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A hereditary angioedema screening on an index case

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Abstract

Background: Hereditary angioedema (HAE) is characterised by recurrent episodes of angioedema and can be fatal.

Objective: The present study aimed to screen HAE.

Methods: A total of 60 individuals were screened. The frequency and severity of symptoms were scored from 0 to 8. Measurements were taken of C4 and C1 esterase inhibitor protein (C1-INH) levels. Mutation in the C1 inhibitor gene was examined in 9 patients with HAE.

Results: A positive correlation between the C1 esterase inhibitor protein levels and C4 level was detected in the group as a whole ($p < 0.001$, $r = 0.725$, $n = 60$). A negative correlation between the C1 esterase inhibitor protein level and severity score was observed in the whole group ($p < 0.001$, $r = -0.486$, $n = 60$). A negative correlation was also detected in the entire group between the C4 level and severity score ($p = 0.002$, $r = -0.389$, $n = 60$). In the patients with HAE, a positive correlation between the C1 esterase inhibitor protein level and C4 levels was detected ($p = 0.034$, $r = 0.705$, $n = 9$). A heterozygous c. 601A > T nonsense variant was identified at the C1 esterase inhibitor gene—SERPING1—in patients with Type 1 HAE.

Conclusion: It is well known that there is a prolonged delay in the diagnosis of HAE. The present study demonstrates that it is very important and even life-saving to screen for HAE on the basis of an index case.

Key words: Hereditary, angioedema, C4, SERPING1, pediatric

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Abbreviations:
Hereditaryangioedema: HAE
DeoxyriboNukleicAcid: DNA
Etilendiamintetraasetikasit: EDTA

Introduction

Hereditary angioedema (HAE) is an inherited disease characterised by recurrent angioedema attacks. This swelling, which has indistinct borders and is not accompanied by pain, develops as plasma leaks between dermal layers of the skin through post-capillary venules. This leakage is mainly due to a deficiency in or inadequate functioning of a protein known as a C1 inhibitor.[1,2] The disease is relatively rare, with a reported incidence of between 1/10,000 and 150,000.[3] The most realistic estimate of incidence is thought to be 1/50,000.[4–6]

This disease is hereditary and transmitted as an autosomal dominant, meaning the disease does not skip generations but rather occurs in every generation.[7] There is no information about difference in propensity for the disease between ethnicities or genders. In a heterozygous individual, C1 esterase

inhibitor protein levelsare around 50% at birth, and symptoms usually do not appear before the C1 esterase inhibitor protein level falls below 35%. As such, patients typically begin to experience angioedema attacks in childhood, especially at the age of 2-3 years. A decrease in C1 esterase inhibitor protein levels and the resulting clinical picture usually occurs within two decades.[8] The disease tends to worsen with puberty, then lasts for the duration of the patient's life. The frequency and severity of the attacks may differ radically between patients or even over the years in the same patient.[9]

Symptoms of the disease tend to be more severe in patients with early-onset symptoms compared to those with late-onset ones.[10] Some patients experience severe attacks requiring emergency care while others experience only mild attacks that are resolved without any treatment. Beyond this vague distinction, how an attack will progress cannot be predicted by looking at the initial symptoms. Therefore, making a diagnosis as early as possible for those patients with mild attacks may prevent patient death due to laryngeal oedema attacks.

While very disturbing, angioedema attacks are usually not life-threatening, with the exception of swelling which occurs in the airways. More than 50% of hereditary angioedema patients experience a laryngeal enema episode at least once in their lifetime. Laryngeal symptoms usually occur in adulthood and are rare before the age of 3 years.[11] If an attack begins in the mouth and affects the respiratory tract, a clinical picture in which it closes the glottis and leads to death by asphyxiation may develop. Mortality in undiagnosed HAE can be up to 50%.[12] The disease is especially dangerous because in addition to the high mortality risk, a diagnosis of HAE can take an average of 8.5 years after the appearance of symptoms.[13] To combat this, examining the family and relatives of an indexed HAE patient for signs of the disease is vital.

