



SHORT COMMUNICATION

Novel homozygous variants in the *SERPING1* gene in two Turkish families with hereditary angioedema of recessive inheritance

Nihal Mete Gökmen¹ , César Rodríguez-Alcalde², Okan Gülbahar¹ , Margarita Lopez-Trascasa³ , Hüseyin Onay⁴ & Alberto López-Lera^{2,5}

¹ Department of Internal Medicine, Division of Allergy and Immunology, Ege University Faculty of Medicine, Izmir, Turkey

² Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain

³ Departamento de Medicina, Universidad Autónoma de Madrid, Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain

⁴ Department of Medical Genetics, Ege University Faculty of Medicine, Izmir, Turkey

⁵ Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain

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Correspondence

Nihal Mete Gokmen, Department of Internal Medicine, Division of Allergy and Clinical Immunology, Ege University Medical Faculty, 35100 Bornova, Izmir, Turkey.
E-mail: enihalmete@yahoo.com.tr

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Abstract

Hereditary angioedema as a result of deficiency of the C1 inhibitor (HAE-C1INH; MIM# 106100) is a rare autosomal disorder and affected individuals are generally heterozygous for dominant negative variants in the *SERPING1* gene. Homozygosity for *SERPING1* pathogenic variants was long considered to be embryonically lethal; however, five nonrelated families with a recessive HAE pattern have been described in the last decade. In this report, we functionally characterized two newly reported nonrelated, consanguineous families with a recessive presentation of HAE attributed to *SERPING1* variants in the reactive center loop (family D; S438F) and gate (family A; I379T) regions. S438F heterozygotes (family D) showed variable levels of intact 105-kDa and cleaved/inactive 96-kDa isoforms of C1INH, whereas their homozygous relative presented only the 96-kDa band. Functional studies showed that S438F reduced C1INH interaction with target proteases in heterozygous (C1s, 32–38% of controls and FXIIa, 28–35% of controls) and homozygous (C1s, 18–24% of controls and FXIIa, 4–8% of controls) carriers, which is consistent with the more severe presentation of HAE in the family and decreased C1q levels in homozygous patients. By contrast, plasma C1INH from I379T heterozygotes (family A) showed normal C1INH/C1s binding (84–94% of controls) and no significant reduction in C1INH/FXIIa complexes (50–70% of controls). However, the homozygote failed to inhibit both C1s (25–42% of controls) and FXIIa (14–18% of controls). This profile is concordant with the less severe presentation of HAE in the family and the conserved C4 and C1q levels in heterozygous and homozygous patients.

INTRODUCTION

The C1-inhibitor (C1INH) protein irreversibly inactivates target proteases of complement, contact, fibrinolytic and coagulation pathways. Hereditary C1INH deficiency (HAE-C1INH; MIM# 106100) is a rare autosomal disorder and characterized by recurrent, spontaneous episodes of nonpruritic edema in the subcutaneous and submucosal layers.¹ Affected individuals are generally

heterozygous for dominant negative variants² in the *SERPING1* gene, which results in quantitative (type I) or qualitative (type II) deficiency of C1INH. More than 700 different *SERPING1* mutations have been identified in heterozygous HAE-C1INH patients.³ Biochemically, these patients present with low complement C4, reduced C1INH function and/or C1INH levels, and conserved C1q. Homozygosity for *SERPING1* mutations was long suspected to be embryonically lethal. However, over the

last decade, complete C1INH deficiency was described in several nonrelated families with recessive HAE inheritance patterns, representing a distinct presentation of the disease with unusual clinical and biochemical features including a significant reduction in C1q levels.^{4–7}

In this report, we functionally characterize two newly reported nonrelated, consanguineous families of Turkish origin with recessive transmission of HAE attributed to *SERPING1* pathogenic variants in the gate (family A; I379T) and reactive center loop (RCL; family D; S438F) regions.⁷

RESULTS AND DISCUSSION

Family A, novel homozygous p.I379T (c.1202T > C) missense variant in exon 7

The proband (patient A10) is a 48-year-old woman with HAE type I attributed to the presence of homozygous p.I379T *SERPING1* variant resulting from a consanguineous marriage. Her angioedema symptoms first started at the age of 27 during pregnancy. She is mildly symptomatic, experiencing peripheral angioedema attacks four times per year, with just one genital and one abdominal episode. She presents with low C1INH levels (10 mg dL⁻¹) and function (18.3%), and normal C4 (10 mg dL⁻¹) and

