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Bradykinin and histamine mediated angioedema

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Bradykinin and histamine mediated angioedema

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Table of contents

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Editorial: Bradykinin and histamine mediated angioedema

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hereditary, angioedema, urticaria, histamine, bradykinin

Editorial on the Research Topic
Bradykinin and histamine mediated angioedema

Angioedema is a condition characterised by localised, potentially life-threatening oedema of the subcutaneous and submucosal tissues. Although the clinical presentations of histamine- and bradykinin-mediated angioedema can be similar, their underlying pathophysiologies are distinct, necessitating precise diagnosis and fundamentally different treatment approaches. This is particularly critical in hereditary angioedemas (HAE), where delays in diagnosis can lead to severe complications, including fatal laryngeal oedema.

Spurred by recent advances in understanding these pathways and the development of novel therapies—from monoclonal antibodies to bradykinin receptor antagonists—this Research Topic was conceived to collate the latest evidence. We are pleased to present this collection of 11 articles from 60 international authors, featuring original research, reviews, and case reports that illuminate the evolving landscape of angioedema management.

Synopsis of the research topic

The collection opens with a foundational review by Lima et al., which elegantly delineates the pathophysiology of both histamine- and bradykinin-mediated angioedema, providing essential context for the subsequent studies.

A significant focus of this issue is on refining diagnostics and understanding the real-world patient experience. Sexton et al. contribute a novel assay for quantifying high-molecular-weight kininogen (HKa), a promising step towards better biomarker development. Highlighting the challenges in clinical practice, Van der Poorten et al., in a nationwide Belgian study, report a median seven-year delay in diagnosing HAE, underscoring the need for greater awareness. Complementing this, Day et al. identify Black genetic ancestry and concomitant calcium channel blocker use as key risk factors for ACE inhibitor-induced angioedema and highlight the long median latency of treatment prior to first angioedema presentation (>5 years) in the majority of a South African cohort.

Several articles evaluate modern therapeutics. Bara et al. demonstrate the real-world efficacy of lanadelumab for long-term prophylaxis in Romanian patients, noting

significant improvements in disease control and quality of life. Delving deeper into the mechanism of such treatments, Sexton et al., in a separate proteomic analysis, identify new potential biomarkers in patients undergoing lanadelumab therapy.

The importance of adaptable care models is addressed in two community case studies. Du et al. propose a standardised diagnostic and treatment workflow for resource-limited settings, while Andarawewa and Aygören-Pürsün emphasise the principle of individualised long-term therapy.

Finally, three compelling case reports illustrate these principles in action. Pinhal et al. successfully used subcutaneous C1-INH during pregnancy and lactation, and Guo et al. stress the value of family screening in identifying asymptomatic patients. Another report by Du et al. details the identification of a novel *SERPING1* gene mutation in a Chinese patient, leading to a definitive diagnosis and effective prophylactic treatment.

This Research Topic collectively underscores the rapid momentum in understanding bradykinin-mediated angioedema, while reaffirming the need for precise diagnosis and personalised care. We believe this compilation will serve as a valuable resource for clinicians, researchers, and students alike, ultimately contributing to improved outcomes for patients worldwide.

Author contributions

AP: Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Project administration, Supervision, Validation, Visualization. JP: Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Supervision, Validation, Visualization. MR: Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Project administration, Supervision, Validation, Visualization.

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Individual approach to long-term therapy in patients with hereditary angioedema (HAE-C1-INH): A case series

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hereditary angioedema, long-term prophylaxis, frequency of attacks, burden of disease, angioedema control test

Introduction

Hereditary angioedema (HAE) is caused by C1-Inhibitor (C1-INH) deficiency leading to recurrent attacks of skin swellings, abdominal pain, and laryngeal edema. HAE can cause severe disability and may be potentially life-threatening. There is considerable variation in the clinical presentation and the severity of HAE, with the frequency of attacks ranging from none to two to three times a week (1). In a recent multinational patient survey conducted before the availability of novel prophylactic regimes for HAE, the attack frequency was reported to be more than one attack per month in 51.6% of patients. Moreover, most participants (82%) showed an insufficiently controlled disease (2). Current guidelines recommend the complete control of the disease and the normalization of the patients' life as the goals of HAE treatment (3). In many cases, this implies the application of long-term prophylaxis (LTP) to prevent attacks from achieving this. The novel preventive therapies, subcutaneous and intravenous plasma-derived human C1 inhibitor concentrate (pdC1INH), lanadelumab, and berotralstat, offer safe and effective prevention of HAE attacks (3–5).

Here, we present a clinical case series of patients who were managed successfully using different types of novel long-term prophylaxis regimes.

Methods

We assessed demographic and clinical data obtained from clinical records of three HAE-C1-INH patients with previously poorly controlled HAE. To assess disease control, we used the angioedema control test (AECT), a validated patient-reported outcome tool used in patients with recurrent angioedema. The maximum AECT score of 16 indicates complete disease control, while scores of less than 10 indicate insufficiently controlled disease (6). Type, dose, and dose intervals of the medicines for LTP as well as the frequency and characteristics of HAE-attacks prior to and

break-through attacks during LTP, if any, were assessed and adverse events and tolerability were documented.

Case 1

A 42-year-old male patient was diagnosed with hereditary angioedema with C1-INH deficiency (HAE-C1-INH) at the age of 5 years. At the age of 25 years, he presented with frequent HAE symptoms, which were predominantly abdominal pain attacks. Along with angioedema of the extremities and three episodes of laryngeal edema, the patient was treated with attenuated androgen danazol, initially in a dose of 400 mg per day for about 6 months followed by a maintenance dose of 200 mg per day for 2 years. At the time of presentation to our HAE center, the patient was experiencing three HAE attacks per month despite this therapy. These episodes usually manifested as abdominal attacks and swellings of extremities and genitals. While the patient's weight at the time of initiation of danazol LTP was 130 kg (height 183 cm, BMI 38.8), after 2 years of danazol LTP, on his first presentation at our center, his weight was 165 kg (BMI 49.3). Additionally, treatment-emergent panic attacks and anxiety were present. Danazol was discontinued, and the patient was treated with on-demand intravenous (i.v.) pdC1INH. The patient later developed psoriasis and obstructive sleep apnea, which stayed untreated due to psychological intolerability of the PAP mask and device.

When the frequency of angioedema increased to three times a week within 1 month after cessation of danazol, at the age of 33 years, LTP with pd-C1INH i.v. every 3 days, which was the available novel LTP at that time, was initiated in this patient. Nevertheless, angioedema attacks occurred once a week, requiring adaptation of the injection interval to every 2 days. However, the attack frequency remained 1–4 per month, presenting as abdominal attacks and swellings in the extremities, genitals, and buttocks.

With the approval of lanadelumab for LTP in patients with HAE, LTP with 300 mg s.c. every 14 days was initiated in this patient. Other than an abdominal attack on day 3 after starting this therapy regime, the patient remained attack-free for 7 months. The SMPC of lanadelumab allows a prolongation of the injection interval to 4 weeks (7). In this case, the interval was gradually extended up to 20 days without break-through attacks. Ten months of lanadelumab LTP were reached at this time. While receiving lanadelumab 300 mg s.c. every 22 days, three abdominal attacks occurred within 4 weeks, requiring interval readjustment to every 20 days followed by a 1-year attack-free period with this injection interval. The interval was then increased to 23 days successfully with no attacks for 4 weeks. The patient had good therapy adherence. The overall observation period under lanadelumab prophylaxis was 33 months.