C4 levels in almost all cases of HAE are low, both during the attacks and between them. Therefore, C4 is a fairly stable screening test for diagnosis. Following an assessment of C4 levels, an examination of the C1 esterase inhibitor protein level and function helps to form a diagnosis and identify whether the HAE is Type I (low C1 esterase inhibitor protein level, decreased C1 inhibitor function) or Type II (normal C1 esterase inhibitor protein level, decreased C1 inhibitor function).[14]

If HAE remains undiagnosed, the mortality rate can rise up to 50%, so early diagnosis and medical treatment is lifesaving. In the present study, having identified a case of Type I HAE, the relatives of the patient were screened to detect any presence of the disease. During this process, the reported mutation in the C1 inhibitor gene was found.

Methods

Subjects

For this study, a total of 60 individuals were screened for HAE as relatives across 5 generations of a confirmed case of HAE Type I. The subjects ranged in age from 1 to 75 years old. Each individual was interviewed and a blood sample was collected and analysed for C4 and C1 esterase inhibitor protein levels. A physical examination of the patients was then performed. The evaluation of all 60 subjects included measurements of C4 and C1 esterase inhibitor protein level, and diagnosis was established according to consensus criteria.[11,14] The examiners identified mutation in the C1 inhibitor gene in 9 patients, indicating the existence of HAE. Genomic DNA was extracted from a peripheral blood sample and complete sequencing of the coding region of the SERPING1 gene was then carried out.

HAE severity score

A simplified HAE severity score was constructed based on the score proposed by the Third C1 Esterase Inhibitor Deficiency Workshop.[15] Episodes of angioedema were classified according to average frequency and intensity of symptoms since onset of the disease. Frequency of symptoms was quantified as follows: > one episode a month, 3 points; between 6 and 11 episodes a year, 2 points; < 6 episodes a year, 1 point; and no symptoms of angioedema, 0 points. The intensity of symptoms was classified as: presence of discomfort but no disruption of daily activity, 2 points; discomfort reducing normal daily activity, 4 points; and inability to work or perform daily activity and/or necessity of hospital care, 5 points. The total of the frequency and intensity scores was used to classify the severity of the disease as: severe (≥ 7 points); moderate (5–6 points); mild (≤ 4 points); and asymptomatic (0 points).

Measurement of the levels of C1-INH and C4

Serum levels of C4 were determined by the Siemens C4 complement reagent—BN prospecnephelometry—with normal values ranging from 15–45 mg/dl. Serum levels of C1 esterase inhibitor protein levelwere determined by the Siemens C1 inhibitor reagent—BN prospecnephelometry—with normal values ranging from 0.15–0.35 g/l.

Identification of DNA mutation

Genomic DNA was extracted from EDTA (Ethylene diamine tetra-acetic acid) containing whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing of the SERPING1 gene for all exons was performed. After preparing the purified PCR products, capillary electrophoresis was run using an ABI 3130 Genetic Analyser system (Applied Biosystems, Cal, US). Sequence data were analysed using the SeqScape software version 2.6 (ABI, US).

Ethics

All study procedures were applied in accordance with a protocol previously approved by the Ethics Committee of Gaziantep University. Written informed consent was obtained from all adults and from the parents of subjects < 18 years of age.

Statistical analysis

All data analyses and comparisons were applied using SPSS v 11.5 software. The data were tested for conformity to normal distribution and differences between the groups were compared with the Mann-Whitney U test. A Pearson correlation coefficient was applied to investigate the correlation between different parameters. A value of $p < -0.05$ was considered statistically significant.

Results

Of the total screened patients, 35 (58.3%) were male, and 25 (41.7%) were female. C4 levels were found to be low in 9 of the 60 screened subjects. C1 esterase inhibitor protein levels were also found to be low in all 9 subjects, and a diagnosis of Type I HAE was made. The age at diagnosis ranged from 9 to 69 years (Median: 32.00 years; range, 18.00–49.00 years), while age of the onset of the first symptoms ranged from 3 to 19 years (Median: 9.00 years; range, 4.25–12.25 years). Of the patients diagnosed with HAE, 2 (22.2%) were ≤18 years of age and 7 (77.8%) > 18 years. While both of the 2 patients aged 18 years or younger were symptomatic, 6 of the 7 patients aged over 18 years were symptomatic and 1 was asymptomatic. No statistically significant difference was determined between the diseased and healthy groups in respect of age (mean 32.00 years; range, 18.00–49.00 years vs mean 22.00 years; range, 11.00–34.00 years, respectively) (p = 0.147).