C1q (248 µg mL⁻¹; Table 1). She has two heterozygous, asymptomatic children, a 20-year-old son with fragile X syndrome (not studied) and a 14-year-old daughter (A170) with type I HAE-C1INH. Her third pregnancy was terminated upon detection of fragile X syndrome in the fetus. The other homozygous member (A20) is the younger sister of A10. She had experienced only one peripheral episode at age 24. Laboratory studies showed low C1INH levels (12.8 mg dL⁻¹) and function (29.6%), normal C4 (21 mg dL⁻¹) and normal C1q (160 µg mL⁻¹). She has two heterozygous, asymptomatic daughters (patients A30 and A50) with HAE type II. Both sisters have near-normal C1INH levels and slightly low diminished C1INH function. The family reported a consanguineous marriage (Figure 1a). The proband's father (A70) was also heterozygous for the variant. He had normal complement parameters, but experienced one peripheral angioedema attack on the legs and hands at age 70 after angiotensin-converting enzyme inhibitor intake. Unfortunately, he died as a result of chronic obstructive pulmonary disease at age 72. The proband's mother (A80) was also heterozygous for the variant and symptom free. Her C1INH function, C4 and C1INH levels were all within normal range. To treat hypertension, she was taking α-1 antagonist, doxazosin 4 mg and angiotensin II receptor antagonists with diuretic and candesartan 16 mg with hydrochlorothiazide 12.5 mg.

Table 1. The clinical and laboratory characteristics of the HAE patients and their relatives.

Patient	Sex	Age	<i>SERPING1</i>	Onset age	Attack localization	Annual attack frequency	C1INH (21–39 mg dL ⁻¹)	C1INH function (70–130%)	C4 (10–40 mg dL ⁻¹)	C1q (100–300 µg mL ⁻¹)	HAE type
Family A											
A10	F	48	I379T hom	27 ^a	P, GIS, G	2	10	18.3	10	248	I
A20	F	45	I379T hom	24	P	<1	12.8	29.6	21	160	I
A30	F	23	I379T het	–	–	–	21.3	60.9	22	137	II
A50	F	20	I379T het	–	–	–	21.2	62.1	27	150	II
A70 ^b	M	72	I379T het	70	P	<1	30.9	97.5	34	138	Normal
A80	F	74	I379T het	–	–	–	24.4	71.1	32	141	Normal
A170	F	14	I379T het	–	–	–	15.6	54.9	14	151	I
Family D											
D10 ^c	F	55	S438F hom	5	P, F, GIS, G	24	2	2	6	5	I
D20 ^d	F	67	S438F hom	7	P, F, GIS, G, L	>60	3	0	6	1.66	I
D40 ^e	M	61	S438F het	21	P, F, GIS, G, L	3	12	15	7	232	I
D50	F	57	S438F het	–	–	–	12.2	42.7	11	177	I
D70	F	27	S438F het	–	–	–	11	36	8	321	I

^aDisease started in pregnancy. F (in the “sex” column), female; F (in the “Attack localization” column), facial; G, genital; GIS, abdominal; L, laryngeal; M, male; P, peripheral.

^bHe died as a result of chronic obstructive pulmonary disease at age 72. He experienced only one peripheral angioedema attack on the legs and hands at age 70 after angiotensin-converting enzyme inhibitor intake.

^cShe was diagnosed with Sjogren's syndrome with positive antinuclear antibody, anti-SSA and anti-SSB.

^dShe died as a result of hypertensive cerebral hemorrhage at age 67. One of her three daughters died of systemic lupus erythematosus at age 20.

^eThe only heterozygous HAE patient reported to be symptomatic among homozygous families published in the literature.

