
Case report

Paternal mosaicism and hereditary angioedema in a Taiwanese family

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Background: Hereditary angioedema (HAE) is a rare disorder characterized by recurrent attacks of localized subcutaneous or submucosal edema. It is inherited in an autosomal dominant fashion and caused by a deficiency of C1 inhibitor (C1 INH). Most patients with HAE have an absolute deficiency of C1 INH (type I HAE), whereas the rest (approximately 15%) synthesize a dysfunctional C1 INH protein (type II HAE). Mosaicism is rare in HAE.

Objective: To describe the clinical manifestations, laboratory findings, and molecular genetic studies in a Taiwanese family with type I HAE with paternal mosaicism.

Methods: A family that included a 34-year-old man (index patient) and his 25-year-old brother who both had recurrent peripheral angioedema was evaluated. A younger sister had died of an unexplained cause at 18 years of age. We analyzed blood levels of C3, C4, and C1 INH and sequenced the *SERPING1* (*C1NH*) gene that codes for C1 INH in 5 family members, including the parents and 3 brothers.

Results: The 4 men in the family had a novel mutation c.3_73del, p.N1fsX34 in exon 3 of the *C1INH* gene, resulting in C1 INH deficiency. Although the father carried this mutant gene, he had normal serum levels of C1 INH. Based on quantitative analysis of allele dosage by DNA fragment analysis (GeneScan), the father was determined to have genetic mosaicism.

Conclusion: Parental mosaicism is a possible explanation for normal C1 INH plasma concentrations in both parents despite clinically apparent HAE in the children.

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INTRODUCTION

Hereditary angioedema (HAE) is a rare autosomal dominant disease caused by a defective gene that produces either no C1 inhibitor (C1 INH, type I) or dysfunctional C1 INH (type II).^{1,4} The major clinical manifestations are recurrent attacks of localized subcutaneous or submucosal edema of the face, larynx, genitalia, intestinal wall, or limbs, occurring either spontaneously or after stress or trauma. If the larynx is involved, life-threatening airway obstruction may ensue.^{1–3}

We describe a Taiwanese family in which 2 symptomatic brothers had very low levels of C4 and C1 INH, consistent with a diagnosis of type I HAE. Surprisingly, the parents were both asymptomatic and had normal levels of C4 and C1 INH. By genetic studies, we found 4 members, including the father and the 3 sons, who had a novel mutation of the *C1INH* gene, thus confirming the diagnosis. Interestingly, gel electrophoresis of the polymerase chain reaction (PCR) products

and direct DNA sequencing from the father's DNA were different from the 3 sons, suggesting genetic mosaicism. Based on quantitative analysis of allele dosage by DNA fragment analysis (GeneScan), the father was determined to have genetic mosaicism, which has not, to our knowledge, been previously reported in HAE.

MATERIALS AND METHODS

Report of Cases

A 34-year-old man had had approximately 30 episodes of angioedema that involved the extremities since the age of 20 years. The edema was not pruritic or tender and was related to trauma or compression. His 25-year-old brother also had 2 episodes of peripheral edema in the previous 2 years. His younger sister had died suddenly of an unknown cause while asleep at the age of 18 years. Other family members had no angioedema episodes. We collected blood samples from the 2 symptomatic brothers, their parents, and 1 apparently unaffected brother (Fig 1).

Complement Studies

We investigated concentrations of C1 INH, C3, and C4 by radial immunodiffusion.

SERPING1 Gene Sequence Analysis

Genomic DNA was extracted from peripheral blood leukocytes with a purification kit (GFX Purification Kit; Amer-

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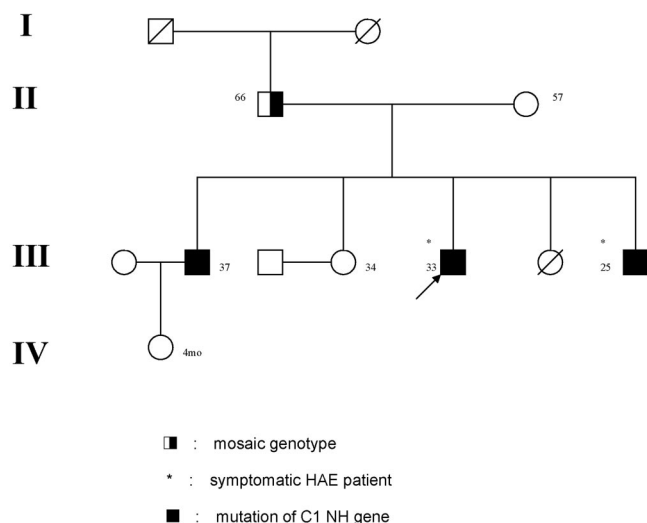
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sham Biosciences UK Ltd, Little Chalfont Buckinghamshire, England). PCR amplification was performed in a volume of 50 μ L with 0.25-mmol/L dNTPs, 1.75-mmol/L magnesium chloride, 0.2 μ M of primers, and 0.1 U of Taq polymerase that amplifies exons 1 to 8 and exon-intron boundaries.⁵⁻⁷ The reaction was performed using a thermocycler (9700 thermocycler; Applied Biosystems, Foster City, California). The amplified product was purified using a PCR DNA and gel band purification kit (GFX PCR DNA and Gel Band Purification Kit; Amersham Biosciences). Sequencing was performed with a DNA sequencing kit (Big Dye Terminator cycle sequencing; Applied Biosystems). The reaction products were purified by Multiscreen-HV plates (Millipore) filled with Sephadex G-50 superfine beads (Amersham Biosciences) and analyzed on an automated DNA sequencer (ABI PRISM 3100; Applied Biosystems). Each DNA sample was sequenced in both directions. All mutations were verified with a second independent PCR product.

