

Letter to the Editor

A Turkish family with a novel mutation in the promoter region of the C1 inhibitor gene*To the Editor:*

Hereditary angioedema (HAE; Online Mendelian Inheritance in Man no. 106100) is a well-defined autosomal dominant trait presented with 3 variants. Type I (HAE-I) and type II (HAE-II) are associated either with a deficiency in or a nonfunctional C1 inhibitor (C1Inh) enzyme, and type III is associated with a different scope of display recently related with the impairment of Factor XII, as noted by Dewald and Bork.¹

The genetic cause of C1Inh deficiency shows a very high allelic heterogeneity on the C1 inhibitor gene (*C1INH*), as noted by Tosi,² and more than 250 dominant mutations have been described thus far, including in the recent publication by Pappalardo et al.³ Autosomal recessive inheritance is very infrequent and accounted for by the homozygous mutations in the promoter region of the gene as noted by Verpy et al.⁴

The proband (Fig 1, A, II:3) was a 53-year-old woman whose symptoms began at the age of 8 years. Since then, she has experienced frequent angioedema attacks. The symptoms randomly affected her face, neck, or extremities and required an emergency treatment at least once a year for severe laryngeal edema. Her 2 sisters had similar clinical findings, both having their first attacks at comparable ages with their sibling (Fig 1, A, II:1 and II:5). Family history revealed no other affected individuals. Routine blood biochemistry analysis of the proband showed normal C1q and C3 levels but decreased levels of C4 and C1Inh, with a decreased C1Inh enzyme activity. Complement profiles of the 2 symptomatic

sisters were similar to that of the proband (Table 1). The C1Inh activities of the nonsymptomatic family members (Fig 1, A, I:1, I:2, and II:4) were found to be slightly decreased, whereas C1Inh, C4, and C1q levels were within normal ranges.

After informed consent was obtained, genetic analysis was performed for all family members, except 2 brothers who refused to be investigated. One healthy volunteer joined our investigation as a control subject. A total of 8 coding and uncoding exons, including deep exon-intron boundaries of the *C1INH* gene, were investigated completely in the proband. Sequencing analyses revealed a novel homozygous c. –101A>G change in the promoter region where the CAAT box is located (Fig 1, B). Two of the symptomatic sisters were also homozygous whereas the other examined family members were heterozygous for the same mutation (Fig 1, B). The control subject had a normal homozygous genotype (Fig 1, B). Because this alteration prevents the consensus sequence for the binding of transcription factors, the consequences on gene expression were planned to be investigated by using the RT-PCR method.

The RT-PCR protocol was designed on simultaneous amplification of partial portions of the complementary *C1INH* (cC1INH; exons 5-7) gene and a housekeeping gene, β -actin (exons 2-6), as an internal control to normalize the quantification of the *C1INH* mRNA level. cDNAs were gained by means of reverse transcribing the total RNA (Mini Blood RNA Isolation Kit; Qiagen, Hilden, Germany) isolated from 200- μ L peripheral blood samples from the healthy control subject and family members (I:1, II:3, II:4, and I:2). At that time, the proband was already undergoing treatment for her attacks.

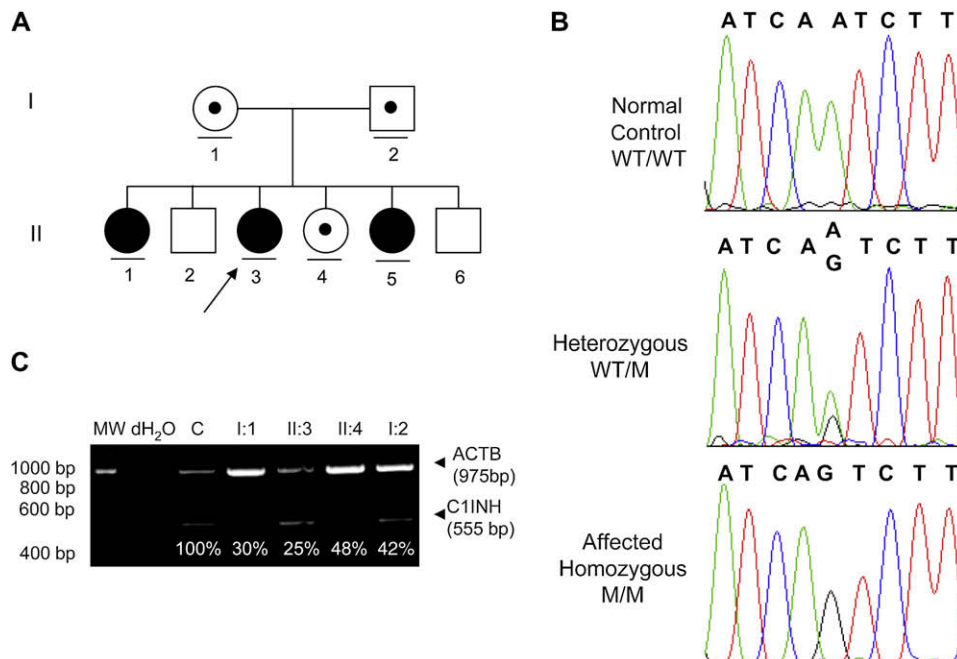


FIG 1. A, Pedigree of the family. Clinically checked individuals are underlined, and the proband is indicated with an arrow. Solid symbols indicate a homozygous subject, and a dot inside a symbol shows a heterozygous subject. B, DNA sequencing chromatograms. C, RT-PCR products on agarose gel. dH₂O excludes background. C indicates the control subject. Other lanes are shown by pedigree numbers. Quantified values for *C1INH* mRNA are listed under the gel photograph. MW, Molecular weight.