In the whole group, a positive correlation was found between the C1 esterase inhibitor protein level(0.26 (0.22–0.29)) and C4 (22.05 (17.17–28.72)) (p < 0.001, r = 0.725, n = 60) (**Figure 1A**) and a negative correlation was found between the C1 esterase inhibitor protein level (0.26 (0.22–0.29)) and the severity score (0.00 (0.00–0.05)) (p < 0.001, r = -0.486, n = 60) (**Figure 1B**). A negative correlation was also detected between the C4 level (22.05 (17.17–28.72)) and the severity score (0.00 (0.00–0.05)) (p = 0.002, r = -0.389, n = 60) (**Figure 1C**).

The C4 and C1 esterase inhibitor protein levels of the patients diagnosed with HAE were significantly lower than those of the healthy group (5.90 (5.90–7.90) mg/dl vs. 24.00 (19.30–

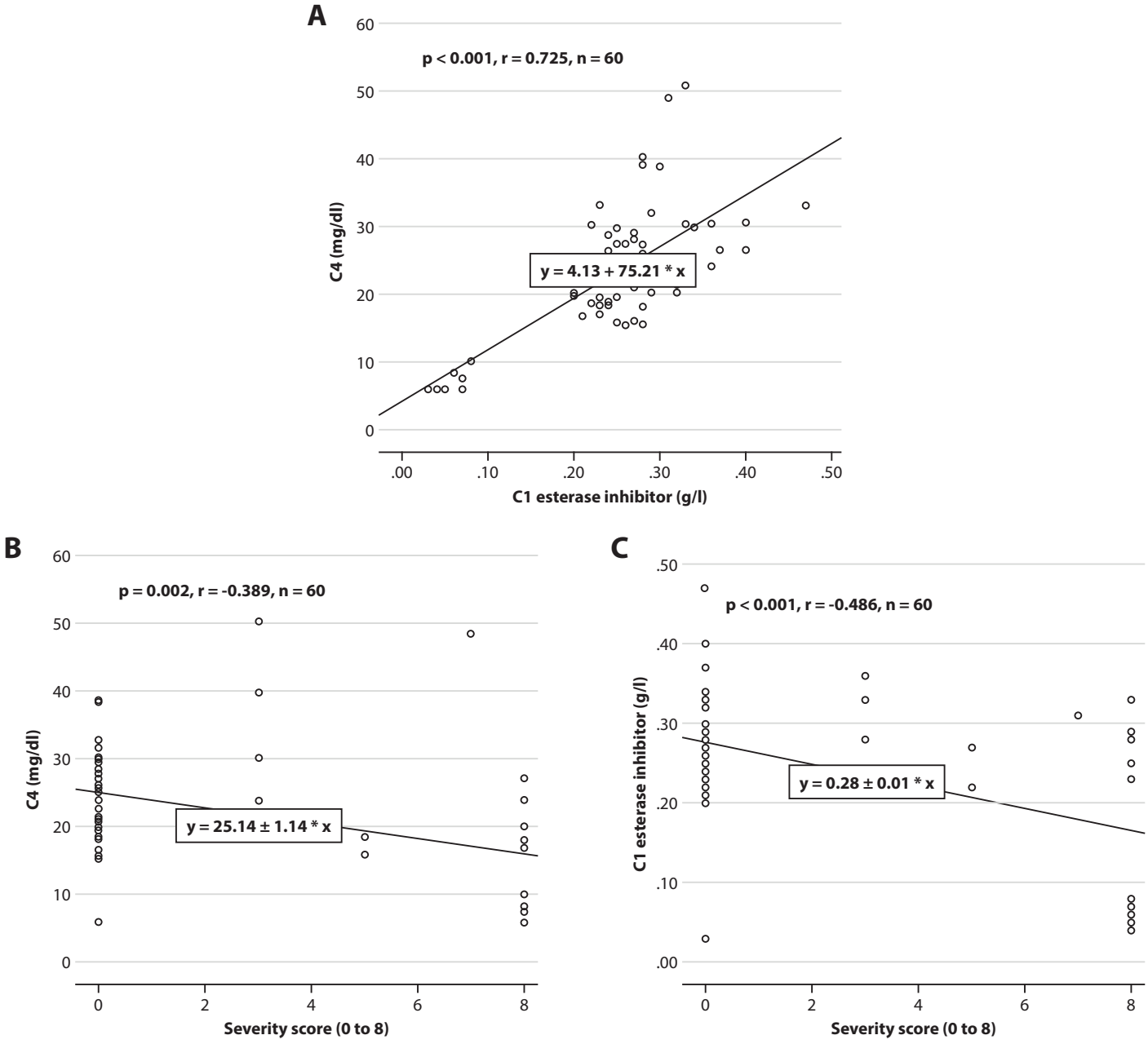

Figure 1. In the whole group; (A) Correlation between C4 level and C1 esterase inhibitor protein level; (B) Correlation between C4 level and severity score; (C) Correlation between C1 esterase inhibitor protein level and severity score (Pearson correlation).

Table 1. Demographic characteristics of all screened individuals.

	Patients with HAE (n = 9)	Healthy (n = 51)	p#
Age*	32.00 (18.00–49.00)	22.00 (11.00–34.00)	p = 0.147
Gender (M/F)	5/4	30/21	----------
C4 level (mg/dl)*	5.90 (5.90–7.90)	24.00 (19.30–29.60)	p < 0.001
C1 esterase protein level (g/l)*	0.050 (0.045–0.070)	0.27 (0.24–0.31)	p < 0.001
HAE Severity Score*	8.00 (8.00–8.00)	0.00 (0.00–0.00)	p < 0.001
Age of the onset of the first symptoms *	9.00 (4.25–12.25)	----------	----------
Age of diagnosis *	32.00 (18.00–49.00)	----------	----------

*median(%25-75); #mann-whitney u test