The treatment was well tolerated with occasional injection site reactions.

The mean attack rate during the 3 months before initiating lanadelumab s.c. prophylaxis was 1.33 attacks per month, while during prophylaxis in the lanadelumab steady state, it was 0.09 attacks per month. This patient showed an attack reduction of 95.48% with his adaptive prophylaxis regime. Disease control was complete as assessed by AECT 16/16 at 33 months after starting prophylaxis. AECT was not available prior to prophylaxis in this patient.

Case 2

A girl who is currently 13 years old was diagnosed with HAE-C1-INH at the age of 2 years due to her positive family history. Her first angioedema attack was a facial swelling that occurred at the age of 2.5 years. Recurrent swelling attacks further affected her face, lips, extremities, GI tract, and larynx. On-demand therapy with pdC1INH to treat attacks was administered by health care personnel. Treatment of these attacks was often delayed due to nocturnal attacks and hospitalization, which caused unnecessary diagnostic procedures despite known HAE before treatment was given. Many days of absence from school was the consequence of these unnecessary procedures and delayed therapy. After a period of reluctance to be trained on i.v. injection of C1-INH, the patient and her parents were successfully trained to self-administer subcutaneous (s.c.) icatibant to treat attacks. However, following treatment of HAE-attacks with s.c. icatibant, she regularly experienced reattacks. This patient reported 9 days of absence from school within 6 months due to HAE shortly before starting long-term prophylaxis. Angioedema attacks occurred 3–4 times a month including two laryngeal attacks. After discussing the approved long-term prophylactic regimes for this age group with the then 12-year-old patient and her parents, it was jointly resolved to start treatment with a subcutaneous C1-inhibitor. In clinical practice, doses applied may deviate from approved doses. The initial dose administered by the patient was 24 U/kg bw twice weekly. Except for an abdominal attack on day 4, the patient remained attack-free with this low-dose regime for 12 months. Dose adaptation was discussed with the patient and her caregivers after two abdominal attacks occured in the following three months, probably due to a weight gain of about 10 kg. The patient showed good compliance with the therapy.

In comparison (printing mistake) this patient experienced 2.67 attacks per month within the 3 months prior to long-term prophylaxis compared to 0.13 attacks per month during the steady state with this well-tolerated prophylactic treatment. The reduction of attack rate with the low dose was 95%. The disease was poorly controlled prior to LTP with an

AECT score of 4/16 and progressed to controlled disease during this prophylactic regime (AECT 14/16 at 3 months, 16/16 at 7 months, and 14/16 at 15 months of pdC1INH prophylaxis).

Case 3

A 79-year-old female was diagnosed with HAE-C1-INH at the age of 40 years despite her first manifestation being at the age of 14 years. Initially, this patient experienced recurrent angioedema of the extremities, face, abdomen, genitals, and urinary tract, as well as signs of laryngeal edema. Following HAE diagnosis, the patient was treated with 300 mg danazol per day for prophylaxis of HAE attacks for about 17 years. Under this treatment, the patient was not free of angioedema attacks, and as a side effect, she experienced amenorrhea. After discontinuation of danazol, there was an increase in attack rate up to 2-3 times per week.

At the time of her first visit to our clinic, the patient was 66 years old, and she was treating her attacks with on-demand C1-inhibitor concentrate intravenously. With gradually decreasing vision due to macular degeneration and difficult venous access, intravenous and subcutaneous self-injection became increasingly difficult for the patient. Therefore, treatment of HAE attacks was often delayed, resulting in delayed complete remission of attacks. With HAE attacks, predominantly abdominal, occurring once a week and poor disease control and loss of vision, long-term prophylaxis with berotralstat was initiated by joint decision, as oral administration was the preferred and optimal route of administration for this patient. Berotralstat 150 mg once daily was administered and observed for a total period of 6 months. Within the observation period, there was a 2-week interruption of berotralstat intake, 4 weeks after the beginning of the prophylaxis, due to nonavailability of the drug, during which the patient experienced two HAE attacks. Following a restart with a regular intake of berotralstat 150 mg once daily, the patient was attack-free for a further observation period of 18 weeks. Good therapy adherence was apparent. Attack reduction was 100% during the regular daily intake of berotralstat 150 mg per os, and the therapy was well tolerated. Disease control moved from poorly controlled (AECT score of 8/16) before prophylaxis to fully controlled (AECT 16/16) at 24 weeks after the beginning of LTP.

Discussion

Hereditary angioedema with C1 inhibitor deficiency (HAE-C1INH) is a rare, disabling, and potentially life-threatening disease. Symptoms range from skin swelling to mucosal swelling of the gastrointestinal system and the upper airways. Due to HAE, patients experience a complex range of physical, psychological, and social impacts (3, 8, 9). For instance, HAE patients show high levels of anxiety and depression (9). Frequent hospitalization and absence from work or school may cause an increased burden of disease not only for the patients but also for their immediate caregivers (8, 9). A substantial burden of disease remains for HAE patients despite introducing new therapies for acute attacks (10). Currently, apart from medicines to treat attacks of HAE, there are several novel, safe, and effective therapy options available for the LTP for patients with HAE to optimize HAE management (3, 4).

This report summarizes observational clinical data of three HAE-C1-INH patients in whom successful long-term prophylaxis was achieved by different therapeutic approaches (**Table 1**). By mutual decision between the physician and patient, individualized approaches to LTP were chosen, considering the clinical needs and preferences of the patients. LTP was further tailored individually when needed. Including the patient in the joint decision on the management of the disease may support therapy adherence, which was optimal in these three patients. The overall goal of HAE management is to achieve total disease control and normalize patients' lives (4). In the cases presented, this goal was achieved by using LTP of different types with individual adaptations.

In case 1, frequent HAE attacks occurred previously despite i.v. C1-INH LTP administered every 2 days, which necessitated an alternative approach to LTP. As long administration intervals were of paramount interest for this patient, the decision was made to initiate LTP with lanadelumab, a monoclonal antibody against plasma kallikrein that is applied every 2 weeks. After reaching the attack-free status, doses of lanadelumab may be applied every 4 weeks (7). In the case presented, however, this interval would not have led to the prevention of attacks as this patients' maximum interval tolerated without attacks was 23 days. With this injection interval, however attack-free status and total control of the disease could be reached.

Case 2 experienced frequent and severe HAE attacks starting early in life. Familiarity with C1-INH reassured this patient and her parents in the decision-making for LTP with SCpdC1INH. Successful LTP could be achieved with low doses of SCpdC1INH, as demonstrated by attack-free status, total control of the disease and elimination of hospitalization and absence from school due to HAE.

Severe burden of disease and patient comorbidities played a major role in the shared decision-making for LTP in case 3. The patients' choice of mode of administration was the oral route that was also commanded by her loss of vision. Other factors

TABLE 1 Demographics, clinical characteristics, and treatment outcomes.

