

Letter to the Editor

Denaturing HPLC in laboratory diagnosis of hereditary angioedema

To the Editor:

Hereditary angioedema (HAE; Online Mendelian Inheritance in Man [OMIM]#106100) is a disorder resulting from the congenital deficiency of C1 esterase inhibitor protein (C1INH), inherited in an autosomal-dominant form. Patients with HAE present different mutation patterns in the *C1INH* gene (C1INH, SERPING1; OMIM# 606860) including deletions, insertions, and point mutations.¹ Eighty-five percent of patients show decreased C1INH antigenic and functional levels (type I HAE); 15% show normal or increased concentrations of a dysfunctional C1INH protein (type II HAE). Clinically, HAE is characterized by episodes of swelling of the extremities, face, trunk, airways, or abdominal viscera, occurring either spontaneously or after stress and/or trauma.¹

Hereditary angioedema diagnosis is made by taking into account the genealogic studies showing the autosomal-dominant pattern of inheritance as well as the clinical characteristics of the acute episodes, and requires laboratory evaluation with a confirmation of C1INH deficiency by means of immunochemical and functional assays.¹ Although this procedure allows diagnosis in most of the cases, in some patients an accurate diagnosis is not quickly or easily reached or even reached at all. This can be a result of missing, unclear, or negative family history, confusing clinical manifestations, borderline or apparently normal laboratory results,² or a combination of these factors. Because HAE is a severe disease and the attacks can be life-threatening, the problems linked to a delayed or missed diagnosis are often dramatic.³ On the other hand, patients with a false diagnosis of HAE may be treated improperly, with the actual underlying disease neglected and untreated. Therefore, to clarify the diagnosis definitely, the best possible approach is to study the *C1INH* gene.

Several mutation-scanning methods have been applied to the detection of sequence variation in the *C1INH* gene.¹ Although these methods would provide the possible detection and characterization of the mutations, they are not suitable for HAE diagnosis because of cost, complexity, and time constraints/limitations. Denaturing HPLC (DHPLC), which is a rapid, inexpensive, and simple method, has been proposed for mutation screening in several genetic diseases.⁴ With these considerations in mind, we developed a DHPLC-based protocol for mutation detection in the *C1INH* gene to improve laboratory diagnosis of HAE.

For this purpose, by using DHPLC (and also direct DNA sequence analysis, which was used to confirm DHPLC results), we studied 49 patients with HAE, 8 patients with an unconfirmed suspicion of HAE, and 50 normal subjects who served as controls. All of the subjects provided informed consent.

Genomic DNA was isolated from peripheral blood lymphocytes using a phenol-chloroform protocol. A PCR amplification of the *C1INH* gene was performed on 200 ng genomic DNA using commercially available primers as reported by Guarino et al.⁵ Each amplicon was analyzed by using DHPLC in a WAVE DNA Fragment Analysis System (Transgenomic Inc, Crewe, United Kingdom). The mobile phase was constituted of 0.05% DHPLC-grade acetonitrile (9017-03; JT Baker, Phillipsburg, NJ) in 0.1 mol/L triethyl ammonium acetate (Transgenomic Inc) and 25% acetonitrile in 0.1 mol/L triethyl ammonium acetate.

The appropriate experimental conditions for running DHPLC have been listed previously.⁵ Three wild-type samples were always used as negative controls. Heterozygous profiles were identified by visual inspection of the chromatograms on the basis of the appearance of additional earlier eluting peaks.

As reported, after DHPLC, direct-DNA sequence analysis was performed to serve as the control. The amplified segments were purified with a GeneDial Seq-Prep Kit (GeneDial, Lucca, Italy) and were sequenced in both directions using CEQ 2000 (Beckman Coulter Inc, Fullerton, Calif).

By means of DHPLC, we detected mutations in 49 of 49 patients with HAE and in 2 of 8 patients with an unconfirmed suspicion of HAE. No mutations were found in the other 6 patients with unconfirmed suspicion of HAE or in any of the 50 controls. Three HAE patients also showed 3 sequence variants detected by means of 1 or more additional peaks indicating the presence of 3 polymorphisms as reported in Table I. DHPLC representative elution profiles regarding mutations falling in exons 3, 5, and 8 are reported in Fig 1. Direct DNA sequence analysis fully confirmed either positive or negative results obtained by DHPLC. The 49 patients with HAE showed 14 different mutations (11 novel and 3 previously described^{6,7}) and 3 polymorphisms, whereas the 2 patients with an unconfirmed suspicion of HAE (2 brothers with unaffected parents but with confirmed paternity) showed another novel identical mutation (Table I). These patients presented a germinal mosaicism (previously reported by us in a separate article⁵). The 6 patients with unconfirmed suspicion of HAE who demonstrated having a wild-type *C1INH* gene were thereafter redirected to another diagnosis (acquired angioedema [1; negative for C1q autoantibodies], endometriosis [1; patient's father was affected with HAE, as confirmed by DHPLC], systemic lupus erythematosus [3], and Sjögren syndrome [1]). The genomic DNA sequence in the 50 controls was found to agree completely with the wild-type sequence described previously.¹⁰