Table 2. Clinical and laboratory characteristics of healthy and diseased individuals.

	Patients with HAE (n = 9)	Healthy (n = 51)	p#
Male/Female	5/4 (55.6%/44.4%)	30/21 (58.8%/41.2%)	
Symptoms of painful swelling in the body	6 (66.7%)	2 (3.9%)	p < 0.001
Symptoms of painful swelling on the face	6 (66.7%)	0 (0%)	p < 0.001
Symptoms of painful swelling due to trauma occurred	6 (66.7%)	0 (0%)	p < 0.001
Symptoms of painful swelling after tooth extraction	2 (22.2%)	0 (0%)	p = 0.001
Symptoms of painful swelling after exercise, sorrow or alcohol intake	5 (55.6%)	0 (0%)	p < 0.001
Symptoms of hoarseness, feeling of obstruction in the throat and shortness of breath	3 (33.3%)	1 (2.0%)	p = 0.001
Symptoms of severe abdominal pain	8 (88.9%)	9 (17.6%)	p < 0.001
Symptoms of severe abdominal pain accompanied by vomiting and diarrhoea	4 (44.4%)	4 (7.8%)	p = 0.003
Symptoms of patients had undergone abdominal operations at any time in their lives	0 (0%)	0 (0%)	---------
Patients who were hospitalized in intensive care due to laryngeal edema	0 (0%)	0 (0%)	---------
Patients with low C4 level	9 (100%)	0 (0%)	p < 0.001
Patients with low C1 esterase protein level	9 (100%)	0 (0%)	p < 0.001

#mann-whitney u test

Table 3. Characteristics of patients with HAE.

ID	Age (years)	Gender (M/F)	Age of the onset of the first symptoms (Year)	Age of diagnosis (Year)	Severity score	C4 (mg/dl)	C1Inh (g/l)
1	26	M	3	26	8	5.90	0.05
2	69	F	5	69	8	10.00	0.08
3	32	M	13	32	8	7.50	0.07
4	34	M	10	34	8	8.30	0.06
5	9	F	4	9	8	5.90	0.07
6	10	F	8	10	8	5.90	0.05
7	37	F	19	37	8	5.90	0.04
8	61	M	-	61	0	5.90	0.03
9	26	M	10	26	8	5.90	0.05

29.60) mg/dl, p < 0.001) and (0.050 (0.045–0.070) g/l vs. 0.27 (0.24–0.31) g/l, p < 0.001, respectively) (**Table 1**).