	Case 1	Case 2	Case 3
Age	42 years	13 years	79 years
Gender	Male	Female	Female
Age at the onset of HAE symptoms	3 years	2,5 years	14 years
Diagnosis of HAE	5 years	2 years	40 years
Past medical history	Hypertension, atrial fibrillation, bronchial asthma, obstructive sleep apnea, chronic gastritis, psoriasis, obesity (BMI 49,3)	Umbilical hernia operation in the first year of life	Hypertension, macular degeneration. (current vision <0,2), past history of breast cancer, past history of Hepatitis C infection
Current type and dose of LTP	Lanadelumab 300 mg s.c. every 23 days	C1-inhibitor concentrate 20U/kg bw s.c. 2x/week	Berotralstat 150 mg p.o. per day
Observation period of current LTP	33 months	15 months	6 months
Previous therapy	PdC1INH i.v. every 2 days	On-demand pdC1INH i.v.	On-demand pdC1INH i.v.
Mean attack rate per month in the 3 months before the current LTP	1.33 (under C1-INH 1,000 U i.v. every 2 days)	2.67	4.33
Mean attack rate per month during LTP during the steady state	0.09	0.13	0[a]
Percentage of attack reduction during LTP	95.48%	95.13%	100%
AECT prior to LTP	Not available	4/16	8/16
AECT with current LTP	16/16	14/16	16/16

[a]Excluding two attacks that occurred during the 2 weeks when the patient missed her berotralstat LTP.

that might have influenced the decision for oral prophylaxis like impaired motor skills or coordination, tremor, or needle phobia were not present in this patient. The use of an oral kalikrein inhibitor for LTP led to an immediate and enduring attack-free status and eventually to a totally controlled disease in this patient.

In all three patients, attacks were reduced by 95–100% compared to prior therapy (Table 1). Also, disease control was improved moving patients from poorly controlled to well-controlled disease with individually adapted LTP.

In summary, a customized approach for each of the patients could be found that led to optimal outcomes.

Conclusion

This case series gives an indication of how HAE patients with a need for long-term prophylaxis may be approached to find the individually best solution. Apart from the efficacy and safety of the various types of approved LTP, the mode and frequency of administration, potential experience with the medicinal product, and pre-existing comorbidities might affect the choice of LTP. These may include impairment of sight, disturbed motor skills, or impaired coordination among others. Shared decision-making considering patients' preference may help optimizing therapy adherence and thereby the outcome of long-term prophylaxis.

Contribution to the field

Hereditary angioedema (HAE) due to C1 inhibitor deficiency is a rare inherited disorder that may cause recurrent swellings of the skin and gastrointestinal tract that may lead to considerable morbidity and potentially lethal upper airway edema. The frequency and severity of HAE attacks can vary from patient to patient and even within an individual over time, ranging from no attacks at all to two to three attacks per week. Therapies for patients with HAE-C1-INH to treat acute attacks have existed for many years, and effective and safe novel therapies to prevent attacks by long-term prophylaxis (LTP) have additionally become available in recent years.

Particularly, the long-term prophylactic regimes require considerable cooperation by the patient; hence, shared decision-making prepares the ground for suitable therapy adherence to ensure a good clinical outcome.

We discuss three patients with previously poorly controlled diseases, who were managed successfully using different types of novel prophylaxis regimes and therapy alterations in two cases. This case series indicates how HAE patients with a need for long-term prophylaxis may be approached to find the individually best solution. Apart from the efficacy and safety of the various types of approved LTP, the mode and frequency of adminstration, experience over medicinal products, and pre-existing comorbidities might affect the choice of LTP. These may include impairment of sight, disturbed motor skills, or impaired coordination, among others.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

SA and EAP contributed to the acquisition of clinical data and wrote (SA) and reviewed the manuscript critically (EAP). All authors contributed to the article and approved the submitted version.

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Hereditary angioedema (HAE) in Belgium: results from a national survey

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Background: Hereditary angioedema (HAE) is a rare heritable disorder that is characterized by recurrent, circumscribed, nonpitting, nonpruritic, often painful subepithelial swellings of sudden unpredictable onset that generally fade during 48–72 h. Epidemiological data of hereditary angioedema patients in Belgium is lacking.

Methods: We set up a nation-wide, multicentric study involving the 8 Belgian hospitals known to follow-up patients with Type I and II HAE. All Belgium HAE patients were asked to fill out questionnaires that mainly covered demographic data, family history, and detailed information about diagnosis, treatment and burden of their Type I and II HAE.

Results: 112 patients with type I or type II HAE could be included. Median delay between first symptoms and diagnosis was 7 years. 51% of patients had experienced pharyngeal or tongue swelling and 78% had experienced abdominal symptoms, both known to cause an important reduction in quality of life. 60% of symptomatic patients reported to receive long term prophylactic treatment. Human plasma-derived C1-esterase inhibitor concentrate was used by 56.3% of patients. 16.7% and 27.1% of patients used a 17-α-alkylated androgen and tranexamic acid as long term prophylactic therapy.

Conclusions: We present the first nation-wide epidemiological study regarding HAE in Belgium. Our data show that the morbidity of HAE is not to be underestimated. Knowledge and dissemination of this data is critical in raising awareness, encouraging development of therapies and optimising nationwide management.

KEYWORDS

hereditary angioedema, epidemiology, nationwide, Belgium, diagnosis, rare disease

Abbreviations
HAE, hereditary angioedema; C1-INH, complement factor 1 esterase inhibitor; C1-INH HAE, low C1-inh function hereditary angioedema, hence Type I and II HAE; pdC1-INH concentrate, plasma-derived C1-esterase inhibitor concentrate.

Introduction

Hereditary angioedema (HAE) is a rare heritable disorder that is characterized by recurrent, circumscribed, nonpitting, nonpruritic, often painful subepithelial swellings of sudden unpredictable onset that generally fade during 48–96 h.

Patients with HAE experience angioedema because of a defective control of the plasma kinin forming cascade (1). Type I and II HAE are characterized by a deficiency in the complement factor 1 esterase inhibitor (C1-INH) and are henceforth designated as C1-INH-HAE. Type I is characterized by low serum levels of C1-INH. In type II, serum levels of C1-INH are normal or even elevated, but the protein is dysfunctional. As recommended in the recently updated WAO/EAACI guidelines (2) and in an international consensus report on HAE in children (3), diagnosis of C1-INH-HAE should start with an evocative personal and familial history complemented with a measurement of complement factor C4 (that is usually decreased) combined with the demonstration of a deficient C1-INH function. To distinguish between type I and type II HAE, additional antigenic quantification of C1-INH could be performed (4, 5).

HAE with normal C1-INH is clinically indistinguishable from type I and II C1-INH-HAE. However, these patients show normal values for C4, C1-INH function and C1-INH quantification. Normal C1-INH HAE will not be the focus of this study. For a state of the art on the pathophysiology of normal C1-INH-HAE, the reader is referred elsewhere (2, 6, 7).

The prevalence of C1-INH-HAE is often estimated in research, but substantial epidemiological studies remain scarce. Population-based epidemiological studies have been conducted in various European countries (8–18), while data regarding the Belgian population remains lacking. This while knowledge of epidemiological data is critical in raising awareness, encouraging development of therapies and optimising management.

Here, we describe epidemiological and clinical data obtained *via* a standardized questionnaire that was completed by 112 Belgian type I and II C1-INH-HAE patients.

Materials and methods

We set up a nation-wide, multicentric study involving the 8 Belgian hospitals known to follow-up patients with C1-INH-HAE. The centers included were: (*information removed due to blinding of the manuscript)*. The study was approved by the local committees of all the participating centers (registration number BE300201734006). Patients or their caregivers provided a written informed consent according to the declaration of Helsinki. Principal investigators and coinvestigators of all centers contributed to the preparation and standardization of the questionnaires that can be found in the repository file. Briefly, criteria for inclusion were a diagnosis of type I or II C1-INH-HAE. Diagnosis of C1-INH HAE type I and II was based upon a decreased C4 and a decreased C1-inhibitor function or a decreased C1-inhibitor antigenic quantification in case of family history (2).