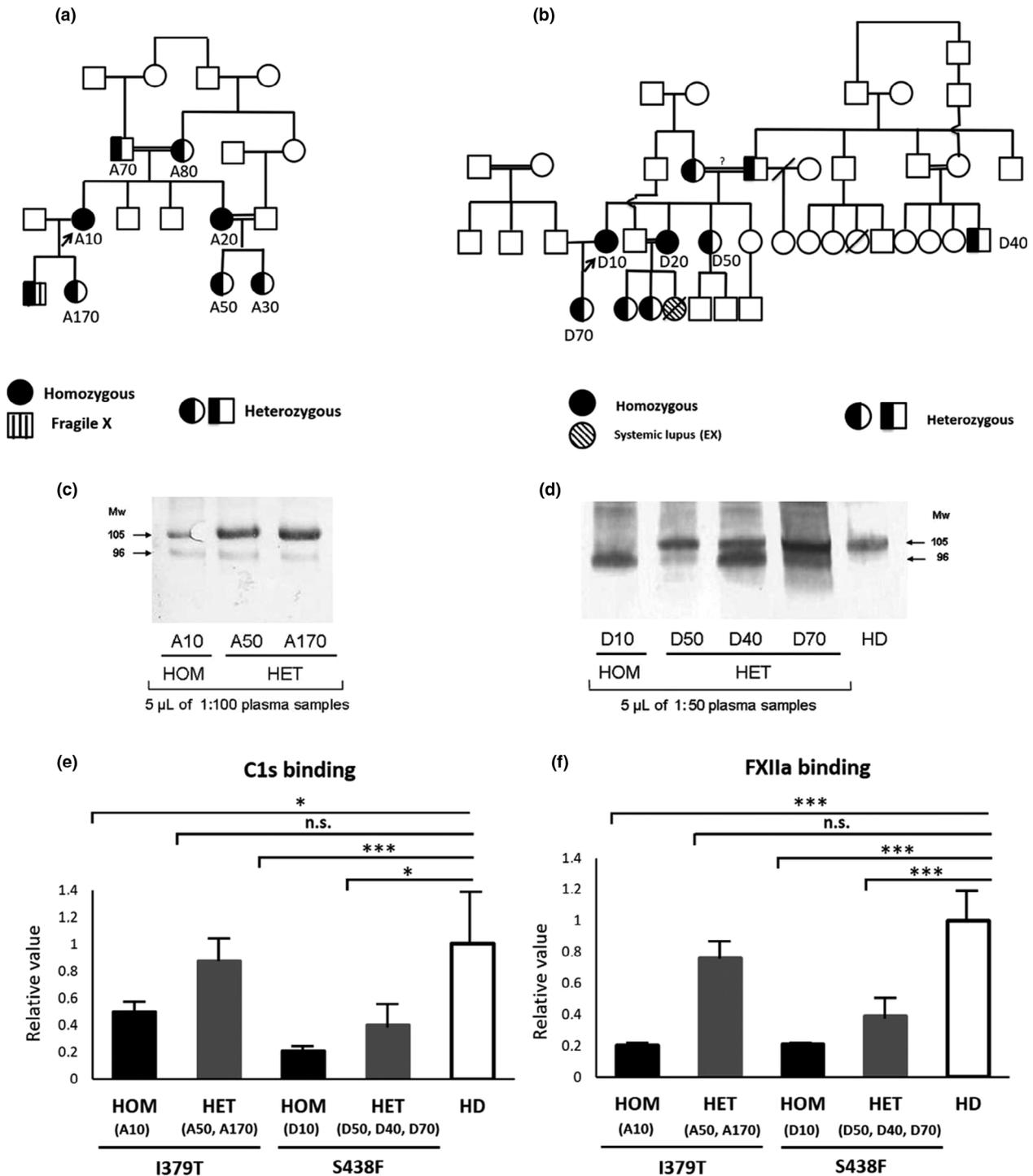


Figure 1. Western blot and functional biochemical studies. **(a, b)** Family trees of the two pedigrees studied. **(c)** Western blot of plasma samples of family members carrying the I379T variant showing the native (105 kDa) and cleaved (96 kDa) forms of C1INH. **(d)** Western blot of plasma samples of the homozygous patient D10 carrying the S438F variant showed only cleaved (96 kDa) forms of C1INH; however, heterozygous patients carrying the S438F variant presented the cleaved and the native forms of C1INH. **(e, f)** C1s- and FXIIa-binding capacity of C1INH in homozygous (HOM; A10 and D10), heterozygous (HET; A50, A170, D50, D40, D70) and healthy donor (HD) citrated plasma samples. The mean \pm s.d. from three replicate experiments is shown. Five different healthy donors were used as controls for functional studies. $***P \leq 0.001$; $*P \leq 0.05$; n.s., nonsignificant; s.d., standard deviation.

complexes more severely than that of C1INH–C1s complexes, which is consistent with conserved C4 and C1q levels in both heterozygotic and homozygotic family members and with the less severe presentation of HAE in the family (Table 1).

