of complement tests: C4, 10 to 40 mg/dL; C1-INH antigenic levels, 21 to 39 mg/dL; C1-INH functional levels, >67% normal, 41% to 67% equivocal, <41% abnormal.

**These values represent the complement tests of the proband at diagnosis (another 2 patients from this family were also analyzed with rather similar clinical and laboratory findings).