RESULTS

Both symptomatic brothers had very low C4 and C1 INH serum levels (C4: 5 and 8 mg/dL; C1 INH: 4 and 7 mg/dL), consistent with a diagnosis of type I HAE. Their asymptomatic elder brother had decreased levels of C1 INH but C4 concentration at the lower normal limit (C4: 16 mg/dL;

Figure 1. Pedigree of a family with hereditary angioedema. The index patient (arrow) was a 34-year-old man with recurrent angioedema. His 25-year-old brother also had symptoms. The younger sister had died suddenly of unexplained causes at 18 years of age. The father had no siblings, and his parents were both dead. No angioedema episodes have been noted among the parents, the elder sister, the elder brother, and his daughter. The elder sister (lives abroad) and the 4-month-old granddaughter are not enrolled in the studies.

Table 1. Serum C3, C4, and C1 Inhibitor (C1 INH) Levels and *SERPING1* Gene Studies in a Family With Hereditary Angioedema

Family member	Manifestations and age at onset	C3 (N:79–152), mg/dL	C4 (N:16–38), mg/dL	CH ₅₀ (N:63–145), CAE units	C1 INH (N:15–35), mg/dL	C1INH Gene Mutation
Father	None	98	22	141.2	32	Exon 3 c.3_73del, p.N1fsX34
Mother	None	124	22	120.0	59	None
Elder brother	None	135	16	133.2	9	Exon 3 c.3_73del, p.N1fsX34
Index patient	Recurrent angioedema since 20 years	107	5	93.6	4	Exon 3 c.3_73del, p.N1fsX34
Younger brother	Recurrent angioedema since 24 years	98	8	110.3	7	Exon 3 c.3_73del, p.N1fsX34

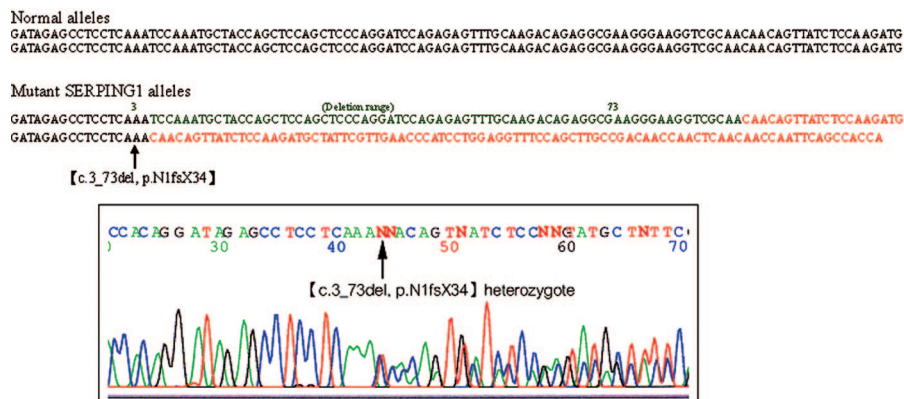


Figure 2. Nucleotide and amino acid sequences of the normal and mutant *SERPING1* alleles. A 70–base pair deletion in exon 3 results in a frameshift mutation.