TABLE 1. Complement profile of the family

Examined individuals	Age (y)	Biochemistry of complement profile (normal ranges)			
		C4 (15-50 mg/dL)	C1INH (14-35 mg/dL)	C1INH function (%)	C1q (100-300 µg/mL)
I:1*	80	28.3	19	46	371
I:2*	80	36.1	19	65	176
II:1†	58	9.47	6	32	184
II:3†	53	7.02	3.84	28	186
II:4*	47	34	15	55	147
II:5†	45	10.1	7	31	191

Abnormal values are shown in bold. Levels for II:1, II:3, and II:5 are pretreatment values.

*Heterozygous.

†Homozygous.

The quantification of the *C1INH* mRNA levels was realized by normalizing the ratio for *C1INH* to the housekeeping gene β -actin of tested individuals to the healthy control subject. This calculation resulted in a reduced level of *C1INH* mRNA in the homozygous patient comparable with the degrees seen in heterozygous individuals and not a lesser degree, as expected (Fig 1, C). It was previously demonstrated by Pappalardo et al⁵ that increased expression of *C1INH* mRNA occurred in patients undergoing treatment with danazol. Supporting this finding, one could rationalize our results by stating that the mRNA level of our homozygous patient would have been lower than that of the heterozygous individuals in proportion to her previously poor C1Inh profile if her blood sample for RNA experimentation were taken before the initiation of her therapy. The proband's C4 and C1Inh levels were 10.5 mg/dL and 6.27 mg/dL, respectively, during treatment, which is comparable with those of the asymptomatic heterozygous individuals of the family.

Affected members of this family displayed a concordant complement profile with HAE-I, whereas heterozygous individuals had mild decrease of C1Inh activities. This could not be classified as HAE-II because the decrease was not more than 50% of normal values, and C4 levels were within normal limits. Although the parents of our proband did not previously mention consanguinity, they originated from the same small village, ultimately sharing a common ancestor.

To our knowledge, only one autosomal recessive case has been reported to date in a family with c. –103C>T mutation, affecting the promoter region of *C1INH*, as noted by Verpy et al.⁴ More recently, another case with a homozygous mutation in the coding region of the *C1INH* gene was described by Blanch et al⁶ with a unique activation and consumption profile of the classical complement activation pathway different from that commonly observed in patients with HAE but similar to that seen in patients with acquired angioedema.

We propose that the homozygous occurrence of c. –101A>G in our patient is pathogenic, not only because it is the only change in the *C1INH* gene but also because its recessive mode segregates with the affected individuals in our family and also because *C1INH* mRNA levels in heterozygous carriers are reduced in parallel to their C1Inh profile. Furthermore, c. –101A>G was not identified in 136 ethnically matched control chromosomes analyzed based on differential band patterns in single-strand conformation polymorphism analysis, which excludes its state for being an infrequent polymorphism.

All 3 homozygous patients of our family responded well to danazol. This matter reflects the genotype-phenotype relationship of the promoter site mutations, which generally do not influence

the quality but rather the quantity of the encoded protein. Therefore unlike the previously reported patients carrying homozygous structural mutations in the coded region of the *C1INH* gene, who could not benefit from treatment with androgens because of the lack of a wild-type allele, patients with homozygous promoter region mutations can benefit from the treatment.⁶

In conclusion, we describe here the second autosomal recessive family with a novel mutation affecting the *C1INH* promoter region (c. –101A>G). Identified sequence alteration in homozygous form causes moderate-to-severe life-threatening HAE symptoms; on the other hand, these symptoms respond well to attenuated androgens.

We thank the family members who participated in this study. We also thank Duzen Laboratories, Istanbul, Turkey, for allowing us to use their DNA sequencer, ABI3130.

Suna Büyükköztürk, MD^a
Belgin Kesim Eroğlu, MD^b
Aslı Gelincik, MD^a
Abdullah Üzümcü, PhD^c
Ferhan Özseker, MD^a
Bahattin Çolakoğlu, MD^a
Murat Dal, MD^a
Z. Oya Uyguner, PhD^c

From ^athe Department of Internal Medicine, Division of Allergy, and ^cthe Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, and ^bSisli Etfal Training and Education Hospital, Medical Genetics Clinic, Istanbul, Turkey. E-mail: gelincik@istanbul.edu.tr.

Supported by the Research Fund of Istanbul University (project no. 502/05052006).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

REFERENCES

- Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. *Biochem Biophys Res Commun* 2006;343:1286-9.
- Tosi M. Molecular genetics of C1 inhibitor. Review. *Immunobiology* 1998;199:358-65.
- Pappalardo E, Caccia S, Suffritti C, Tordai A, Zingale LC, Cicardi M. Mutation screening of C1 inhibitor gene in 108 unrelated families with hereditary angioedema: functional and structural correlates. *Mol Immunol* 2008;45:3536-44.
- Verpy E, Biasotto M, Brai M, Misiano G, Meo T, Tosi M. Exhaustive mutation scanning by fluorescence-assisted mismatch analysis discloses new genotype-phenotype correlations in angioedema. *Am J Hum Genet* 1996;59:308-19.
- Pappalardo E, Zingale LC, Cicardi M. Increased expression of C1-inhibitor mRNA in patients with hereditary angioedema treated with Danazol. *Immunol Lett* 2003;86:271-6.
- Blanch A, Roche O, Urrutia I, Gamboa P, Fontán G, López-Trascasa M. First case of homozygous C1 inhibitor deficiency [published erratum appears in *J Allergy Clin Immunol* 2007;119:745]. *J Allergy Clin Immunol* 2006;118:1330-5.

doi:10.1016/j.jaci.2008.12.022