



prolong the duration of the treatment before considering a shift to more toxic drugs in case of relapse. The study was approved by the Internal Review Board of the Clinic.

The baseline features of the study patients along with the clinical response to the treatment are shown in Table 1. Seven of 14 patients (50%) showed an ongoing complete control of the disease (UAS7 < 6) despite halving the monthly dose of omalizumab. In the remaining seven patients, the dose reduction was associated with a worsening of the disease (UAS7 score between 7 and 21), although in no case, it returned to the baseline severity. The partial loss of response to the drug did not depend on disease duration, the presence of thyroid autoimmunity, or baseline ESR, CRP, or D-dimer, whereas it was associated with the baseline severity of the disease ($P < 0.05$). Also, a history of angioedema was much more frequent among those who worsened following the reduction in omalizumab dosage (6/7, 86%) than in patients who maintained the control of the disease (2/7; 29%), but due to the small number of patients, the difference did not reach the statistical significance.

Although studies on larger number of patients are needed to confirm these observations and despite the limitation of being non-randomized, the present real-life study suggests that it is possible to halve the costs of omalizumab maintenance treatment in about one half of patients with severe CSU showing a prompt response to the drug at the dose of 300 mg/mo without any loss of clinical efficacy. Patients who worsen with a reduced dosage of omalizumab are in general those showing a more severe diseases at baseline as well as those with a history of angioedema associated with the wheals. This is in keeping with previous observations that in patients with angioedema, only the dosage of 300 mg/month seems to be effective.² Whether the reduction in omalizumab dosage as a maintenance therapy will work also in patients showing a delayed response to the drug remains to be established. In conclusion, this study confirms previous observations in a very small number of patients¹⁰ that a maintenance monthly dose of 150 mg of omalizumab may be sufficient to guarantee an ongoing control of the disease in a large proportion of patients with CSU showing an early response to the drug.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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A missense mutation of the plasminogen gene in hereditary angioedema with normal C1 inhibitor in Japan

To the Editor,

Hereditary angioedema with normal C1 inhibitor (HAEnCI) is a novel type of HAE, whose genetic background is heterogeneous.¹

Mutations in the factor XII (F12) gene have been reported almost exclusively in Caucasians,²⁻⁶ whereas none have been reported for other races, including Asians. The genetic abnormalities that cause

HAEnCI in Asians were therefore unknown. Recently, Bork et al⁷ and others⁸ reported a missense mutation, pLys330Glu (K330E), in exon 9 of the plasminogen (PLG) gene in German patients with HAEnCI that did not exhibit *F12* gene mutations. We report here that this new genetic abnormality in the *PLG* gene was identified in 4 members of two unrelated families with HAEnCI in Japan.

Twenty unrelated Japanese families have been registered in our patient registry as having HAEnCI. The criteria for HAEnCI were as follows: (a) two or more patients per blood-related family with recurrent angioedema; (b) normal C1 inhibitor activity in plasma; (c) no urticaria at present or in the patient's history; and (d) no treatment efficacy with antihistamines and corticosteroids. All of the patients were Japanese. These patients were registered in the HAE registry of Kyushu University. The detailed data regarding patient clinical information and genetic analysis from our HAE registry will be published elsewhere. This study was approved by Institutional Review Board of Kyushu University Hospital. Twenty probands from 20 unrelated families with HAEnCI were subjected to direct sequencing of exon 9 of the *PLG* gene. From composite analysis using PCR/SSCP and direct sequencing, these patients did not carry any mutations in the coding regions or the flanking introns of the *F12* gene (Supporting information). In 2 families, two members each carried a heterozygous missense mutation, K330E. The K330E *PLG* gene mutation was

absent in the 100 healthy Japanese controls. The characteristics of the members of Family 1 (Patient 1 and Patient 2) and Family 2 (Patient 3 and Patient 4) are shown in Table 1.