When the clinical characteristics of the diseased group and the healthy group were compared, the complaints of painful swelling in the body, painful swelling on the face, painful swelling after trauma, painful swelling after tooth extraction, painful swelling after exercise/alcohol intake or when upset, hoarseness or feeling of obstruction in the throat and shortness of breath, severe abdominal pain and vomiting associated with abdominal pain and diarrhoea were significantly statistically higher in the diseased group (**Table 2**). The characteristics of the diseased group are shown in **Table 3**.

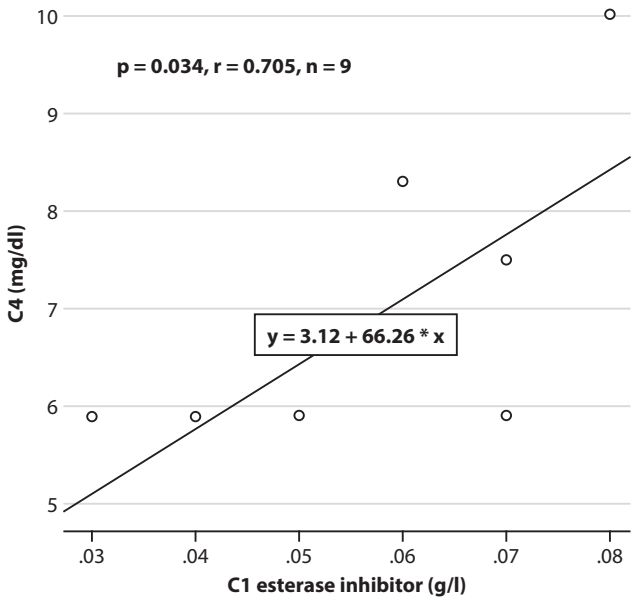

Figure 2. Correlation between C4 level and C1 esterase inhibitor protein level in patients diagnosed with HAE (Pearson correlation).

In the patients with HAE, a positive correlation was detected between the C1 esterase inhibitor protein leveland C4 levels (p = 0.034, r = 0.705, n = 9) (**Figure 2**).

Mutation analysis of the SERPING1 gene

In the light of the clinical findings, the SERPING1 coding region (ENST00000278407) was analysed in the present study index case (VI-1), and subjects X2, IX-4, IX-5, VIII-7, VIII-5, VIII-2, VII-3, VII-1 and a novel heterozygous c. 601 A > T nonsense variant was identified in patients with Type 1 HAE, confirmed by truncation in the serpin protease inhibitor domain of the protein (p.Lys201X) (**Figure 3B**). This nonsense mutation was previously described by Gösswein et al. (Ref: PMID: 18758157). This variant (K201*) causes a premature stop codon before the 300 amino acid interval from the original end point. The truncated protein is likely to be the cause of the disease phenotype. In addition, in silico programmes, SIFT (Sorting Intolerant From Tolerant) (http://sift.jcvi.org) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) have predicted that this mutation is significant in the cause of the disease. The mutation is listed in the Human Gene Mutation

