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Deletions in *SERPING1* Lead to Lower C1 Inhibitor Function: Lower C1 Inhibitor Function Can Predict Disease Severity

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Keywords
C1 inhibitor function · C1q · Disease severity · Hereditary angioedema · Novel mutations · *SERPING1* gene

Abstract
Background: How genotype affects phenotype in hereditary angioedema with C1 inhibitor deficiency (C1-INH-HAE) has not been totally clarified. In this study, we investigated the relationship between different types of mutations and various phenotypic characteristics. ***Methods:*** Clinical data from 81 patients from 47 families were recorded. Complement proteins were analyzed from 61 untreated patients. The coding exons and the exon-intron boundaries of the *SERPING1* gene were sequenced, and deletion/duplication analysis with multiple ligation dependent probe amplification was performed. The relationship of complement protein with the mutation type was analyzed by using generalized estimating equations. ***Results:*** Thirty-five different mutations (15 novel and 2/15 homozygous) were identified. There was no causative mutation in 6 patients (7.4%). Patients with deletion and large deletion had the lowest (5.05%, 0–18.7; 5.8%, 0–16.5%, respectively), and the none mutation group had the highest C1 inhibitor function (23.3%, 11–78%, $p < 0.001$). C1 inhibitor function levels decreased as the age of the disease progressed ($r = –0.352$, $p = 0.005$). Lower C1 inhibitor function levels caused severer disease ($r = –0.404$, $p = 0.001$) and more frequent annual attacks ($r = –0.289$, $p = 0.024$). In the off-attack period, C1q levels were lower than normal in 9.8% of the patients. ***Conclusion:*** Deletion mutations may represent the most unfavorable effect on C1 inhibitor function. The earlier disease onset age could be a sign for lower C1 inhibitor function levels in adult life. C1q levels could also be low in C1-INH-HAE patients, as in acquired angioedema. Lower C1 inhibitor function can predict disease severity and may have negative impacts on the course of C1-INH-HAE.

Introduction

Hereditary angioedema with C1 inhibitor deficiency (C1-INH-HAE) (HAE; Online Mendelian Inheritance in Man; OMIM #106100) is a relatively rare autosomal dom-

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inant disorder that manifests as recurrent episodes of swelling involving the face, tongue, extremities, gastrointestinal tract, genitalia, and upper airways [1, 2]. C1-INH-HAE results from mutations in the C1 inhibitor gene, *SERPING1* (MIM #606860; GenBank NM_000062), a gene located on the chromosome locus 11q12–q13.1, consisting of 8 exons. However, patients without any mutation in *SERPING1* have also been reported [3–5]. There are two types of C1-INH-HAE, type I and type II. In type I HAE, mutations are located on any exon in the *SERPING1* gene leading to low C1 inhibitor antigenic levels, whereas in type II HAE, characterized by normal or elevated levels of C1 inhibitor but dysfunctional protein, mutations are located in *SERPING1* exon 8. The phenotype of the disease varies widely ranging in severity from negligible to life-threatening attacks, even among members of the same family sharing the same mutation [1, 2]. Up to 500 mutations associated with C1-INH-HAE have been identified in *SERPING1* so far (http://hae.enzim.hu/). Most of these mutations are missense, followed by deletion, insertion, nonsense, and large deletion [6]. Several studies have been conducted to clarify a possible association between mutation types, and clinical phenotypes. Some data showed no close association between mutation type and the phenotypic expression of C1-INH-HAE [7, 8], but others reported that missense mutations had favorable outcomes on C1 inhibitor antigenic levels, clinical severity score, and age at onset of symptoms, respectively [4, 9–11]. Although the genetic background of the disease has already been determined from various populations, there are limited data about genetic properties of C1-INH-HAE from Turkish patients [12]. In the present study, we first determined the genetic database of 81 Turkish patients from 47 unrelated families with C1-INH-HAE. Then we focused on the relationship between the different types of mutations in *SERPING1* and various phenotypic characteristics and laboratory parameters.

Methods

Patients

Eighty-one symptomatic patients from 47 unrelated Turkish families with C1-INH-HAE, 72 (89%) with HAE type I and 9 (11%) with HAE type II, were recruited from two major medical centers for HAE: Departments of Internal Medicine, Divisions of Allergy and Clinical Immunology from Ege University Faculty of Medicine (Izmir), and Istanbul Faculty of Medicine (Istanbul). All patients were diagnosed as having C1-INH-HAE according to medical history and laboratory values (at least on two different occasions). None of the patients were on angiotensin-converting enzyme inhibitor treatment or estrogen-based therapy. The symptom severity was assessed by a well-defined scoring system in the pretreatment period [13]. This study was approved by the Ethics Committee of Ege University (B.30.2.EGE.0.20.05.00/OY/1128/456) and was conducted according to the principles of good clinical practice and adhered strictly to the ethical standards outlined in the Declaration of Helsinki [14]. Written informed consent forms were obtained from all patients.