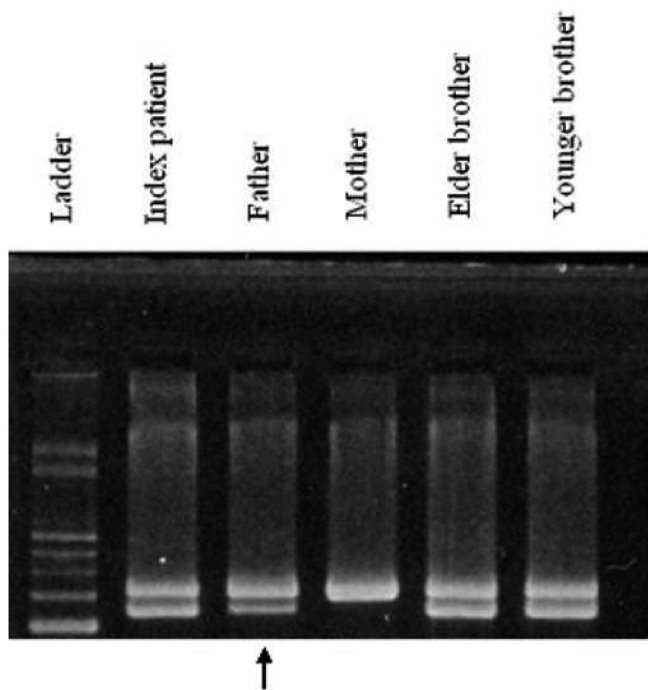


Figure 3. Gel electrophoresis of polymerase chain reaction products in a family with hereditary angioedema shows an aberrant band (arrow) in the blood of the father that is lower in intensity than the wild-type band, suggesting mosaicism.

reference range, 16–38 mg/dL; C1 INH: 9 mg/dL). The asymptomatic parents both had normal levels of C4 and C1 INH. All 5 had normal serum C3 levels (Table 1). Direct DNA sequencing of the coding regions of the *SERPING1* gene showed the 2 symptomatic brothers and the asymptomatic father and brother had a 70–base pair deletion in exon 3 that caused a frame shift (c.3_73del, p.N1fsX34) (Fig 2).

Interestingly, gel electrophoresis of the PCR products from the father's DNA showed an aberrant band that was lower in intensity than were the wild-type bands, suggesting genetic mosaicism (Fig 3). We then used DNA fragment analysis (GeneScan) to perform quantitative analysis of allele dosage. The relative allele dosage of the father's specimens was different: 0.22:0.78 in blood and 0.33:0.67 in buccal cells. Thus, we can confirm the diagnosis of paternal mosaicism (Table 2 and Figure 4).

DISCUSSION

The mutation that accounted for HAE in this family, a 70–base pair deletion in exon 3 (c.3_73del, p.N1fsX34), has not, to our knowledge, been previously reported. The *C1INH* gene is located on chromosome 11, which is composed of 8 exons and 7 introns and is 17,159 kb long.^{8–10} At present, nearly 150 different mutations have been reported.^{11,12}

It is estimated that up to 25% of HAE cases are the result of spontaneous mutations in persons with no family history of the disease.² What is particularly interesting in this family, however, is that the mutation was clearly transmitted to the offspring by the father, but he himself was not affected with HAE because of genetic mosaicism.

Mosaicism refers to a condition in which an individual has 2 or more cell populations that differ in genetic makeup. It occurs as a result of an error in cell division very early in fetal development. This error can affect any type of cell, including both germ and somatic cell lines. Mosaicism is suspected, in the father's case, if electrophoresis shows an aberrant band that is lower in intensity than the wild-type bands. Because affected and unaffected cells are usually not equal in number, the DNA patterns in mosaicism differ from those of both healthy individuals and individuals with a mutated gene. We used DNA fragment analysis (GeneScan) to perform quantitative analysis of allele dosage to confirm the diagnosis of mosaicism. Healthy individuals had a relative allele dosage

Table 2. Results of Quantitative Analysis of Allele Dosage by DNA Fragment Analysis (GeneScan)

Family member	Specimen	Allele	Size, base pairs	Peak area	RD ^a
Father	Blood	Deletion	280	14,630	0.22
		Normal	350	52,686	0.78
	Buccal swab	Deletion	280	4,674	0.33
		Normal	350	9,413	0.67
Mother	Blood	Deletion	NA	0	0.00
		Normal	351	83,432	1.00
Elder brother	Blood	Deletion	280	54,995	0.51
		Normal	351	52,419	0.49
Index patient	Blood	Deletion	280	65,425	0.51
		Normal	350	63,090	0.49
	Buccal swab	Deletion	280	10,367	0.54
		Normal	350	8,844	0.46
Younger brother	Blood	Deletion	280	32,855	0.53
		Normal	351	29,660	0.47

Abbreviation: NA, not applicable; RD, relative dosage.

^a The RD of each allele was calculated as follows: (peak area of deletion allele/peak area of total alleles: peak area of normal allele/peak area of total alleles).

