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Hereditary Angioedema: A Taiwanese Family with a Novel Gene Mutation

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SUMMARY Hereditary angioedema (HAE) is an autosomal dominant disorder caused by a deficiency of C1 esterase inhibitor (C1-INH). Affected individuals have attacks of swelling involving almost any part of the body. We studied a family with 15 living members, including a 16-year-old girl who had 3 attacks of angioedema in 2 years. Her paternal uncle had died of asphyxiation during an attack 15 years previously. We analyzed the blood of each family member for C3, C4, and C1-INH levels and sequenced the SERPING1 (formerly C1NH) gene that codes for C1-INH. Six individuals had decreased serum levels of C4 and C1-INH, and they were all found to have a single nucleotide A deletion at codon 210 of the gene, I210fsX210, a novel mutation that accounts for the HAE in this family.

Hereditary angioedema (HAE) is a rare, life-threatening condition manifested by acute attacks of facial, laryngeal, genital, or peripheral edema. There may be associated abdominal pain secondary to intra-abdominal edema. Current estimates of the prevalence of HAE are between 1 in 10,000 and 1 in 150,000 people.[1,2] The attacks last 2 to 5 days, after which they regress spontaneously. The clinical characteristics were first fully described by William Osler.[3] The pathophysiologic basis of HAE, namely a deficiency of C1 inhibitor (C1 INH), was discovered by Donalson and Evans in the early 1960s.[4] HAE is an autosomal dominant inherited disease caused by a defective gene that produces either no C1 INH (type I HAE) or a dysfunctional C1 INH (type II HAE). A third type has also been reported, occurring exclusively in women who have normal C1 INH levels and function.[5] It is estimated that 20% to 25% of HAE cases are the result of spontaneous mutations in persons with no family history of the disease.[6,7] In this paper, we report a family in Taiwan with a novel mutation of the gene encoding for C1 INH.

PATIENTS AND METHODS

Subjects

A 16-year-old girl presented with 3 attacks of angioedema of the face and neck within a 2-year period. When asked about her family history, she reported that her paternal uncle had had recurrent laryngeal edema with facial angioedema and had died of asphyxia 15 years earlier. We therefore investigated serum levels of C3, C4, C1 INH and the SERPING1gene that encodes for C1 INH gene in all 15 members of the family.

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C3 and C4 analysis

We used the Beckman Array® automatic system to measure C3 and C4 serum levels by rate nephelometry.[8]

C1 inhibitor analysis

C1 esterase inhibitor concentrations were measured by radial immunodiffusion assay.

C1 inhibitor gene (SERPING1) sequencing

The 15 living members of the family, including the 16-year-old index patient, were screened for a SERPING1 gene mutation by automatic direct sequencing. Genomic DNA was extracted from peripheral blood leukocytes with a GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech) according to the manufacturer's instructions. Genomic DNA (1 μg) was subjected to 35 cycles of PCR amplification in a final volume of 50 μl using a AmpliTaq Gold DNA polymerase kit (Applied Biosystems), 0.25 mM dNTPs, 1.75 mM $MgCl_2$ and 0.2 μM of the primers that amplified exons 1 to 8 with exon-intron boundaries by primers with slight modification in the presence of 0.1 U of T*aq* polymerase.[9,10,11] PCR was performed using an Applied Biosystems 9700 thermocycling kit under the following conditions: denaturation of genomic DNA at 95°C for 10 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 10 minutes. The amplified product was purified using a GFR PCR DNA and Gel Band Purification Kit (Amersham Biosciences). The sequence reactions were performed by using a DNA sequencing kit (Big Dye Terminator cycle sequencing, Applied Biosystems). The sequencing products were precipitated with ethanol/sodium acetate and analyzed on an automated DNA sequencer (ABIPRISM 3100, Applied Biosystems). Each DNA sample was sequenced in both directions. All mutations were verified with a second independent PCR product.