Complement Studies

Blood samples were taken from 61 untreated patients, as the remaining 20 patients were on medication. C4 (reference range, 10–40 mg/dL) and C1 inhibitor antigen levels (reference range, 21–39 mg/dL) were analyzed by immunonephelometry (Siemens, Marburg, Germany), and C1 inhibitor function levels (reference range, 70–130%) were measured by chromogenic assay (Berichrom Siemens, Marburg, Germany). Semiquantitative C1q levels (reference range, 100–300 μg/mL) were tested in patients and in 100 healthy controls by radial immunodiffusion using the C1q Binarid Radial Immunodiffusion kit (Binding Site, Birmingham, UK).

Genetic Analysis

Mutation analysis of the 81 C1-INH-HAE patients was conducted in the Medical Genetics Department of Ege University Hospital. The coding exons and the exon-intron boundaries of the *SERPING1* gene were sequenced to detect mutations. Genomic DNA was isolated from peripheral blood cells using standard techniques. All PCR products were sequenced by the dye termination method using a DNA sequencing kit (Perkin-Elmer, Foster, CA, USA) and analyzed using the ABI Prism 3100 sequence analyzer (Applied Biosystems, Foster, CA, USA). The multiple ligation dependent probe amplification (MLPA) (P243-MRC Holland, the Netherlands) technique was performed in order to detect exon or gene level large deletions which could not be detected with sequencing analysis of the *SERPING1* gene. Results were evaluated via Coffalyser software. The pathogenicity of the novel mutations was evaluated according to the American College of Medical Genetics standards and guidelines [15].

Statistics

Quantitative variables were summarized by their means and standard deviations (or medians with minimum and maximum values in case of deviations from normality), and qualitative data were expressed with observed frequencies and percentages. The IBM SPSS Statistics 23 program was used for statistical analysis and graphics were prepared by using R software (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria; http://r-project.org). The Shapiro-Wilk test was used to assess normality of quantitative variables. According to this test, the Mann-Whitney U test was applied to compare the group of patients and Spearman correlation analysis was performed to evaluate binary associations. For relationships between qualitative variables, the χ^2 test or Fisher's exact test was implemented. Since our data consisted of related sets of individuals (family members), this relation among family members was taken into consideration by using generalized estimating equations (GEEs) when modeling each complement protein (C1 inhibitor, C1 inhibitor function, and C1q) and each clinical characteristic separately with the mutation type. All GEE models were fitted under exchangeable correlation structure, and the quasi likelihood information criterion was used to evaluate the

Table 1. Clinical characteristics of 81 patients and 47 families with C1-INH-HAE

	HAE type I	HAE type II	Total
Patients, *n*	72	9	81
Family[1], *n*	40	7	47
Gender female	40 (55.6)	2 (22.2)	42 (51.9)
Age, years	41.5±14.8	37.7±9.1	41.1±14.3
Age at disease onset, years	12.5±7.1	8.5±3.9	12.1±6.9
Diagnostic delay, years	22±13.3	23.8±9.9	22.2±12.9
Erythema marginatum	24 (33.3)	2 (22.2)	26 (32.1)
Misdiagnosis			
FMF	12 (16.7)	2 (22.2)	14 (17.3)
Unnecessary surgery	12 (16.7)	1 (11.1)	13 (16)
Symptom severity[2]	7.1±2.1	7.5±1.7	7.2±2.1
Upper airway edema	48 (66.7)	6 (66.7)	54 (66.7)
Abdominal attacks	62 (86.1)	9 (100)	71 (87.6)
Annual attack frequency	27.4±24.8	34±31.3	28.1±25.5
Consanguinity[1]	12 (30)	5 (71.4)	17 (36.2)
Family history of HAE[1]	31 (77.5)	6 (85.7)	37 (78.7)
Death in the family due to LE[1, *]	14 (43.8)	5 (83.3)	19 (50)

Figures in parentheses are percentages. FMF, familial Mediterranean fever; LE, laryngeal edema. * $p = 0.023$. [1] Analyzed based on family numbers. [2] Adapted from Freiberger et al. [13] recorded based on localization and frequency of attacks and also disease onset age.

goodness of fit of these models. In these models, complement proteins were included in log-transformed form, and negative binomial distribution was selected for annual attack frequency, abdominal attack frequency and total laryngeal attacks due to the overdispersion, also logistic distribution was chosen for binary responses. Additionally, the none mutation type was selected for the reference category in the models. A p value of <0.05 was considered to indicate statistical significance.

Results

Demographic and Clinical Characteristics

Demographic and clinical characteristics of 81 patients from 47 families with C1-INH-HAE are depicted in Table 1.

Patients with earlier disease onset age suffered from more frequent annual attacks (in every localization and abdominal attacks) ($r = -0.420$, $p < 0.001$, and $r = -0.371$, $p < 0.001$, respectively). Total laryngeal attacks were more frequent in patients with delayed diagnosis ($r = 0.324$, $p = 0.003$). There were no clinical differences between type I and type II HAE patients, except that history of death in the family due to laryngeal attacks was more common in type II patients (87.5 vs. 42.2%, respectively, $p = 0.023$).