Patient 1 was a 97-year-old woman. She first experienced angioedema at the age of 94. Her tongue was swollen for 2 days, which gradually subsided after several days without any treatment. She was taking an angiotensin-converting enzyme inhibitor (ACE inhibitor) and was ordered to stop taking it immediately after the episode. Her second attack occurred 3 months later. She had severe tongue swelling and airway obstruction. Intranasal intubation was performed using a fibroscope. The CT scan was performed to examine the extent of edema. Her airway had been occluded from the remarkable edema of the tongue (Figure 1). Intestinal or skin edema, due to the angioedema, was not observed after the onset of the disease. Her two sisters had several, similar attacks that affected their tongues and lips. As both sisters had already died, their medical records were unclear.

Patient 2 was a 65-year-old man, and a son of Patient 1. He experienced lip angioedema at the age of 35, after he underwent surgery for a duodenal ulcer. Among the 8 siblings of Patient 2, a female experienced an episode of eyelid angioedema. However, none of the siblings gave consent for the genetic analysis.

Patient 3 was a 46-year-old woman. She first experienced angioedema at the age of 26. Her symptoms were mainly recurrent

TABLE 1 Characteristics of four patients with HAEnCI that carry a missense mutation in the *PLG* gene

	Family 1		Family 2	
	Patient 1	Patient 2	Patient 3	Patient 4
Age/sex	97/female	65/male	47/female	52/female
Age of onset	94	35	26	34
Age of diagnosis	96	64	47	51
Total number of lifetime attacks	2	1	Many (>50 attacks)	2
Number of attacks affecting each organ				
Tongue	2	0	Common	0
Face	0	1	Common	2
Larynx	0	0	2	0
Extremities	0	0	0	0
Abdomen	0	0	Several	0
Other organs	0	0	0	0
Inducer	None	Surgery	Travel, lack of sleep	Dental treatment
Drugs ^a	ACE inhibitor at the 1st attack	None	None	None
C1-INH activity (reference range: 70%-130%)	104	84	112	ND
C4 concentration (reference range: 13-35 mg/dL)	28	21	19	ND
PLG activity (reference range: 70%-130%)	ND	118	ND	ND
Treatment ^b	Intubation for the 2nd attack	None	Tranexamic acid (effective for 2 y)	None
PLG gene mutation	K330E hetero	K330E hetero	K330E hetero	K330E hetero
F12 gene mutation	None	None	None	None

^aDrugs known to aggravate HAE (ACE inhibitor or estrogen-containing drugs).

^bIncludes all specific treatments for angioedema including emergency and prophylactic treatments.

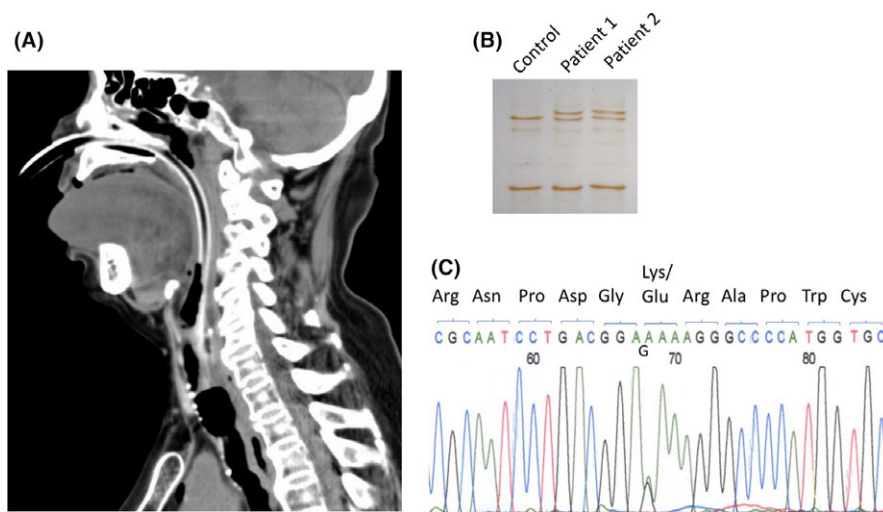


FIGURE 1 A, The CT scan of patient 1 showed remarkable swelling of the tongue. B, PCR-SSCP analysis of exon 9 of the PLG gene. In Patient 1 and Patient 2, an additional abnormal band is visible. C, Direct sequencing of exon 9 of the PLG gene of Patient 1 shows that a c.988A>G transition caused a missense mutation that changed the codon AAA for Lys to GAA for Glu at amino acid residue 330. Residue numbering is based on the primary translation product.