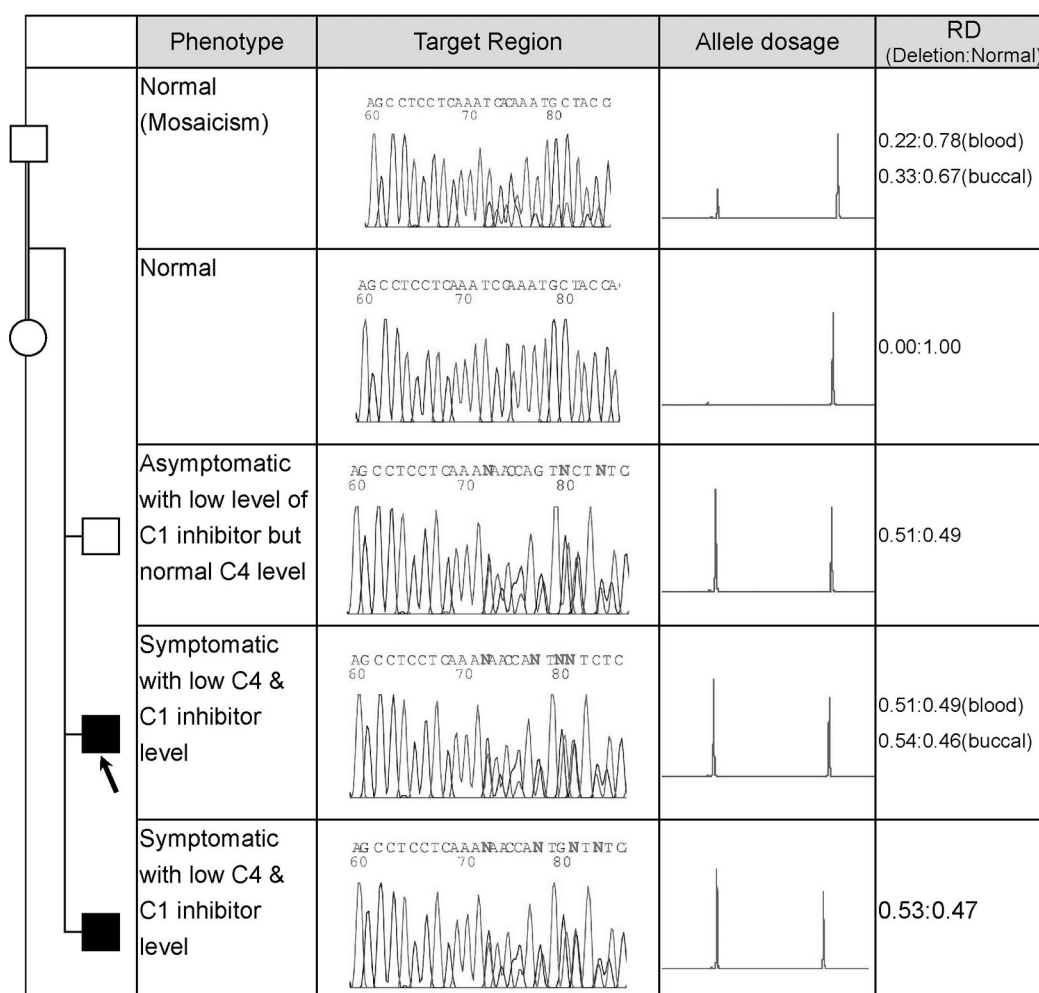


Figure 4. Summary of results of clinical manifestations, DNA sequencing, and allele dosage quantitative analysis by GeneScan. RD indicates relative dosage.

(deletion allele/normal allele) of approximately 0:1. Heterozygous patients had a relative allele dosage close to 1:1; however, the relative allele dosage of mosaic patients varied in different tissues and was not close to 1:1. Thus, we can make the diagnosis of mosaicism.

Symptoms are difficult to predict in a person with mosaicism. The presence of some percentage of normal cells may result in less severe disease than that seen in those with only the defective gene. Gonadal mosaicism, in which some gametes are affected, can, of course, be transmitted to the offspring, in whom it will appear as a new mutation.

All 3 sons of this family had the novel mutation c.3_73del, p.N1fsX34 in exon 3 of the *SERPINC1* gene, but only the 2 younger have thus far developed frank HAE beginning in the third decade of life. However, the eldest son in this family had no symptoms despite having a low level of C1 INH, clearly caused by the same mutation as his affected brothers. His father was asymptomatic because of mosaicism, but the

son's lack of symptoms may instead suggest variable penetrance. Asymptomatic carriers have been reported, estimated as approximately 5% of individuals with C1 INH abnormalities, but the reason for this is unknown.² In conclusion, the findings in this Taiwanese family with familial HAE demonstrate that mosaicism may explain the absence of symptoms in the parents of individuals with familial HAE.

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