When the effect of mutation type on each clinical characteristic was evaluated with the GEE model, this effect was found statistically significant only in annual attack frequency and total laryngeal attack frequency ($p = 0.006$ and $p < 0.001$, respectively). Although all mutation types had a negative effect on the annual attack frequency compared to the none mutation type, this negative effect was statistically significant only with large deletion ($\beta = -0.85$ and $p = 0.04$). Nonsense, deletion, and p.R466 mutation types had lower total laryngeal attack frequency than the reference category ($\beta = -2.08$ and $p = 0.02$, $\beta = -1.54$ and $p = 0.08$, $\beta = -2.08$ and $p = 0.03$, respectively).

Complement Proteins

For type I HAE patients, serum C1 inhibitor antigenic levels (mean 5.57 ± 2.81 mg/dL [min.-max.: 2–14 mg/dL]) were positively correlated with disease onset age ($r = 0.307$; $p = 0.025$). Positive correlation was also present between C1 inhibitor levels, and C4 levels ($r = 0.334$; $p = 0.015$), C1 inhibitor function ($r = 0.362$; $p = 0.008$), and C1q levels ($r = 0.456$; $p = 0.001$) in type I HAE patients. The mean of C1 inhibitor antigenic levels was 42.15 ± 6.62 mg/dL (33–50 mg/dL) for type II HAE patients. There was no correlation between C1 inhibitor antigenic levels and symptom severity score in all patients and in type I HAE patients.

In all patients, the mean C1 inhibitor function level was 11.73 ± 11.58% (min.-max.: 0–78%). All patients ex-

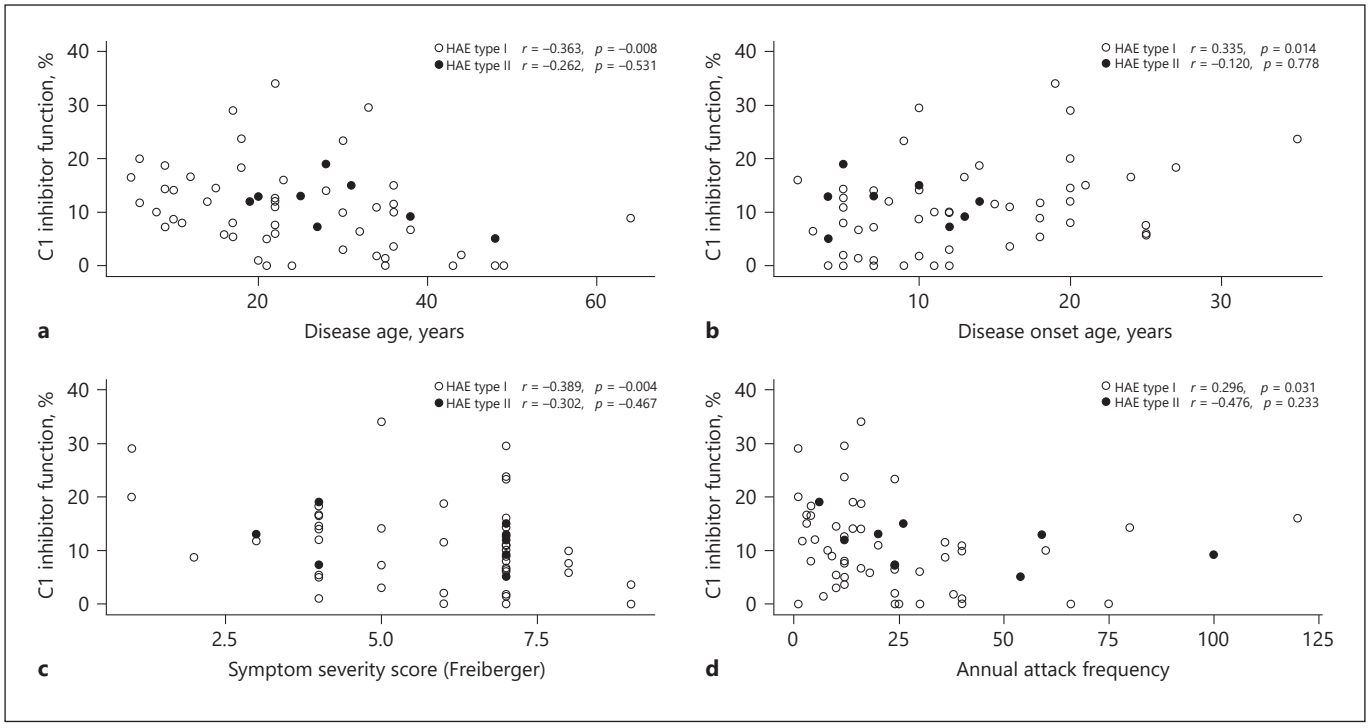

Fig. 1. C1 inhibitor function and various clinical parameters. **a** For all patients, as the disease age increases, C1 inhibitor function levels diminish ($r = -0.352$, $p = 0.005$). **b** HAE started earlier in patients who had lower C1 inhibitor function levels ($r = 0.278$, $p = 0.03$). **c**, **d** Patients whose C1 inhibitor function levels are lower have higher symptom severity scores ($r = -0.404$, $p = 0.001$) (**c**) and more frequent annual attacks ($r = -0.289$, $p = 0.024$) (**d**).