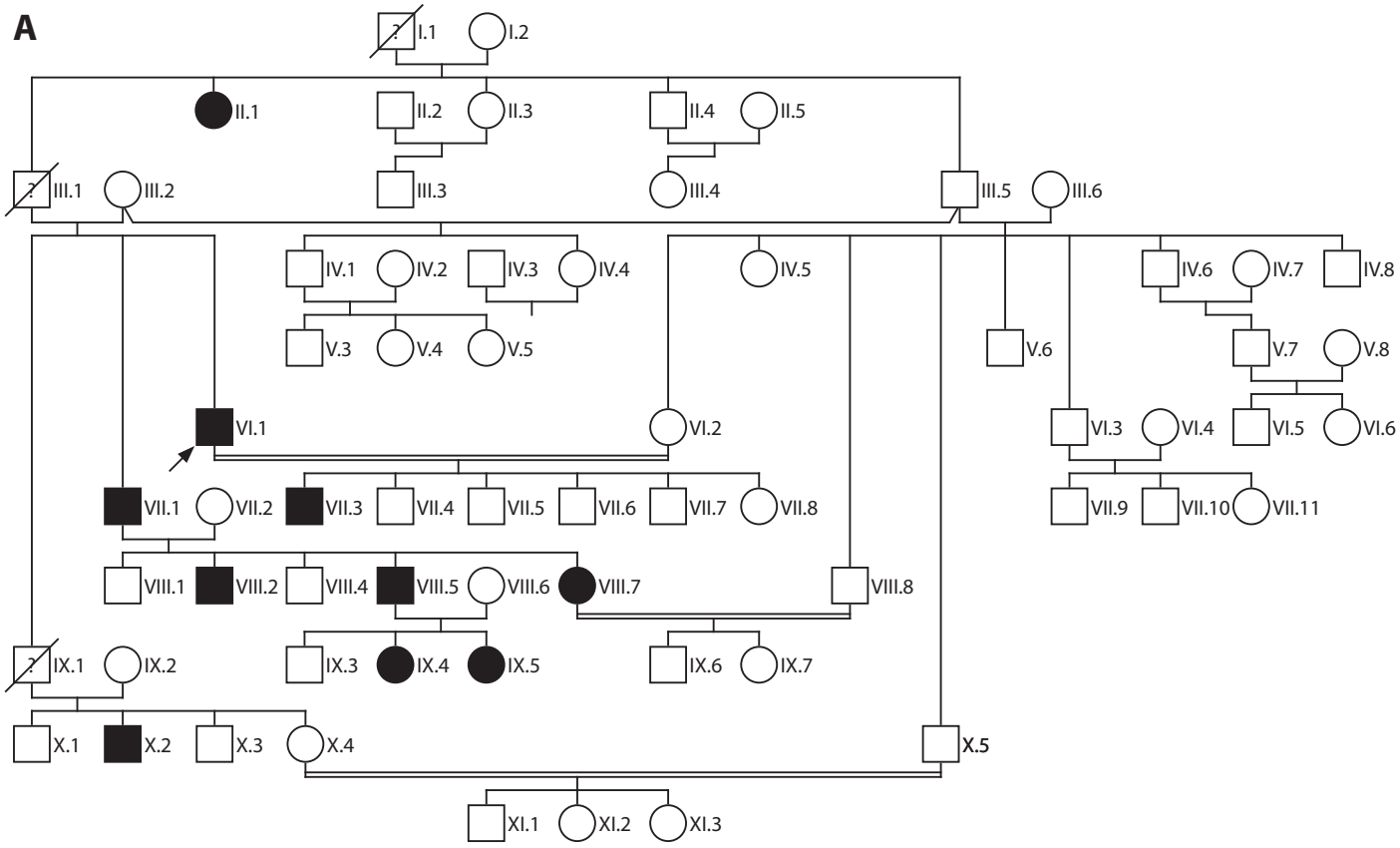

Figure 3.
A. The hereditary angioedema family pedigree. All black boxes indicate the patients analysed for SERPING1.