All participants who agreed on participation were included after they completed an electronic questionnaire (see repository file). The questionnaire was encrypted and anonymous, with a decoding table only accessible by the treating physician.

Questionnaires mainly covered demographic data, family history, and detailed information about diagnosis, treatment and burden of their C1-INH-HAE. Duplicates were avoided by checking the patients' medical history in their electronic medical file upon first presentation. This includes previous contacts at other reference centers. Moreover, before analysis of data, we checked for potential duplicates by checking dates of birth combined with the patient sex. No potential duplicates were identified.

Next, we performed descriptive statistics. Data are presented as numbers, percentages and median with range.

Results

Inclusion

Registration of patients is shown in **Figure 1A**. By February 3rd 2022, a total of 125 patients (50 (40%) male, 75 (60%) female), completed the questionnaire.

Another 55 C1-INH-HAE patients who received the questionnaire did not answer or stated they were not willing to participate.

6/125 (4.8%) patients who filled out the questionnaire, were diagnosed with normal C1-INH-HAE. In 7/125 (5.6%) patients, the type of HAE was unknown (no lab data available). These patients were excluded.

Hence, as shown in **Table 1** and **Figure 1B**, 112 patients with type I or type II C1-INH-HAE could be included.

Type I C1-INH-HAE was diagnosed in 86/112 (76.8%) patients, type II C1-INH-HAE was diagnosed in 10/112 (8.9%) patients. In 16/112 (14.3%) patients, C1-INH function was low, but additional antigenic C1-INH quantification was not performed, hence no distinction can be made between type I and type II.

Symptoms

As shown in **Figure 1B**, 100/112 (89.3%) type I/II C1-INH-HAE patients experienced symptoms at least once in their lives.

The median age of onset of symptoms was 13.5 years (range 0–57 years). The median age at diagnosis was 20 years (range 0–71 years). The median time between onset and diagnosis was 7 years (range 0–49 years). Twelve patients who never experienced angioedema were diagnosed with the condition after a family member was affected (first degree relative). The diagnosis in these patients was based upon a low C1-INH function and/or low quantitative levels of C1-INH.

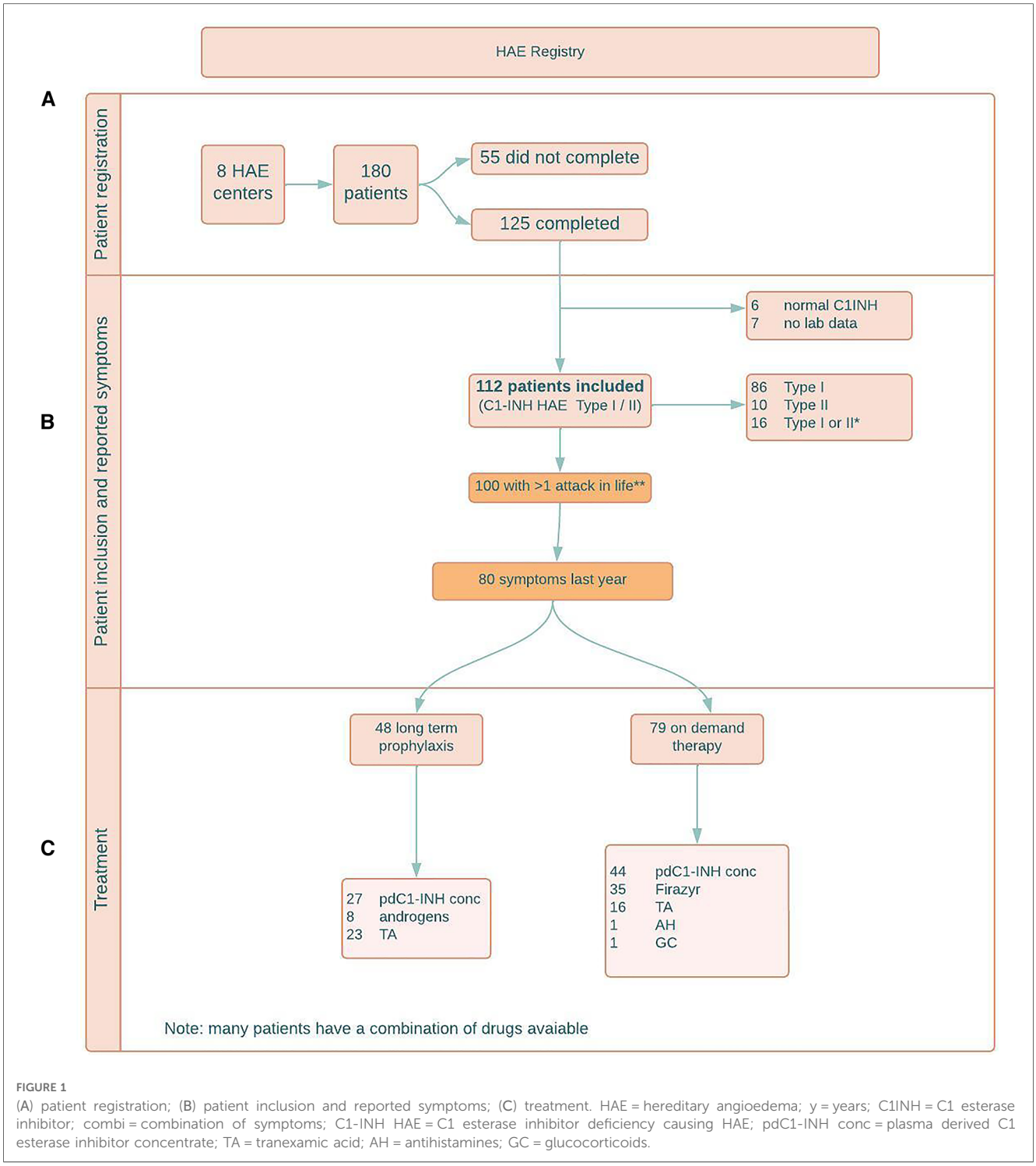

FIGURE 1
(A) patient registration; (B) patient inclusion and reported symptoms; (C) treatment. HAE = hereditary angioedema; y = years; C1INH = C1 esterase inhibitor; combi = combination of symptoms; C1-INH HAE = C1 esterase inhibitor deficiency causing HAE; pdC1-INH conc = plasma derived C1 esterase inhibitor concentrate; TA = tranexamic acid; AH = antihistamines; GC = glucocorticoids.

During the previous year, 80/112 patients experienced symptoms.

Of the 80 patients that experienced symptoms last year, the median number of attacks was 6 (range 1–60) and 29 patients visited the emergency department with an HAE attack at least once.

As shown in Figure 2, the most reported symptoms were swelling of the extremities and abdominal symptoms (pain, nausea, vomiting, diarrhea, constipation), with respectively 95/100 (95%) and 78/100 (78%) patients that ever experienced symptoms, reporting to have experienced these at least once in their lives and respectively 74 (74%) and 56 (56%) patients during the last year.

70 (70%) patients experienced facial swelling at least once in their live, of whom 37 (37%) patients during the last year. Genital swelling was reported by 64 (64%) patients to have occurred at least once in their lives and by 38 (38%) patients during the last year.