swelling of the upper lip and tongue, which subsided within several days. At most, her attacks occurred once a week. She experienced a feeling of suffocation twice during the course of her disease. Travel and/or lack of sleep induced the attacks in this patient. After she began to take 0.75 g/day of tranexamic acid orally 2 years ago, her angioedema attacks terminated completely, except for one attack episode. Patient 3 has two older sisters. One of the sisters (Patient 4) experienced angioedema during dental treatment, which disappeared without any treatment. The other sister did not experience any angioedema attacks. Patient 3 and her older sisters were not married and had no history of pregnancy. Their mother experienced one angioedema attack, whereas their father did not. Genetic analysis has not yet been performed for their parents. We were unable to obtain genetic data from the other family members, so the exact penetrance of the disease could not be estimated.

Our present study might contribute to a detailed understanding of the clinical features and pathogenesis of HAE-PLG. First, HAE with a PLG gene mutation (HAE-PLG) might be a disease condition common to all ethnic groups, for which HAE-PLG has not yet been reported. It is of note that genetic abnormalities in the *F12* gene^{2,6} and angiotensin-1 (*ANGPT1*) gene⁹ have not been reported in patients with HAE-PLG in an ethnic group other than Caucasians and their descendants. These two gene mutations might be caused by a founder effect in Caucasians. Second, as Bork et al⁷ have already noted, tongue swelling seems to be a characteristic clinical feature of HAE-PLG. Third, tranexamic acid may be a treatment option for HAE-PLG. In 1 of our patients, whose angioedema was so severe that attacks occurred at most once a week, prophylactic administration of tranexamic acid led to an almost complete inhibition of the attacks for 2 years. Bork et al⁷ reported that long-term prophylaxis with tranexamic acid completely inhibited attacks in two patients with HAE-PLG for 12 and 4 years, respectively.

In conclusion, we identified a missense mutation, K330E, in the PLG gene in four patients from two unrelated families with HAE-PLG in Japan. To our knowledge, the *F12* mutations, as well as other genetic abnormalities, have not been reported in Asian patients. We believe

that this is the first report to identify the genetic basis of HAE-PLG in an ethnic group other than Caucasians. The accumulation of additional HAE-PLG cases from different ethnic groups will contribute to understand the pathophysiology of HAE-PLG in more detail.

CONFLICTS OF INTEREST

Dr. Horiuchi reports speaker fees from CSL Behring. The other authors have no conflict of interests to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Maternal pregestational or gestational diabetes and childhood wheezing: A population-based cohort study

To the Editor,

Early-life exposures that modify intrauterine environment may influence the development of wheezing and asthma in children. Diabetes may be one of these compromising factors, possibly through mechanisms related to alterations in fetal allergen sensitization and lung maturation disruption.^{1,2} Previous studies on diabetes and offspring wheezing/asthma have revealed contradicting findings.^{3,4} Until now, no studies have examined whether the association between maternal diabetes and childhood wheezing/asthma varies by both types of diabetes and wheezing/asthma phenotypes.

1 | METHODS

We conducted a register-based cohort study, utilizing data from all Danish residents. We identified 569 649 liveborn singletons during 1996-2004 from the Danish Medical Birth Registry. We excluded 3954 children with missing or gestational age <22 weeks or >45 weeks, 18 244 children who emigrated, and 2774 children who died before their 6th birthday.

We retrieved information on diabetes diagnoses from the Danish National Diabetes Register, which contains information on patients with diabetes treated in either primary or hospital care in Denmark. We additionally identified mothers with gestational diabetes (ICD-10 code O24.4) in the Danish National Patient Register (DNPR). Type of diabetes was categorized as pregestational or gestational diabetes according to the timing of first treatment. We further classified pregestational diabetes into type 1 or type 2 diabetes (Figure S1):