cept one had C1 inhibitor function below 50%. One patient had a mean C1 inhibitor function level of 78% (analyzed 6 times in treatment-free periods, but all higher than 50%), he had C1 inhibitor antigenic and C4 levels of 3 and 7 mg/dL, respectively. C3 and C1q levels were normal all the time. He had experienced recurrent abdominal and peripheral swelling attacks at most twice a year since he was 15 (for more than 45 years), responsive to on demand therapy with C1 inhibitor concentrate. His mother was also reported to have similar abdominal pain and peripheral swelling attacks in her youth but died before having a diagnosis. Therefore, this patient has been accepted as having C1-INH-HAE, despite having a normal C1 inhibitor function.

HAE symptoms started earlier in the patients with lower C1 inhibitor function. C1 inhibitor function levels decreased as the age of the disease progressed. Lower C1 inhibitor function levels caused severer disease and more frequent annual attacks (Fig. 1). C1 inhibitor function levels were positively correlated with C1 inhibitor antigenic levels ($r = 0.344$; $p = 0.007$) and C1q levels ($r = 0.503$; $p < 0.0001$) in all patients ($n = 61$), and patients with type I HAE ($r = 0.362$, $p = 0.008$, and $r = 0.517$, $p < 0.0001$, respectively). There were no such correlations for type II HAE patients.

For all patients ($n = 61$), the mean C1q level was 173.34 ± 81.23 μg/mL (min.-max.: 1.66–333 μg/mL). Six patients (9.8%) had C1q levels less than or equal to 50 μg/mL (10.77 ± 6.91 μg/mL; min.-max.: 1.66–18 μg/mL). None of the healthy controls had C1q levels lower than the reference value. Patients with C1q levels less than or equal to 50 μg/mL had a longer disease duration and had a significantly lower C1 inhibitor function and C1 inhibitor antigenic level in comparison to patients with high C1q levels ($p = 0.015$, $p = 0.001$, and $p = 0.002$, respectively). All the 6 patients with low C1q levels had also very low C1 inhibitor function level (≤3.6%).

Genetic Results and Novel Mutations

Seventeen out of the 47 families (36.2%) reported consanguineous marriage. Thirty-five different mutations were identified. Of these, 27 (36%) were missense, 12

Table 2. Mutations and clinical characteristics

Mutation type	Patients, *n*	HAE type	Onset age, years	Severity score [13]	Annual attacks, *n*	Abdominal attacks, *n*	Laryngeal attacks, *n*/years of disease duration	Laryngeal death in the family, %
Missense	27	I	10 (3–27)	8 (1–9)	24 (1–101)	9 (0–52)	0.03 (0–12)	40
Deletion	12	I	9.5 (5–17)	8.5 (4–11)	26 (1–80)	6 (0–52)	3 (1–4.7)	83.3
Insertion	7	I	7 (5–20)	6 (4–10)	14 (3–56)	12 (1–20)	0 (0–18)	none
Intronic	2	I	19 (15–23)	4 (1–7)	20 (4–36)	5 (0–10)	0.015 (0–0.03)	none
Large deletion	7	I	16 (7–25)	8 (4–9)	14 (4–30)	4 (0–24)	0.1 (0–24)	50
Nonsense	11	I	15 (5–25)	7 (1–9)	24 (1–52)	18 (0–52)	0.03 (0–0.75)	25
p.R466	9	II	8 (4–14)	8 (5–9)	24 (5–100)	10 (1–60)	0.04 (0–0.3)	83.3
None	6	I	13.5 (2–35)	7 (5–9)	24 (12–120)	12 (1–24)	0.06 (0–10)	25

(16%) were deletions, 11 (14.7%) were nonsense, 9 (12%) were p.R466, 7 (9.3%) were large deletion, 7 (9.3%) were insertions, and 2 (2.7%) were intronic. There was no causative mutation in the sequencing region of *SERPING1* gene in 6 out of 81 (7.4%) patients. Half of these patients had a family history. Clinical and laboratory characteristics of the mutations are given in Table 2.

Fifteen (42.8%) of these mutations were novel; 13 out of 15 novel mutations were heterozygous, and 2 were homozygous [p.I401T (c.1202T>C) missense mutation in exon 7, and p.S460F (c.1379C>T) missense mutation in exon 8]. Localizations of novel *SERPING1* mutations and the characteristics of the patients with novel mutations are given in Table 3.