Plasma from family D patients carrying the S438F variant demonstrated significant reduction in C1INH levels. Figure 1d shows anti-C1INH western blot of 1:50 plasma dilutions. S438F heterozygotes D40, D50 and D70 exhibited variable levels of the 105- and 96-kDa isoforms, whereas their homozygous relative D10 presented only the cleaved/inactive 96-kDa band. Functional studies showed that S438F reduced C1INH interaction with both C1s and FXIIa in the heterozygous (C1s, 32–38% of controls and FXIIa, 28–35% of controls) and homozygous (C1s, 18–24% of controls and FXIIa, 4–8% of controls) carriers (Figure 1e, f). Of note, the large variation in proteases binding between S438F carriers approximately correlated with the intensity of the 105-kDa isoforms in the samples analyzed, as measured by band densitometry (data not shown), which is consistent with the very diminished functionality of the S438F allele.

Functionally, the variants I379T and S438F resemble the closely located R378C and I440S recessive variants also found in C1INH-HAE homozygous patients.^{5,8,9} (Figure 2). Caccia and colleagues proposed that the reduction in protease control by variant R378C is a result of a nonoptimal presentation of the RCL and/or unproductive turnover of the inhibitor and the release of an RCL-cleaved serpin and an active protease.⁸ It is possible that an aberrant positioning of the RCL differently affects C1s and PKa or FXIIa inhibition (because of the different protease sizes, structures or charge distributions), rendering C1INH variants near p.R378 prone to inefficient control of contact system activation while partially preserving that of the classical complement pathway.

The two homozygotes studied are symptomatic and present HAE-C1INH type I phenotypes, whereas their heterozygous relatives have either type II (I379T), type I (S438F) or even normal phenotype (A70 and A80) and have not yet presented clinical manifestations of HAE except for mild symptoms in patient D40 (S438F). Homozygotes for S438F had early disease onset, autoimmune diseases (Sjögren's syndrome and a daughter who died of systemic lupus erythematosus) and very low levels of C4 and C1q at diagnosis, whereas the homozygous carriers of I379T reported late disease onset, both coinciding with pregnancies, and have conserved C4 and C1q levels with no signs of autoimmunity. This suggests that the autoimmune manifestations in S438F carriers may be associated with their reduced classical pathway activation control, resulting in long-term C1q

and C4 hypocomplementemia; however, further research is needed to address this question in detail.¹⁰

Taking into account the absence of HAE symptoms in the majority of individuals carrying nondominant *SERPING1* variants in heterozygosis cases described so far and the rarity of homozygous situations, their incidence is likely underestimated. Moreover, the autoimmune profile here reported is not at all a constant finding in recessive HAE pedigrees (as exemplified here by the I379T and S438F variants) but seems to be in any case restricted to homozygous carriers of RCL variants, leading to chronic and severe C1q hypocomplementemia.^{4,5} Further research is needed to better address this question.

Under-recognition is the main difficulty for undertaking more extensive studies on this rare presentation of the disease and hinders yet important questions. Do other parts of the molecule potentially harbor additional, yet undescribed recessive variants or is the phenomenon restricted to certain RCL mobile regions? Which are the specific molecular mechanisms responsible for the shift from dominant to recessive inheritance for each of these pathogenic variants?

To summarize, despite the established autosomal dominant inheritance of most HAE-causing variants and the distinction between type I and type II phenotypes, the evidence obtained in this study of the I379T and S438F variants considered together with previously reported data on the R378C and I440S variants (Figure 2) supports the existence of recessive or incomplete dominant pathogenic variants in C1INH which lead to variable HAE biochemical profiles associated with recessive presentations of the disease.