1. According to the DNPR diagnosis if the mother had one type of diabetes diagnosis recorded.
2. For those first diagnosed after 1995 and with no record in the DNPR, or those with both type 1 and type 2 diabetes records: according to first-time antidiabetic prescription, insulin for type 1 diabetes, and oral antidiabetics for type 2 diabetes.
3. For those remaining, with undetermined diabetes type, we used age at first-time diabetes treatment <30 and ≥30 years as the cutoff for type 1 and type 2 diabetes, respectively.⁵

Our outcomes of interest were childhood wheezing phenotypes determined by wheezing treatment (i.e. hospital treatment for wheezing or anti-asthmatic drug treatment) during 0-3 years of age and 4-6 years similar to a previous study from our group.⁶ Information on hospital treatment was based on ICD-10 codes J45 and J46 from the DNPR and anti-asthmatic drug treatment based on Anatomical Therapeutic Chemical classification codes (Table S1) from the Danish National Prescription Registry. We defined wheezing treatment at age 0-3 years as at least two anti-asthmatic drug prescriptions within 1 year period or at least one hospital treatment for wheezing during 0-3 years. Wheezing treatment at age 4-6 years was similarly defined. We categorized children into four mutually exclusive groups:

1. *No wheezing*: no wheezing treatment;
2. *Early-onset transient wheezing*: wheezing treatment during 0-3 years of age only;
3. *Early-onset persistent wheezing*: wheezing treatment both during 0-3 years and 4-6 years of age;

Additional online supporting information

Methods of genetic analysis

Written informed consent was obtained from all of the participants. Genetic analysis of exon 9 of the *PLG* gene and all the exons of the *F12* gene was performed by composite analysis using PCR/single-strand conformation polymorphism (SSCP) analysis of the genomic DNA, and direct sequencing was performed. PLG-9F(forward)CAAAGCGTGGTTCCTAGAC and PLG-9R(reverse)ACTTGGGCCCTCAGTAAGCT were used as intron-based, exon 9-specific primers. The sequences of the primers for the exons of the *F12* gene are available upon request. Briefly, 50 ng of genomic DNA was amplified with 0.2 μ M of each primer, 25 μ M of deoxyribonucleoside triphosphate, 0.125 U of Taq polymerase, and the standard buffer supplied by the manufacturer (TaKaRa, Kyoto, Japan) in a total volume of 12.5 μ L. Thirty reaction cycles were performed consisting of 30 s at 95°C, 1 min at 62°C, and 1 min at 72°C with a thermal cycler (Gene Amp PCR System 9700, Applied Biosystems). After denaturation at 96°C for 5 min, the PCR products were subjected to electrophoresis on 12% polyacrylamide gels, followed by silver staining for visualization. The PCR products were also purified and sequenced with a BigDye Terminator V3.1 Cycle Sequencing Kit (ThermoFisher Scientific, Tokyo, Japan). Electrophoresis of the sequenced products was performed on an Applied Biosystems 3700 Genetic Analyzer (ThermoFisher Scientific)¹.

Additional discussion

Details on how the mutation of the *PLG* gene causes angioedema remains unknown. K330E is located in the third of the five kringle domains of the PLG protein. The kringle domains are thought to facilitate PLG binding to other proteins and to influence its function and/or the functions of counterpart ligands². The finding that the plasma PLG activity in 1 of our patients

with HAE-PLG was normal is in line with a previous report that described normal plasma PLG activity in 3 patients with HAE-PLG. Considering that PLG activity is assayed in vitro in an artificial system, this is conceivable. In order to clearly demonstrate the functional consequences of the K330E mutation in vivo, it would be helpful to use animal models, such as human K330E PLG knock-in mice. However, the amino acid alignment of the PLG protein showed that residue 330 is a Lys in humans, while this residue is conserved as E330 in all other species³. It is therefore not simple to study the impact of the K330E mutation using animal models. We suppose that collecting data on the clinical symptoms, biomarkers, responses to treatment, and aggravating factors will contribute to the clarification of the pathophysiology of the K330E mutation in humans. In fact, a bradykinin-2 receptor antagonist has been reported to be effective in two patients with HAE-PLG, which suggests the importance of bradykinin in the pathogenesis of HAE-PLG⁴. The accumulation of additional HAE-PLG cases from different ethnic groups is needed to understand HAE-PLG in more detail.

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