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TABLE 1 Data of included patients with type I and/or II HAE.

Included patients	
Total (*n*)	112
Type I HAE (*n*)	86
Type II HAE (*n*)	10
Unknown (Type I or II) (*n*)	16
Symptoms	
Age of onset (y) (median, range)	13.5 (0–57)
Diagnostic delay (y) (median, range)	7 (0–49)
Symptoms at least once/live (*n*)	100
Symptoms last year (*n*)	80
Prophylactic treatment	
Total (*n*)	48
pdC1-INH conc (*n*)	27
Androgens (*n*)	8
Tranexamic acid (*n*)	23
On demand treatment	
Total (*n*)	79
Firazyr	35
Tranexamic acid (*n*)	16
Antihistamines	1
Glucocorticoids	1

n, number; HAE, hereditary angioedema; y, years; pdC1-INH conc, plasma derived C1 esterase inhibitor concentrate; AH, antihistamines; GC, glucocorticoids.

51 (51%) patients experienced pharyngeal or tongue swelling at least once in their lives, of whom 31 (31%) patients during the last year.

As shown in Figure 3, the most common triggers were mental stress (69/100; 69%) and physical activity (49/100; 49%). 38 out of 63 (60.3%) adult women who ever reported symptoms, experienced more attacks whilst taking estrogen containing contraceptives. In 32 patients, an attack was triggered by a dental or surgical procedure.

Sick leave

49/100 (49%) symptomatic patients, reported absenteeism of work/school during their last year.

The median number of absenteeism periods was 3 (range 1–20).

13 patients reported that their school/work environment were unsupportive of their situation.

35 patients missed out on activities they considered as important in their lives, because of HAE attacks (e.g. physical exercise, writing or travelling).

Treatment

54 patients had used long term prophylactic therapy at some point in their lives and 48 patients were using long term prophylactic therapy at the time they responded to the questionnaire. This is 60% (48/80) of the 80 patients who experienced symptoms last year.

As shown in Figure 1C, the most common used long term prophylactic therapy was a human plasma-derived C1-esterase inhibitor concentrate (pdC1-INH concentrate), used by 27/48 (56.3%) patients. The median number of attacks during the last year in the patients taking a pdC1-INH concentrate was 5, compared to 3 in the patient group not on appropriate long-term prophylaxis.

8/48 (16.7%) patients used a 17-α-alkylated androgen as long term prophylactic therapy.

13/48 (27.1%) patients reported daily off-label use of tranexamic acid as only long term prophylactic therapy and another 10/48 (20.8%) patients used tranexamic acid in combination with pdC1-esterase INH concentrate or androgens.

Finally, 2 patients did not specify the long term prophylactic therapy they were actually taking.

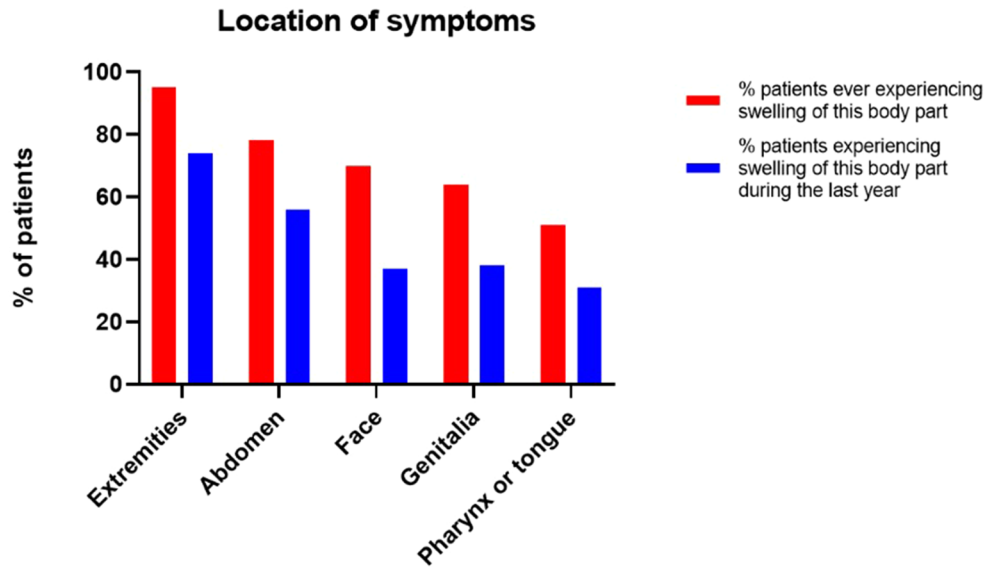

FIGURE 2
Location of symptoms.

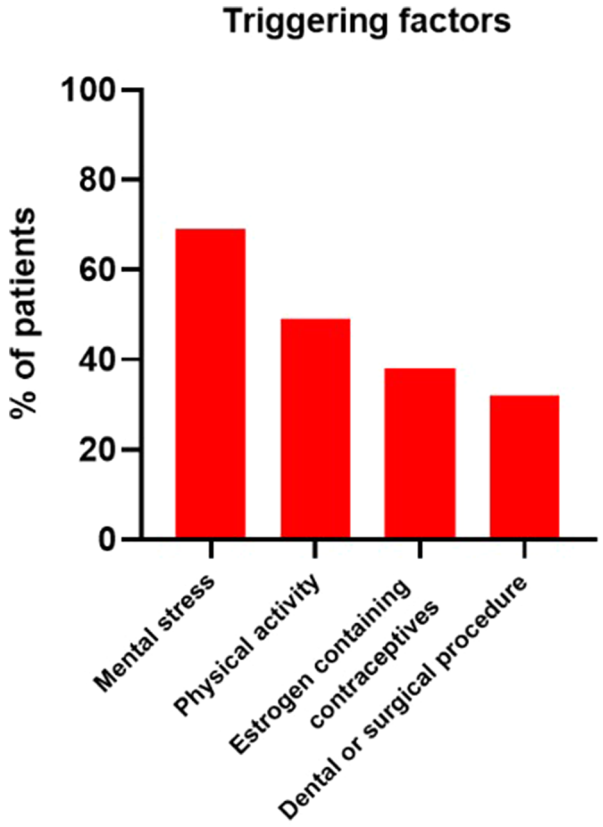

FIGURE 3
Four most frequently mentioned triggering factors. Other triggers included specific types of food, alcohol consumption, weather changes, viral infection and sleep deprivation.

As shown in Figure 1C, 79 (70.5%) patients reported that they had an on-demand treatment available in case of attacks; 44/79 (55.7%) in the form of pdC1-esterase INH concentrate, 35 (44.3%) in the form of the bradykinin 2 receptor antagonist icatibant and 5 of them had a combination of both available. Another 16 patients reported to use a pdC1-esterase INH concentrate or icatibant in combination with tranexamic acid, one patient in combination with antihistamines and another patient in combination with glucocorticosteroids.

Only 5/79 patients had no pdC1-esterase INH concentrate or icatibant available, 4 of them only had on-demand treatment in the form of transexamic acid, 1 patient in the form of H1-antihistamines.

2/79 patients reported to take butylscopolamine for abdominal cramping.

8/79 patients had an epinephrine auto-injector available.

Many patients had multiple drugs available, hence the total number of drugs does not add up to the total number of patients.