RESULTS

Six of the 15 family members investigated had decreased C4 and C1 INH serum levels, consistent with a diagnosis of type I HAE (Table 1). Of these, only 2 had a history of recurrent episodes of edema of the face and neck with compromise of the airways, including the 16-year-old index patient and

Table 1 Serum C3, C4, and C1 inhibitor levels and SERPING1 gene studies in a family with hereditary angioedema

Family member	C1 inhibitor (mg/dl)	C3 (mg/dl)	C4 (mg/dl)	SERPING1 gene mutation
1	53.7	134	28	None
2	9.9	120	12	**I210fsX210**
3	6.5	118	<10	**I210fsX210**
4	53.7	126	40	None
5	42.5	103	18	None
6	40.3	97	22	None
7	8.5	118	11	**I210fsX210**
8	6.5	81	10	**I210fsX210**
9	53.7	121	30	None
10	49.1	105	42	None
11	53.7	118	36	None
12	6.5	118	<10	**I210fsX210**
13	6.5	110	<10	**I210fsX210**
14	49.1	112	27	None
15	44.6	94	16	None

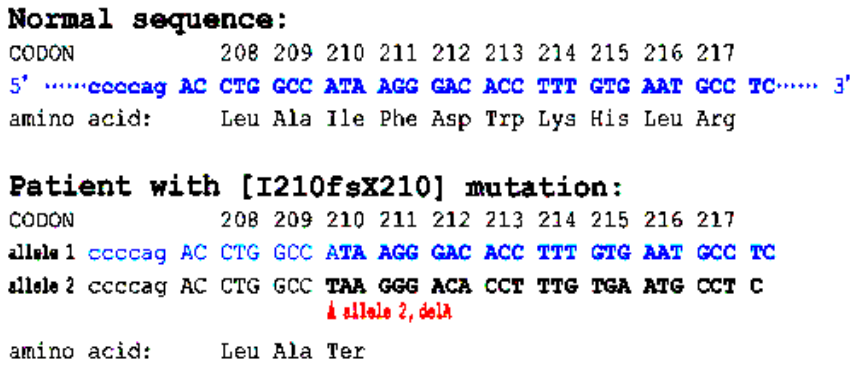

Fig 1. Direct DNA sequencing of the polymerase chain reaction product of exon 5 of the SERPING1 gene from a normal control (a) and from a patient with hereditary angioedema (b). There is a single-base deletion (del A, vertical arrow) in one allele in the patient, causing a frameshift mutation at codon 210, with a normal ATA becoming TAA, a stop codon.

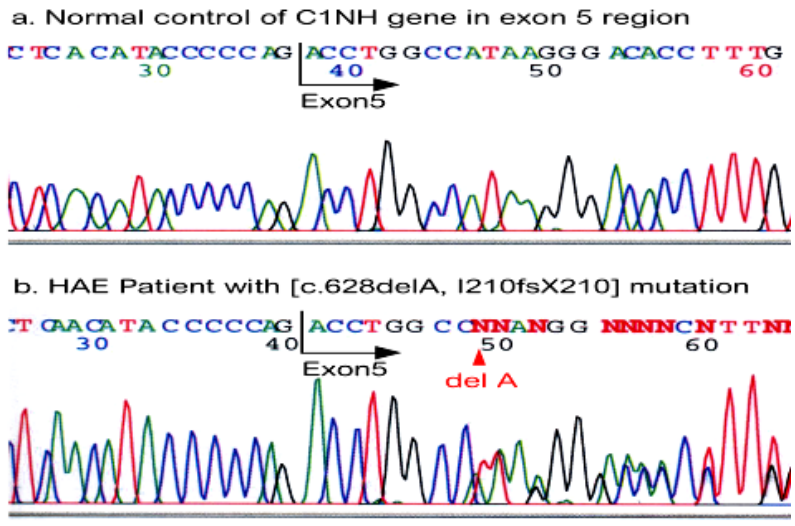

Fig. 2 Nucleotide and amino acid sequences of the normal and mutant SERPING1 alleles and their product. The deletion of nucleotide A at codon 210 in exon 5 results in a frameshift mutation generating a TAA stop codon instead of the normal ATA. The alanine translated from codon 209 thus becomes the C-terminal residue of the abnormal C1-INH molecule.

her 12-year-old sister. These attacks were often associated with trauma or an emotional episode. Nine family members had normal serum C1-INH and C4 concentrations, and none had a history compatible with HAE. Among the 6 with a low C1 INH level, only one also had a low serum C3 level (a son of the man who had died). Direct DNA sequencing analysis of the PCR products of the SERPING1 gene revealed that the 6 family members with decreased serum C1 INH levels were all heterozygous for a single-base deletion of A at codon 210 in exon 5 causing a frame shift (I210fsX210)(Fig. 1). This deletion resulted in a non-sense mutation at the codon 210, ATA to TAA (Fig. 2), thereby truncating the