Missense and In-Frame Mutations

Thirteen missense mutations were identified. Five were novel: p.I401T (c.1202T>C), p.S460F (c.1379C>T), p.N426I (c.1277A>T), p.P360L (c.1079C>T) and p.L151P (c.452T>C). All novel missense mutations were predicted to be disease causing by PolyPhen and SIFT prediction softwares. These variants except p.L151P (allele frequency <1/10,000) were not found in the ExAC Database. There was no family history in the patients who carried p.N426I (c.1277A>T), p.P360L (c.1079C>T), and p.L151P (c.452T>C) mutations. These three mutations were classified as likely pathogenic, and p.I401T (c.1202T>C) and p.S460F (c.1379C>T) mutations were classified as pathogenic, according to American College of Medical Genetics guidelines [15]. One in-frame deletion mutation detected (c.1296–1304 del GGACCCAGA) was described before [12]. According to all this information together with clinical findings and family history, we concluded that these mutations are disease-causing.

Frameshift and Nonsense Mutations

Twelve frameshift and 4 nonsense mutations were identified. Eight frameshift mutations, (p.L12AfsX8 [c.32–33insT], p.F313LfsX15 [c.936–937insC], p.D144VfsX4 [c.431delA], p.P98TfsX35 [c.291–292insA], p.R286AfsX10 [c.854delC], p.P376QfsX50 [c.1122–1123insCTCT], p.Y330FfsX7 [c.989–1001del], and p.S422FfsX3 [c.1259–1260insT]) and 1 nonsense mutation (p.E164X [c.490G>T]) were novel.

Splicing Mutations

One previously reported (HAEdb, hae.enzim.hu) and 1 novel mutation, IVS2 + 3A>T (c.51 + 3A>T), were identified in the study. The novel mutation affected the position +3 of the donor splice site of intron 2 and was evaluated using the Human Splicing Finder in silico prediction tool (URL http://www.umd.be/HSF/). Human Splicing Finder analysis predicted a broken wild-type donor site in consensus values compatible with a splicing defect (–13.69%). Considering the consensus value variation, it is possible to evaluate this novel splicing mutation as pathogenic.

Large Deletions

Three large deletions were identified. All of the large deletions have been previously reported. When evaluated together with the laboratory findings, the presence of the lowest C1 inhibitor antigenic level and lower C1 inhibitor function in patients with large deletions were also compatible with their pathogenicity (Fig. 2).

None Mutation Group

After sequencing of the coding exons, the exon-intron boundaries and the promoter region of the *SERPING1* gene, and deletion/duplication analysis with the MLPA

Table 3. The characteristics of the patients with novel mutations

Family	Patient	Gender	Age, years	Onset age, years	Exon/intron	Mutation type	Mutation		Severity	EM	C1-INH (21–39 mg/dL)	C1-INH function (70–130%)	C4 (10–40 mg/dL	CIq (100–300 μg/mL)	Attack location	Annual attack frequency
							cDNA	protein								
1	1[1]	F	46	27	exon 7	missense	c.1202T>C	p.I401T	4	–	10	18.3	10	248	P, GIS, G	4
	2[1]	F	45	23					1	–	n.d.	n.d.	n.d.	n.d.	P	<1
	3[1]	F	67	7					8	–	3	0	6	1.6	P, F, GIS, G, L	66
	4[1]	F	51	5					8	–	2	2	6	5	P, F, GIS, G	24
	5	F	37	20					1	–	14	29	18	258	P	1
2	6	F	40	–	exon 8	missense	c.1379C>T	p.S460F	0	–	13	34	21	215	–	–
	7	F	21	–					0	–	11	36	8	321	–	–
	8	M	57	21					7	–	12	15	7	232	P, F, GIS, G, L	3
3	9	F	25	13	exon 2	insertion	c.32-33insT	p.L12AfsX8	5	–	6	16.6	8	221	P, GIS	3
	10	M	54	5					9	–	6	15.2	6	297	P, F, GIS, G, L	56
4	11[2]	M	69	13	exon 6	insertion	c.936-937insC	p.F313LfsX15	10	–	5	19.7	5	337	P, F, GIS, G, L	20
5	12	M	36	17	exon 3	deletion	c.431delA	p.D144VfsX4	7	–	7	11.6	6	341	P, F, G, L	8
	13	M	47	11					9	+	5	10	6	237	P, F, G, L	8
6	14	F	35	7	exon 3	insertion	c.291-292insA	p.P98TfsX35	6	–	4	14	6	173	P, GIS	16
	15	M	33	5					6	–	7	19	6	262	P, GIS, G	14
7	16	M	25	7	exon 8	missense	c.1277A>T	p.N426I	6	–	6.9	18.5	6.7	180	P, GIS	12
8	17	F	54	23	intron 2	splicing	IVS2+3A>T	splicing defect	1	–	11.9	29.3	11	149	P	4
	18	F	57	9					9	+	6.5	0	4	81	P, F, GIS, L	40
9	19	M	54	5	exon 5	deletion	c.854delC	p.R286AfsX10	11	+	9.7	0	10	139	P, F, GIS, L	75
	20	M	23	14					7	+	4	18.7	4	102	P, F, GIS, L	16
10	21[2]	M	27	5	exon 7	insertion	c.1122_1123 insCT	p.P376QfsX50	9	+	5	12.6	5	141	P, F, GIS, L	12
11	22	F	35	18	exon 7	missense	c.1079C>T	p.P360L	4	–	5.1	5.4	4	94	P, F, GIS, G	10
12	23	M	47	25	exon 3	nonsense	c.490G>T	p.E164X	7	+	4	6	6	215	P, F, GIS, G, L	30
13	24	M	42	12	exon 3	missense	c.452T>C	p.L151P	9	+	7.6	9.9	5	333	P, F, GIS, G, L	40
14	25	F	35	20	exon 6	deletion	c.989-1001del	p.Y330FfsX7	4	+	3	1.4	4	14	P, GIS, G	10
15	26	F	41	6	exon 8	insertion	c.1259_1260 insT	p.S422FfsX3	9	–	5.4	14.5	4	273	P, F, GIS, G, L	7