Discussion

C1-INH HAE is a rare hereditary disease characterized by recurrent subepithelial swelling of sudden onset due to the generation of the highly potent vasodilator bradykinin. This is the first nationwide survey of C1-INH HAE in Belgium. 112 patients with HAE Type I and II could be included. Moreover, we know of 55 C1-INH-HAE patients who were sent, but did regrettably not complete the questionnaire.

This gives a minimum prevalence of 1.56/100.000 in Belgium, largely similar to other European countries like Sweden (1.54/100.000), Denmark (1.41/100.000), Italy (1.54/100.000) and Spain (1.09/100.000) (8, 10–12). However, we believe for this to be an underestimation. Patients were selected to participate through the reference centers, and a lack of clinical recognition by general physicians and limited referral to these centers, might make the real prevalence of HAE in Belgium to be higher.

Despite HAE being a rare disease, diagnostic error and delay comes with serious consequences such as reduced quality of life and, in case of laryngeal and/or tracheal angioedema, even risk of rapid asphyxiation and death (19, 20).

Hence, correct management and knowledge of the current guidelines, is of utmost importance. Even though the patients were included through HAE reference centres, the participating physicians reported for their patients to consult only once every one to two year(s), in the mean time being followed by their general physician. Non-specialist physicians might not be aware of the guidelines of such a rare pathology.

It is evident that our data disclose many areas needing urgent improvement in terms of recognition, disease management and correct treatment.

First, we report a median diagnostic delay of 7 years. This once again underlines the importance to raise more awareness among general physicians and specialists.

Second, in many patients, the disease was not properly controlled. 51% of patients had experienced pharyngeal or tongue swelling and 78% had experienced abdominal symptoms, both known to cause an important reduction in quality of life. Moreover, 70% had experienced facial swelling, which can be stigmatizing and a cause of a low self-esteem. Nearly half the patients reported absenteeism during their last year at school or work.

A limitation of this study is that the quality of life was only assessed informally, by rating the number of attacks, the type of symptoms and absenteeism. However, this also depicts an important gap in our current management of HAE. After all, current guidelines recommend that a formal quality of life assessment is performed at least yearly.

Moreover, we report the use of non-recommended treatment options, potentially explaining the high disease burden. As recommended in the WAO guidelines, plasma-derived C1 inhibitor should be a first-line choice. However, still almost half of patients on long term prophylactic therapy, reported the daily off-label use of tranexamic acid. Neither the new revised guidelines, nor the 2017 guidelines, in use at the start of this study, recommend antifibrinolytics for long-term prophylaxis. The reason for this probably relates to the fact that many patients are primarily followed by non-specialist physicians who might continue (off-label) treatments that are not standard care anymore. Moreover, patients might be reluctant to start (seemingly invasive) intravenous or subcutaneous treatment and patients are often not compliant to the recommended usage frequency.

As was mentioned in the results, the median number of attacks during the last year was higher in the patients taking a pdC1-INH

concentrate compared to the patients not on appropriate long-term prophylaxis. However, this is assumed to be biased by the fact that patients experiencing more severe symptoms, would likely be considered sooner for prophylactic treatment. A follow-up study regarding the longitudinal effect of this treatment on the patients' symptoms, could be interesting for future research.

In the new guidelines, the use of a monoclonal antibody kallikrein inhibitor is also recommended as first-line prophylaxis. However, in Belgium, reimbursement for this drug was only approved in June 2022.

A final gap we could identify in the management of Belgian HAE patients, it the fact that only 70.5% of patients have on demand treatment available, while guidelines recommend this for all patients.

However, these numbers are high compared to other European studies. In Sweden, only 27% of patients had a recommended on-demand treatment option. It is of note that icatibant was not available at the time of the Swedish survey (11).

Almost all of these patients report to use pdC1-INH concentrate and/or icatibant. However, there is still room for improvement, since 5 patients reported tranexamic acid or H1-antihistamines to be the only treatment they had available during an acute attack, while neither of them should be considered on-demand therapy.

In conclusion, this paper provides the opportunity to emphasize the recently updated guidelines (2) for the management of HAE. We believe that our study provides a representation overview of the type I and II C1-INH-HAE patients in Belgium. From our data, it emerges that many physicians seem not to be up to date about the current guidelines for the treatment of HAE (the study was performed in H with the highest levels of expertise/please formulate differently). Hence the importance of this study and the dissemination of these data, which we believe will raise awareness about the disease and will benefit the recognition and management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Primary Ethics Committee: Faculty of Medicine and Health Science, Antwerp University Hospital. All Ethics Committee of all participating centres also approved this study. Namely: University Hospital Leuven, Saint-Luc University Hospital, Brussels, Hôpital Sient-Pierre, Brussels, Ghent University Hospital, C.H.U. de Liège Hospital, C.H.U. Brugmann, Brussels. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Drafting of manuscript: All authors contributed to the drafting of the manuscript. Design of the study: DE, MV, AV, and VS. Data collection: All authors contributed to the inclusion of patients and collection of data. Creation figures: MV. All authors approved the final text.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Pathophysiology of bradykinin and histamine mediated angioedema

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Angioedema is characterized by swelling localized to the subcutaneous and submucosal tissues. This review provides an overview of angioedema, including the different types, triggers, and underlying pathophysiologic mechanisms. Hereditary and acquired angioedema are caused by dysregulation of the complement and kinin pathways. In contrast, drug-induced and allergic angioedema involve the activation of the immune system and release of vasoactive mediators. Recent advances in the understanding of the pathophysiology of angioedema have led to the development of targeted therapies, such as monoclonal antibodies, bradykinin receptor antagonists, and complement inhibitors, which promise to improve clinical outcomes in patients with this challenging condition. To accurately diagnose and manage angioedema, an understanding of this condition's complex and varied pathophysiology is both necessary and critical.

KEYWORDS

angioedema, pathophysiology, complement and kinin pathways, vasoactive mediators, targeted therapies

1. Introduction

Angioedema (AE), a condition characterized by sudden, self-limiting, localized swelling of the skin and mucosal tissues, presents a complex clinical challenge. Though isolated angioedema is possible, particularly in bradykinin-induced angioedema, angioedema is much more commonly associated with urticarial disorders such as chronic spontaneous urticaria (CSU) (1, 2).

AE is principally categorized into histamine-mediated angioedema and the rarer, but clinically significant, bradykinin-mediated angioedema. The pathophysiology of these types differs fundamentally and understanding these differences is essential to devising accurate diagnostic and therapeutic strategies (3).

Histamine-mediated angioedema is the more common variant of AE. It often manifests as an immediate type I hypersensitivity reaction, affecting the face, lips, tongue, and throat, and may be accompanied by urticaria or hives. Histamine-mediated angioedema typically responds well to standard allergy treatments such as antihistamines, corticosteroids, and epinephrine (4). In contrast, bradykinin-mediated angioedema can be precipitated by stress, trauma, or certain medications, often without any identifiable trigger (5).

Bradykinin-mediated angioedema is a condition marked by fluid extravasation due to vasodilation and increased vascular permeability, stimulated by bradykinin, a potent vasodilator. Its pathophysiology revolves around the complex interplay between bradykinin, high molecular weight kininogen (HMWK), and kallikrein (6). Disruption in this balance

leads to characteristic symptoms such as nonpitting, nonpruritic, asymmetrical and localized swelling or the skin and/or mucosa; gastrointestinal mucosa involvement, for instance, may result in abdominal pain, nausea, vomiting, or diarrhea (7). Bradykinin-mediated angioedema's pathogenesis, pathophysiology, and clinical manifestations are still not fully comprehended, and mismanagement can lead to fatal outcomes (8).