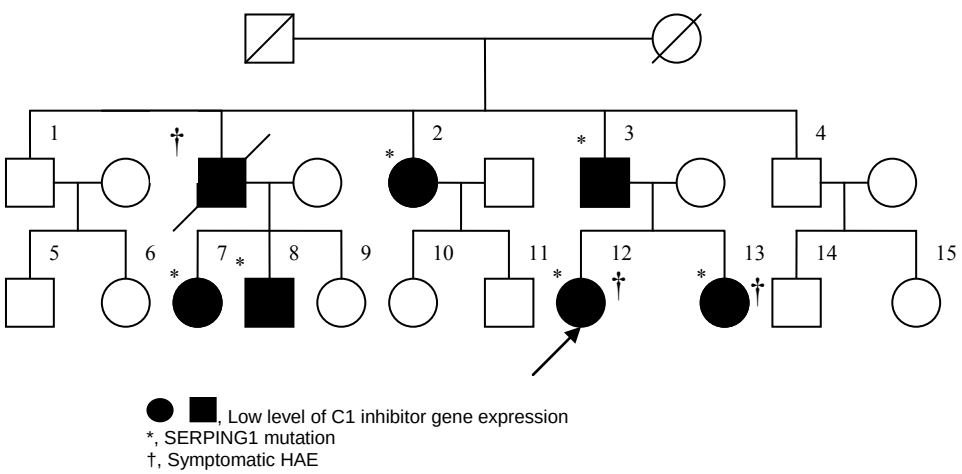

Fig. 3 Pedigree of a family with hereditary angioedema. The index patient (arrow) is a 16-year-old with recurrent angioedema of the face and neck. Her sister has also had 2 episodes of angioedema. A paternal uncle died during an attack 20 years previously.

C1-INH C-terminus by 270 amino acids. None of the 9 family members with normal C1-INH levels had this point mutation (Fig. 3).

DISCUSSION

The mutation found in these 6 family members, I210fsX210, has not been previously reported. Jasnson *et al.*[12] mapped the SERPING1 gene to 11q12-11q13.1. It has 8 exons distributed over a 17-kb DNA stretch with introns containing multiple Alu repeat sequences.[13] More than 150 mutations have been reported in this gene,[14,15] ranging from subtle changes affecting 1 or several nucleotides to large deletions or duplications.[10,15,16] Members of the family we investigated had type I HAE caused by a novel point mutation in SERPING1. This result plus inspection of the pedigree allowed us to make a retrospective diagnosis of the HAE in the index patient's uncle who had died some years earlier. His wife had a normal gene, but 2 of his 3 children had the I210fsX210 mutation, which must therefore have been transmitted from their father.

The edema in acute HAE can be diffuse, involving all layers of the skin and of the walls of hollow and solid viscera.[17] Asymptomatic carriers have been reported, estimated at about 5% of individuals with C1 INH abnormalities, but the reason for this is unknown.[7] It is interesting, therefore, that of the 6 living individuals in our study with the I210fsX210 mutation and resulting in low C1 INH levels, only 2 have thus far had attacks of HAE.

Treatments for an acute attack of HAE include C1-INH concentrate and fresh frozen plasma, although the former is not currently available in Taiwan. Long-term prophylaxis involves attenuated androgens, antifibrinolytic agents, or C1-INH concentrate. We treated the index patient with danazol, which succeeded in improving her serum C4 and C1-INH levels. However, she was unable to tolerate the virilizing side effects. Nine months after stopping the medication, she had another episode of angioedema. We therefore instituted prophylaxis with the antifibrinolytic agent tranexamic acid.

This is the first investigation of a family with HAE reported in Taiwan. We have demonstrated a novel mutation, I210fsX210, of SERPING1, the gene that encodes for C1-INH. In addition to identifying this mutation in 6 of 15 living family members who also have low C1-INH levels, we could demonstrate by the pedigree that a family member who died years earlier must have had the same mutation. Four living members are as yet asymptomatic, but they are

at risk for HAE. This knowledge should be helpful in minimizing trigger factors and immediately initiating appropriate treatment should they develop symptoms.

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