EM, erythema marginatum; P, peripheral; GIS, abdominal; G, genital; F, facial; L, laryngeal; n.d., not done. [1] Homozygous. [2] No family history.

technique in order to detect exon or gene level large deletions, there was no causative mutation in the sequencing region of the *SERPING1* gene in 6 out of 81 (7.4%) patients. All of the patients with none mutation had overt HAE clinic, normal C1q levels, and very low levels of complement proteins (C1-INH, C4). Half of these patients had a family history of HAE.

Mutation Types and Complement Proteins

Complement proteins varied in patients with different types of mutations (Fig. 2). In GEE models, mutation type was found significant ($p < 0.001$). For the C1 inhibitor antigenic levels, the large deletion group and the nonsense mutation group had the lowest C1 inhibitor antigenic levels (3.41, 2.93–8, and 3.5, 2–8.4 mg/dL, respectively). There were no differences in C1 inhibitor antigenic levels in patients with HAE type I for various mutation types except the p.R466 mutation (Fig. 2). With regard to C1 inhibitor function, patients with deletion and large deletion had the lowest C1 inhibitor function (5.05%, 0–18.7; 5.8%, 0–16.5%, respectively). All of the mutation types led to a significant negative effect on C1 inhibitor function compared to the none mutation type ($p < 0.001$), except insertion type mutation (Fig. 2). Patients with deletion had the lowest C1q levels (120.5 μg/mL; 14–237 μg/mL), followed by the large deletion group (164 μg/mL; 8–221 μg/mL) (Fig. 2). Mutation carriers as a group had lower C1q levels compared to the reference none mutation type in the model (Fig. 2). Comparisons between each of the mutation carriers and none mutation were significant, except for insertion and p.R466 mutations. Six out of 61 (9.8%) patients had very low levels of C1q (less than or equal to 50 μg/mL). Two of them were sisters and they had a novel p.S460F (c.1379C>T) homozygous missense mutation. Of the remaining 4 patients with low C1q levels, 2 had heterozygous deletion