This review focuses on a simple overview that aims to describe bradykinin- and histamine-mediated angioedema's pathophysiology.

2. Histamine-mediated angioedema pathophysiology

Histamine-mediated angioedema is a commonly encountered condition in emergency departments, accounting for nearly 40%–50% of angioedema cases. Though this reaction is mostly self-limited, laryngeal involvement in severe acute reactions such as anaphylaxis can be life-threatening due to the risk of asphyxiation (9).

The most well-characterized mechanism of histamine-mediated angioedema is a type I hypersensitivity reaction. During the sensitization phase of a type I hypersensitivity reaction, exposure to allergens (including food allergens such as milk or wheat) prompts an increase in the secretion of antigen-specific immunoglobulin E (IgE) molecules by plasma cells. During this asymptomatic reaction, IgE molecules bind to high-affinity FcεRI receptors which are constitutively expressed on mast cells and basophils (10, 11).

Re-exposure to the same allergen leads to IgEs cross-linking with the allergen, thereby triggering the degranulation of mast cells and basophils. This signals the "early-phase" of a type I hypersensitivity reaction (10). Inflammatory mediators such as biogenic amines (e.g., histamine) and serine proteases (e.g., tryptase and chymase) are released, and disrupted vascular integrity ensue via dilation and opening of endothelial cell junctions (10, 12). The resultant vasodilation and capillary permeability results in fluid accumulation in interstitial tissue spaces, causing non-pitting edema; predominantly, the face, ears, throat, tongue, lips, hands, feet, and genitalia are affected (10, 13).

The "late-phase", on the other hand, is not necessarily dependent on IgEs. In contrast to early-phase reactions, cutaneous manifestations of a late-phase reaction involve accumulation and infiltration of eosinophils, neutrophils, CD4+ T cells, and basophils. Late-phase reactions occur more slowly than early-phase reactions, typically occurring hours rather than minutes after re-exposure to the antigen (14).

In addition to type I hypersensitivity reactions, histamine-mediated angioedema can also be caused by direct mast cell or basophil activation, resulting in the release of inflammatory mediators. Such direct activation can be caused by either endogenous or exogenous factors. Anaphylatoxins (complement fragments C3a, C4a, and C5a) are examples of such endogenous factors; they cause direct mast cell activation and degranulation by binding to non-FcεRI receptors on mast cells' cell membrane. Iodine- and gadolinium-based contrast agents, on the other hand, are examples of exogenous factors which act directly on mast cells' and basophils' cell membranes to cause degranulation (15, 16).

Furthermore, disruption of the arachidonic acid pathway may cause histamine-mediated angioedema. The most notable example is NSAIDs-induced urticaria/angioedema (NIUA). Nonsteroidal anti-inflammatory drugs (NSAIDs) strongly inhibit cyclooxygenase-1 enzymes (COX-1), disrupting the arachidonic acid pathway. Such a disruption causes increased production of eosinophils, mast cells, and proinflammatory mediators; this, in turn, results in increased production of cysteinyl leukotrienes (a family of inflammatory lipid mediators) (17). Cysteinyl-leukotrienes increase vascular permeability, and in-vitro studies suggest that cysteinyl leukotrienes can induce histamine hyperresponsiveness by increasing the expression of histamine receptors (18, 19). Altogether, angioedema ensues.

3. Bradykinin-mediated angioedema pathophysiology

The pathogenesis of bradykinin-mediated angioedema was initially believed to be dependent on C1-inhibitor (C1-INH) deficiency and complement activation. A peptide called "C2-kinin" derived from C2 was proposed as the cause of angioedema (20). However, further research showed that this peptide could not be generated from purified components, and it was discovered that bradykinin was the only vasoactive peptide produced in the plasma of patients with hereditary angioedema (HAE) (21, 22). Elevated levels of bradykinin were found in HAE patients during angioedema attacks, confirming it as the mediator of swelling (22, 23).

The key enzymes involved in bradykinin formation are activated factor XII and plasma kallikrein, both of which are inhibited by C1-INH (24, 25). C1-INH has multiple functions relevant to bradykinin formation, including the inhibition of factor XIIa, plasma kallikrein, and coagulation factor XIa. It is also involved in the regulation of complement activation (26). In the absence of C1-INH, there is overproduction of bradykinin due to the uncontrolled activity of these enzymes (**Figure 1**) (25, 27).

Factor XII has a small amount of enzymatic activity, which is sufficient to initiate the bradykinin-forming cascade when it encounters negatively charged macromolecules (28). Once activated, factor XIIa can activate factor XI, which continues the intrinsic coagulation cascade, and can also convert plasma prekallikrein to kallikrein. Kallikrein then digests high molecular weight kininogen (HMWK) to release bradykinin (25, 28). There is a positive feedback loop in which plasma kallikrein rapidly converts factor XII to factor XIIa, amplifying the activation of the cascade (29). Additionally, there is a fibrinolytic pathway that involves the conversion of plasminogen to plasmin by kallikrein, factor XIa, and factor XIIa. This pathway is important in the context of bradykinin-mediated angioedema (specifically hereditary angioedema with normal C1-INH activity), as plasmin can cleave and activate factor XII, thereby feeding the bradykinin-forming cascade (30).