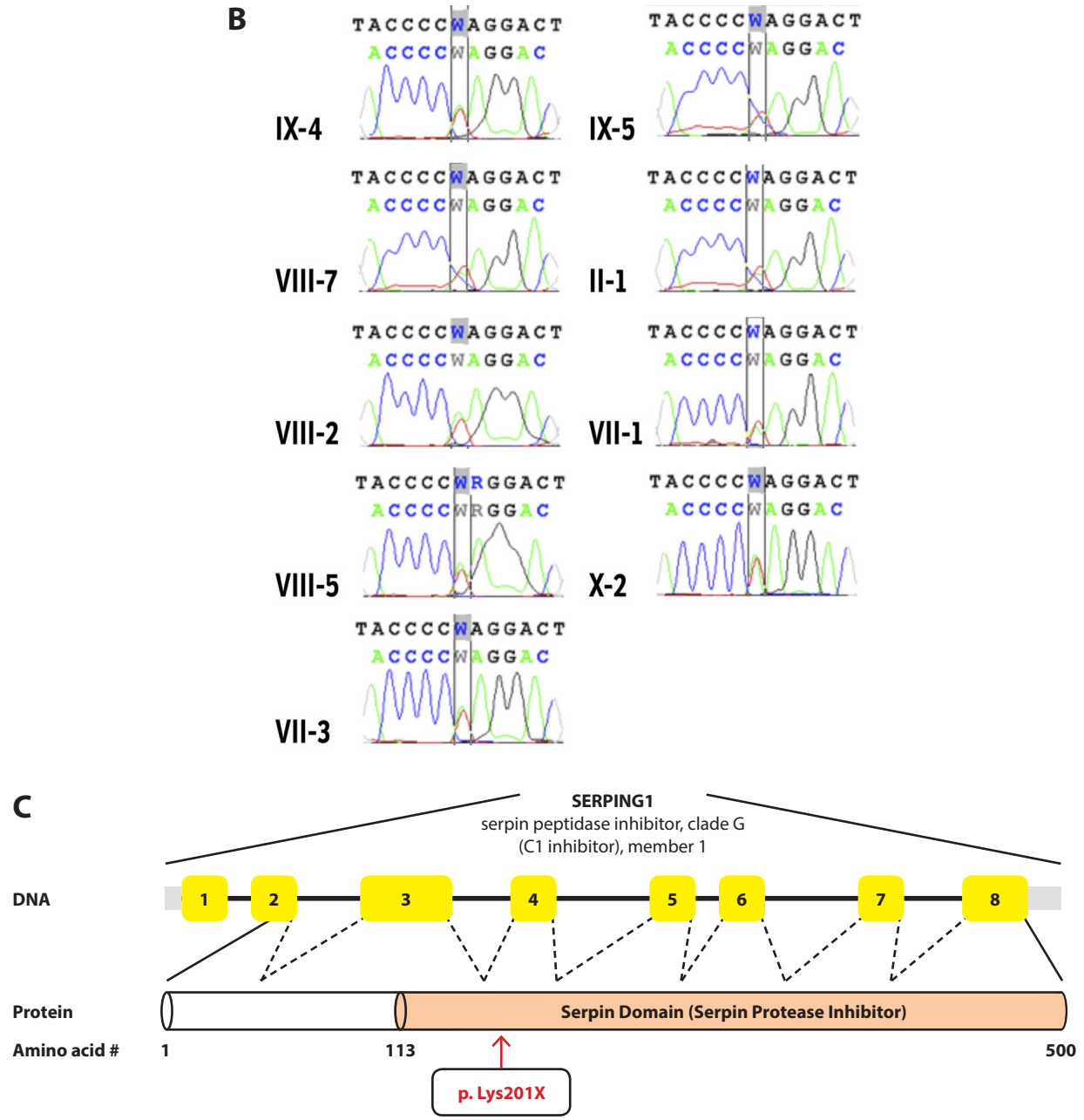

Figure 3. (Continued)
B. Sanger sequencing of the pro-band and all patients. The first two lines show the DNA sequences of patients. The mutated nucleotide is confined in box. Sanger sequencing reveals the heterozygous c.601A > T nonsense mutation in all patients segregated in the family.
C. The SERPING1 gene structure and the location of the functional domain (serpin protease inhibitor) are indicated. Reported mutation in SERPING1 causing HAE p.K201X here is written in red.

Database (HGMD) (https://portal.biobase-international.com/hgmd/pro/mut.php) as a disease-causing mutation. All nine patients with Type I HAE were positive for 601 G > T mutation. The family genetic background is shown in **Figure 3A**.

Discussion

Based on the index case, a total of 60 people were screened for this study. As a result of this screening, 9 (15%) individuals with no previous diagnosis of HAE were diagnosed and given information regarding the disease, and appropriate treatments (such as C1 esterase protein inhibitor concentration during HAE attack periods) were recommended in the form of HAE patient cards. This was of great importance for 2 of the 9 Type I HAE patients diagnosed in the present study screening, as they were were 18 years old or younger. The examiners believe that for paediatric patients with a long life ahead of them, early diagnosis of HAE can both improve quality of life and prolong its duration.