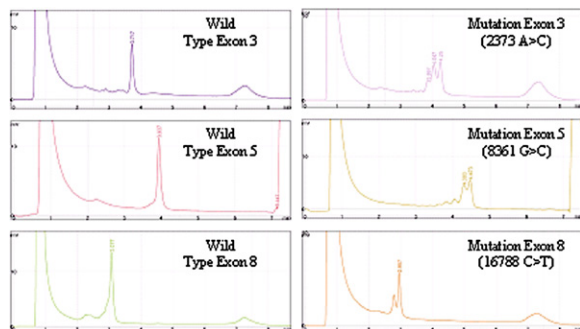
Our data demonstrate the superior efficacy of DHPLC in HAE diagnosis. In fact, DHPLC was effective in detecting the mutations present in the *C1INH* gene in all the patients with HAE studied, whereas profiles deriving from the controls were demonstrated to be normal. Direct DNA

TABLE I. C1INH mutations and polymorphisms identified by DHPLC

Family no.	Patient no.	Intron/exon	Traditional DNA numbering*	Predicted effect on protein or splicing alteration		Type
				Traditional numbering	Systematic numbering	
Sporadic	1	Exon 3	2373 A>C	Lys55Gln	Lys77Gln	Missense
1	2-5	Exon 3	2558 C>T	Ser115Ter	Ser137Ter	Nonsense
1	4	Exon 8	16655 T>C	Phe399Phe	Phe421Phe	Polymorphism
2	6-9	Exon 3	2586 G>T	Asp126Tyr	Asp148Tyr	Missense
3	10-12	Exon 4	4390 T>A	Leu175His	Leu197His	Missense
Germinal mosaicism	13,14	Exon 4	4397 C>G	Tyr177Ter	Tyr199Ter	Nonsense
4	15,16	Exon 5	8361 G>C	Arg219Pro	Arg241Pro	Missense
5	17,18	Exon 5	8424 T>G	Ile240Ser	Ile262Ser	Missense
6	19-21	Exon 8	16662 G>T	Asp402Tyr	Asp424Tyr	Missense
6	19	Exon 8	16830 G>A	Val458Met	Val480Met	Polymorphism
Sporadic	22	Exon 8	16672 T>C	Leu405Pro	Leu427Pro	Missense
Sporadic†	23	Exon 8	16788 C>T ⁶	Arg444Cys	Arg466Cys	Missense
Sporadic†	23	Exon 6	8488 C>T	Ser261Ser	Ser283Ser	Polymorphism
7	24,25	Exon 8	16823 C>A	Phe455Leu	Phe477Leu	Missense
Sporadic	26	Exon 8	16834 T>C ⁷	Leu459Pro	Leu481Pro	Missense
8	27-29	Exon 8	16870 delG	Frameshift471	Frameshift493	Nonsense
9	30-35	Exon 8	16872 C>T ⁷	Arg472Ter	Arg492Ter	Nonsense
10	36-51	Intronic	4350 (c.551-1 G>A) G>A	Donor splicing site?	Donor splicing site?	Splicing

*The traditional genomic DNA nomenclature follows the numbering currently used in the hereditary angioedema database.⁸ For amino acids, both traditional and systematic numberings are shown.⁹

†HAE type II.

**FIG 1.** Specimen chromatograms produced by DHPLC analysis.

sequence analysis confirmed all the results obtained by DHPLC. The 15 mutations found and studied correspond with nearly the 10% of those already reported in the literature, and this fact gives strength to our data. We did not find any false-positive or false-negative results, nor did the presence of polymorphisms invalidate the DHPLC results.

Once the efficacy of DHPLC in HAE diagnosis is acknowledged, the advantages of DHPLC in terms of time, cost, and complexity compared with direct DNA sequence analysis make it an appropriate method for a quick and accurate diagnosis of HAE. In fact, after the preparation of the amplicons, which is a common procedure for both methods that takes a mean time of about 3 hours, the DHPLC run-time is very short (about 10 minutes), the overall costs are significantly lower, and

the method is very simple. We did not have any problem with the set-up of the DHPLC protocol because the amplicons were linear and homogeneous, the identification of the various elution profiles was easy, and no problems arose during the analysis of the different mutations. Furthermore, the fact that DHPLC runs in a fully automated instrument (which is going to be widely available in genetic diagnostic laboratories) allowed us to gain time and to standardize our protocol easily, thus avoiding errors.

In conclusion, this study found DHPLC to be the valid complementary method in the laboratory diagnosis of HAE.

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