METHODS

Complement studies

HAE was diagnosed based on medical history and complement values. C1INH levels (reference range, 21–39 mg dL⁻¹) were analyzed by immunonephelometry (Siemens, Marburg, Germany) and C4 levels (reference range, 10–40 mg dL⁻¹) were analyzed by turbidimetry (Roche Diagnostics, Rotkreuz, Switzerland). Semiquantitative C1q levels (reference range, 100–300 µg mL⁻¹) were tested in patients by radial immunodiffusion using the C1q BINARID Radial Immunodiffusion kit (Binding Site, Birmingham, UK). For the functional studies, serum, ethylenediaminetetraacetic acid (Vacusera 9 ml K2/Edta, Disera, Izmir, Turkey) plasma and citrated (Vacuette, Greiner Bio-One, Kremsmünster, Austria) plasma samples from seven patients [one homozygous patient from each family (A10 and D10), two heterozygous patients from family A (A50 and A170) and three heterozygous patients from family D (D40, D50 and D70)] and one healthy individual were collected at Ege University Medical Faculty after obtaining signed informed consent. Additional serum, ethylenediaminetetraacetic acid (Sigma-Aldrich, St Louis, MO, USA) plasma and citrated (Vacuette,

Greiner Bio-One, Madrid, Spain) plasma samples from healthy donors were obtained at University Hospital La Paz upon informed consent. The studies were approved by the local Ethical Committees at Ege University and University Hospital La Paz and were performed in agreement with the Declaration of Helsinki and its later amendments.

Genetic analysis

Sequence analysis of the 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 variants. 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). Results were evaluated via Coffalyser software (MRC, Holland, Amsterdam, Netherlands).

Functional studies

We studied plasma C1INH by western blot and the capacity to form *de novo* inhibitory sodium dodecyl sulfate-resistant complexes with complement C1s and contact system FXIIa proteases. Western blots were performed with 5 μ L of either 1:50 (family D) or 1:100 (family A) phosphate-buffered saline–diluted ethylenediaminetetraacetic acid plasma samples. Detection was performed with a 1:2000 dilution of monoclonal antihuman C1INH (sc_80631; Santa Cruz Biotechnologies, Heidelberg Germany) as primary antibodies and 1:2000 dilutions of alkaline phosphatase-labeled polyclonal goat antimouse immunoglobulin G (Jackson ImmunoResearch, Cambridgeshire, United Kingdom) as secondary antibodies.

For the C1INH–protease interaction studies, plasma samples at dilutions ranging from 1:2000 to 1:64 000 in phosphate-buffered saline were first incubated with biotinylated C1s (Calbiochem, Madrid, Spain) for 1 h at 37°C. C1INH–C1s complexes were captured with goat anti-C1INH (Dako/Agilent Technologies, Madrid, Spain) antibodies and detected using an in-house ELISA as described previously.⁴ C1INH–FXIIa complexes formed in 30 μ L aliquots of citrated plasma after 15 min of incubation at 37°C with 5 μ L of 100 μ g mL⁻¹ high-molecular-weight dextran sulfate (Sigma Aldrich) were captured with monoclonal antihuman C1INH antibodies (sc_80631; Santa Cruz Biotechnologies), detected with 1:1000 dilutions of goat antihuman FXII antibodies (GAFXII-IG170; Enzyme Research Laboratories) and horseradish peroxidase-labeled monoclonal antigoat immunoglobulin G as secondary antibodies. The reactions were developed with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) for 20 min and the end-point values were measured in an automated spectrophotometer.

Statistical analyses

The statistical analyses of C1INH–protease complexes were performed with the Mann–Whitney *U*-test using the

GraphPad Prism version 7 software package (GraphPad Software, La Jolla, CA, USA). Data are statistically significant for *P* values ≤ 0.05 .

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AUTHOR CONTRIBUTION

Nihal Mete Gökmen: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing—original draft; Writing—review and editing. **César Rodríguez-Alcalde:** Data curation; Formal analysis; Methodology. **Okan Gülbahar:** Data curation; Funding acquisition; Investigation; Project administration; Resources. **Margarita Lopez-Trascasa:** Data curation; Formal analysis; Methodology; Writing—review and editing. **Huseyin Onay:** Formal analysis. **Alberto López-Lera:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing—original draft; Writing—review and editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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