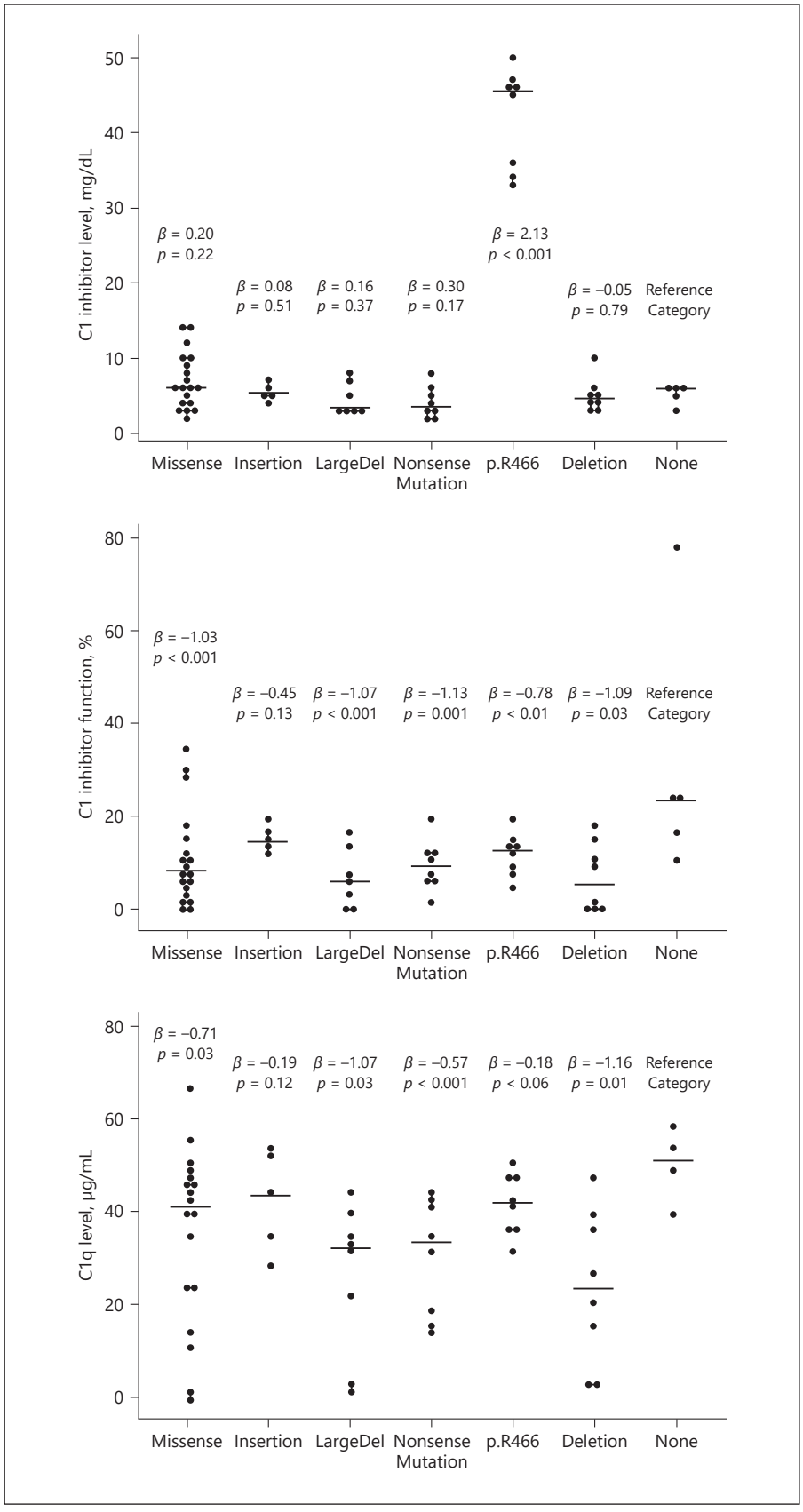

Fig. 2. Mutations and complement proteins. The distribution of each complement protein according to mutation types has been visualized with dot plots. The horizontal lines on these dot plots represent the medians. The GEE estimates for each type of mutation (compared to the reference category; the none mutation group) have been displayed along with the *p* values.

(p.Y330FfsX7 [c.989–1001del] and p.282LfsX22 [c.845_846delCC]), and 2 had a heterozygous large deletion of exon 4 in the same family.

Discussion

In this study, we focused on the clinical, laboratory, and genotypical properties of C1-INH-HAE that might determine the severity of this disease. We found that patients with earlier disease onset age had more frequent annual attacks. C1-INH-HAE symptoms started earlier in patients who had lower C1 inhibitor antigenic levels and C1 inhibitor function (Fig. 1). The earlier disease onset age could be a sign for lower baseline C1 inhibitor antigenic and function levels. As the disease age increased, the C1 inhibitor function level decreased (Fig. 1). However, there are no data reported on this issue, so far. The onset age of angioedematous episodes of C1-INH-HAE is generally within the first two decades of life. The age of onset of symptoms was reported to be important to foreseen disease severity of C1-INH-HAE. Bork et al. [16] showed that the early-onset group (5 years or earlier) had twice more frequent episodes per year than the late-onset group (15 years or later). Farkas [17] reported that the earlier the onset of symptoms, the severer the subsequent course of C1-INH-HAE.

Up to 17% of the patients were initially misdiagnosed as familial Mediterranean fever, a hereditary autoinflammatory disorder with episodic abdominal pain, mimicking abdominal attacks of C1-INH-HAE [18]. Keeping in mind the lack of knowledge of physicians about HAE, overdiagnosis of familial Mediterranean fever in this population should not be surprising [19]. To the best of our knowledge, this is the first study reporting the ratio of familial Mediterranean fever misdiagnosis in C1-INH-HAE patients.

In the current study, we identified 35 different mutations in 81 C1-INH-HAE patients from 47 families. Fifteen (48%) were novel mutations (Table 3). Two of the novel mutations were homozygous, a very rare situation reported only in 4 C1-INH-HAE patients so far [20–22]. Both families had consanguineous marriage, but the disease's phenotype varied largely. The homozygous sisters in the family with p.I401T (c.1202T>C) missense mutation in exon 7 had a very late disease onset age and less severe course of the disease (Table 2). However, the disease was far severer in the homozygous sisters with p.S460F (c.1379C>T) missense mutation in exon 8, and their C1-INH function was extremely low (Table 2). Despite meticulous sequencing analysis of the coding exons, the exon-intron boundaries and the promoter region of the *SERPING1* gene, and deletion/duplication analysis with the MLPA technique, there was no causative mutation in the sequencing region of the *SERPING1* gene in 6 out of 81 (7.4%) patients. Nearly 5% of the C1-INH-HAE patients have been reported to have no mutation in the *SERPING1* gene [4–6]. It is not fully understood how C1-INH-HAE occurs in patients with no *SERPING1* alterations. Factors leading to increased posttranslational consumption of C1 inhibitor or defects located in the untranslated region of the *SERPING1* gene might be possible explanations of the disease pathogenesis in these patients [4, 6].