When the clinical characteristics of the diseased group and the healthy group were compared, the complaints of painful swelling in the body, or on the face, after trauma, after tooth extraction and after exercise, alcohol intake or when upset,

hoarseness or feeling of obstruction in the throat and shortness of breath, severe abdominal pain and vomiting associated with abdominal pain and diarrhoea were statistically significantly higher in the diseased group. These results suggested that questions related to these symptoms might be of immense help during screenings.

Symptoms of painful swelling in the body, painful swelling on the face and painful swelling due to trauma occurred in 66% of the diseased group; painful swelling after tooth extraction in 22% of the patients; painful swelling after exercise, alcohol intake or when upset in 55% of the patients; and hoarseness, feeling of obstruction in the throat and shortness of breath in 33% of the patients at some point in their lives. It was determined that 88% of the patient group had severe abdominal pain at some point in their lives, and 44% had experienced severe abdominal pain accompanied by vomiting and diarrhoea. None of the patients had undergone abdominal operations at any time in their lives. These rates were considerably higher than the rates of 25% reported by Kasamatsu Y et al. and 18% by Lei at al.[16-17] In addition, 33% of the diseased group reported hoarseness or a feeling of obstruction in the throat at some time in their lives. The rate reported by Lei at al., in contrast, indicated a 45.5% incidence for laryngeal oedema.[17]

In the present screening, it was note worthy that none of the diagnosed patients had received a diagnosis previously. This emphasises the importance of screening conducted through an index case. Although the patients showed the typical HAE symptoms that were present in the index case, they did not suspect that they themselves had the disease and wanted to avoid diagnosis.

Of the patients diagnosed with HAE through the screening, 88% were symptomatic. This was much higher than the Lei et al. reported rate of 57.9% symptomatic patients.[17] As most of the diagnosed patients in the present study were over 18 years old, it could be that symptoms become more apparent with age. Of the symptomatic patients, 66% had a medical history of skin manifestations, whereas all the symptomatic patients in the Lei et al. study had skin manifestations.[17]

Delayed diagnosis of HAE is a major problem. Lei et al. reported the delay to be 8.45 years.[17] In the present study, the average age at the onset of symptoms was determined as 9 years, the age at diagnosis 32 years and therefore a delayed diagnosis of 23 years.

Screening tests should be performed for the relatives of patients with HAE, and a diagnosis should be attempted at an early age. Ferraro at al. emphasised that the first symptoms might not occur until after the age of 40 and therefore, especially in young individuals, the absence of symptoms cannot rule out the disease.[18] Although there were strong clinical symptoms in the patients on whom screening was performed in the present study, the patients had not received any diagnoses prior to the screening.

In the whole group, a positive correlation was observed between C4 levels and C1 esterase inhibitor protein levels, and negative correlations were observed between C4 levels and severity scores and the C1 esterase inhibitor protein level and severity scores. In the patients diagnosed with HAE, a positive correlation was observed between C4 levels and the C1 esterase inhibitor protein level. While C4 levels and C1 esterase inhibitor protein levels were significantly lower in the diseased group compared to the healthy group, the severity scores were significantly higher. These results once again revealed how accurate and powerful the use of C4, a simple test, can be in HAE screening.

The present study reports a nonsense heterozygous c.601A > T mutation (p.K201X) at the SERPING1 gene in patients with HAE Type I.

Conclusion

The results of the present study confirm the prolonged delay in the diagnosis of HAE and therefore, if there is an index case, the screening of family members is very important and can be life-saving. Therefore, all physicians, especially those working in emergency rooms, should be well aware of the clinical findings of HAE. Patients with recurrent edema should be asked to take a C4 test. In patients with low C4 levels, the C1 esterase inhibitor pretein level and activity test should be requested. It may also be useful to require a gene test to confirm the diagnosis. Once diagnosed, asymptomatic patients need to be identified early and family screened to protect their lives. Performing the scans; it is suggested by us as a new strategy for fighting against disease.

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None

Conflict of Interest Statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Trial registration

Not applicable.

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