How genotype affects the phenotypical appearance in C1-INH-HAE is not well defined. In the last couple of years, several studies focused on the possible role of the mutations in the phenotypical heterogeneity of C1-INH-HAE patients. A clear correlation between *SERPING1* mutations and disease phenotype was not affirmed by previous reports [7, 23]. Recent studies reported that C1-INH-HAE was started later in those with missense mutations and they experienced a significantly low frequency of attacks [4, 9, 11]. All these studies have mainly focused on missense mutations, and the groups were divided as "missense" and "other type of *SERPING1* defects." Unlike these studies, we defined every mutation type as a different group and tried to examine the effects of each mutation type on the clinical phenotype. Although the mutation type had no effect on the symptom severity, patients with missense mutation had the highest C1 inhibitor antigenic level, as reported by Xu et al. [10]. Patients with large deletion and nonsense mutation had the lowest C1 inhibitor antigenic level (Fig. 2). Patients with deletion and large deletion had less favorable outcomes for C1 inhibitor functions, whereas patients with none mutation had the highest C1 inhibitor function levels, followed by the insertion group (Fig. 2). To our knowledge, no study has reported any relationship between C1 inhibitor function levels and different type of mutations, so far. In addition, we showed that C1 inhibitor function levels strongly correlated with disease severity determined by a well-defined symptom severity score (Fig. 1) [13]. C1 inhibitor function has also been reported to be positively correlated with another disease severity index [24].

Serum C1q values are expected to remain normal in C1-INH-HAE and are known to be depressed in the acquired form of angioedema [24]. However, some exceptions do exist. Homozygous C1-INH-HAE patients with undetectable C1q levels have been reported [20, 21]. In

the present study, we found that C1q levels could be lower than normal in heterozygous C1-INH-HAE patients, as well as in homozygous patients, in the off-attack period. There were no autoantibodies against C1-INH in homozygous patients with low C1q levels. We could not analyze autoantibodies against C1-INH in 4 heterozygous patients with low C1q levels. Reduced C1q levels had been previously reported during the acute attack period in heterozygous C1-INH-HAE patients [25]. In our study, all the patients with low C1q levels had also very low C1 inhibitor function which might lead not only to inadequate inhibition of C1 complex autoactivation, but also to increased activation of the classical complement pathway as seen in homozygous C1-INH-HAE patients [26]. In patients with very low C1 inhibitor function, excessive activation of the complement cascade might lead to a decrease in C1q levels.

In conclusion, earlier disease onset age and lower level of C1 inhibitor function may have negative impacts on the course of C1-INH-HAE. What is more, the earlier disease onset age could be a sign for lower baseline C1 inhibitor function levels in the adult life. Deletion and large deletion mutations might lead to the most unfavorable, and the none mutation group to the most favorable C1 inhibitor function levels. In the off-attack period, C1q levels could also be low in C1-INH-HAE patients, as in acquired angioedema patients. A limitation of our study is that patients were categorized by mutation type. Mutation type is not necessarily a direct reflection of a mutation's effect on translation. Mutations classified together based on DNA sequence may have not the same effect at the protein level. Conversely, mutations classified in different groups may have the same effect at the protein level in terms of amino acid sequence, tertiary structure, extracellular excretion, and function. This area of uncertainty should be investigated with functional validation studies of *SERPING1* mutations in hereditary angioedema.

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Author Contributions

Contributions: (1) conception and design of the study; (2) data generation; (3) analysis and interpretation of the data; (4) preparation or critical revision of the manuscript; (5) the writing of this work to take public responsibility for it; (6) believe it represents valid work and approve it for publication. Contributions of each author are specified in parentheses: Prof. Nihal Mete Gökmen, MD (1, 2, 3, 4, 5, 6); Prof. Okan Gülbahar, MD (1, 2, 3, 4, 5, 6); Associate Prof. Hüseyin Onay, MD (3, 4, 5, 6); Zeynep Peker Koc, MD (1, 2, 3); Semiha Özgül, PhD (1, 2, 3, 4); Associate Prof. Timur Köse, PhD (1, 2, 3, 4); Ass. Prof. Aslı Gelincik, MD (1, 2, 3); Prof. Suna Büyüköztürk, MD (1, 2, 3); Prof. Aytül Zerrin Sin, MD (1, 2, 3).

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