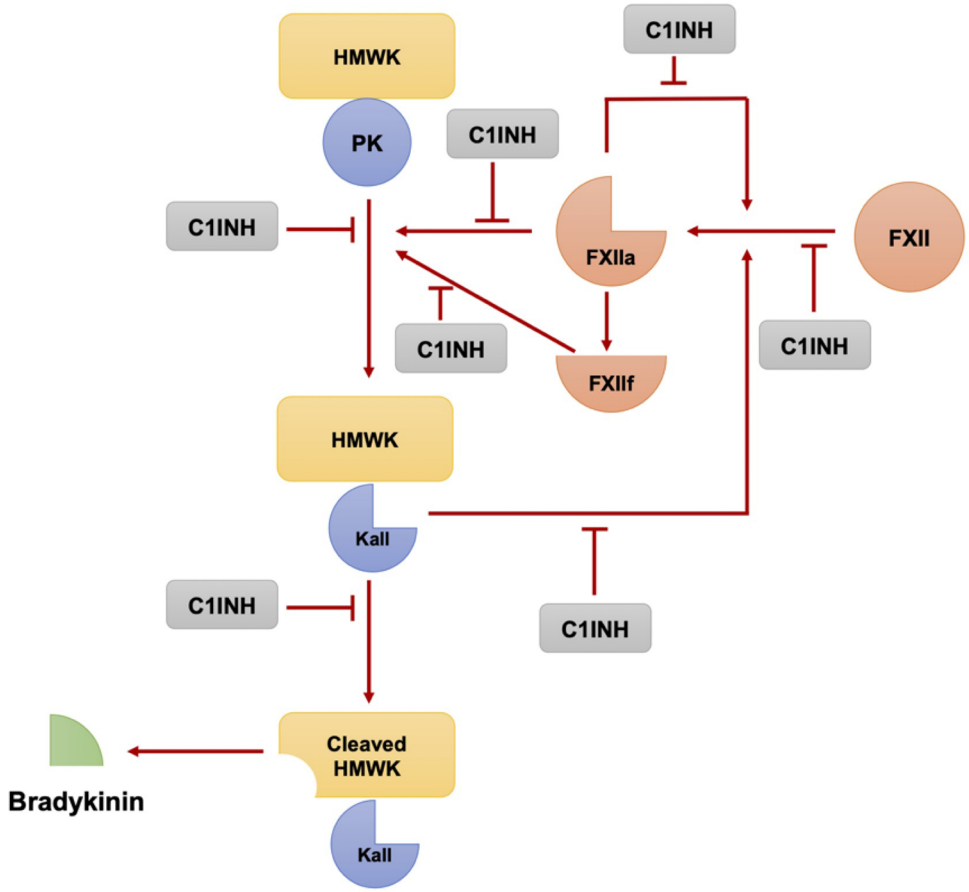

FIGURE 1
The plasma kallikrein-kinin and contact activation systems involve a series of reactions where Factor XII and plasma prekallikrein autoactivate to form factor XIIa and plasma kallikrein respectively. These, in turn, can further activate each other, amplifying the pathway. Moreover, XIIa creates XIIf, which can also convert prekallikrein to kallikrein. This cascade leads to the production of bradykinin from high molecular weight kininogen, a process regulated by the C1 esterase inhibitor. (Modified from Pathophysiology of Angioedema. Dennis Wong, Hermenio Lima, Susan Waserman, Gordon L. Sussman, 2023 in print).

The binding of all the components of the bradykinin-forming cascade to endothelial cells suggests that the endothelium may play a role in the initiation of angioedema attacks (31). Factors such as heat shock protein 90 (HSP-90) and prolylcarboxypeptidase, released by endothelial cells, can activate the HMWK-prekallikrein complex and contribute to the generation of bradykinin (32, 33).

It is important to consider bradykinin-mediated angioedema as a differential diagnosis, and hereditary angioedema (HAE) and certain drug-induced angioedemas (such as ACE-inhibitor-induced angioedema) are possible causes to be considered (34, 35).

HAE is characterized by impaired C1-INH activity, either due to a deficiency (type I) or dysfunction (type II) of the C1-INH protein (36, 37). There are also forms of HAE with normal C1-INH activity, which can be associated with mutations in various genes, including factor XII (38, 39).

In addition to HAE, bradykinin-mediated angioedema can be caused by certain medications, such as angiotensin-converting enzyme inhibitors (ACE inhibitors) and dipeptidyl peptidase-4 inhibitors (gliptins) (40, 41). These drugs decrease the breakdown of bradykinin, leading to its accumulation and the development of angioedema (42, 43). Differentiating between these causes may require genetic testing and evaluation of family history (44).

In summary, the pathogenesis of C1-INH deficiency involves uncontrolled activation of the bradykinin-forming cascade, leading to elevated levels of bradykinin and angioedema (45). Factors such as factor XII, plasma kallikrein, and endothelial cell components play important roles in this process (7). Understanding the underlying mechanisms of bradykinin-mediated angioedema is crucial for accurate diagnosis and appropriate management of affected individuals (46).

4. Discussion

Angioedema, a condition causing localized swelling in subcutaneous and submucosal tissues, can be classified into histamine-mediated angioedema and the less common but clinically significant bradykinin-mediated angioedema. Histamine-mediated angioedema can be either IgE-dependent (type I hypersensitivity reaction) or IgE-independent (e.g., direct mast cell and basophil activation, disruption of the arachidonic acid

pathway). Bradykinin-mediated angioedema, on the other hand, results from an imbalance in the interplay of bradykinin, HMWK, and kallikrein. Understanding the pathophysiology of these types of angioedema is critical for diagnosis and management of this condition.

The differentiation between bradykinin-mediated angioedema and histamine-mediated angioedema is challenging due to an overlap of symptoms. Nonetheless, certain differences in clinical characteristics may guide physicians towards the correct underlying pathophysiology. The presence of pruritus is an important differentiating factor: with histamine-mediated angioedema, the sensation of pruritus is caused by excitation of certain histamine-sensitive unmyelinated C-fibers by histamine (47). Bradykinin-mediated angioedema, on the other hand, is typically non-pruritic. In addition, while patients with bradykinin-mediated angioedema may present with abdominal symptoms such as vomiting and diarrhea, such presentations are rare with histamine-mediated angioedema. Furthermore, while patients with histamine-mediated angioedema respond to treatment by antihistamines, corticosteroids, or epinephrine, patients with bradykinin-mediated angioedema do not respond to such treatments (48).

Differentiating between bradykinin-mediated angioedema and histamine-mediated angioedema is also challenging due to a lack of clear-cut, reliable diagnostic markers. However, research aims to bridge this gap in understanding. For instance, high levels of cleaved high-molecular-weight kininogen (HK) in plasma may be sensitive for detecting type I HAE (49), and threshold-stimulated kallikrein activity assays may allow for differentiation of histamine-mediated and bradykinin-mediated angioedemas (50). This exploration is crucial, not just as an academic exercise, but as a vital step towards developing effective treatment and management strategies for these conditions (51, 52).

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A novel assay of excess plasma kallikrein-kinin system activation in hereditary angioedema

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Background: Cleaved high-molecular-weight kininogen (HKa) is a disease state biomarker of kallikrein-kinin system (KKS) activation in patients with hereditary angioedema due to C1 inhibitor deficiency (HAE-C1INH), the endogenous inhibitor of plasma kallikrein (PKa).
Objective: Develop an HKa-specific enzyme-linked immunosorbent assay (ELISA) to monitor KKS activation in the plasma of HAE-C1INH patients.
Methods: A novel HKa-specific antibody was discovered by antibody phage display and used as a capture reagent to develop an HKa-specific ELISA.
Results: Specific HKa detection following KKS activation was observed in plasma from healthy controls but not in prekallikrein-, high-molecular-weight kininogen-, or coagulation factor XII (FXII)-deficient plasma. HKa levels in plasma collected from HAE-C1INH patients in a disease quiescent state were higher than in plasma from healthy controls and increased further in HAE-C1INH plasma collected during an angioedema attack. The specificity of the assay for PKa-mediated HKa generation in minimally diluted plasma activated with exogenous FXIIa was demonstrated using a specific monoclonal antibody inhibitor (lanadelumab, IC_{50} = 0.044 μM).
Conclusions: An ELISA was developed for the specific and quantitative detection of HKa in human plasma to support HAE-C1INH drug development. Improved quantification of the HKa biomarker may facilitate further pathophysiologic insight into HAE-C1INH and other diseases mediated by a dysregulated KKS and may enable the design of highly potent inhibitors targeting this pathway.

KEYWORDS

biomarkers, phage display, bradykinin, plasma kallikrein, hereditary angioedema

1 Introduction

The plasma kallikrein-kinin system (KKS) lies at an interface between *coagulation* through the activation of the intrinsic coagulation pathway, and *inflammation* via its ability to generate bradykinin, a potent mediator of vascular permeability, inflammation, and pain (1). The KKS consists of prekallikrein (PK), high-molecular-weight kininogen (HK), and coagulation factor XII (FXII) and is activated by FXII

Abbreviations
HK, high-molecular-weight kininogen; HKa, cleaved high-molecular-weight kininogen; LK, low-molecular-weight kininogen; KKS, kallikrein-kinin system; PK, prekallikrein; PKa, plasma kallikrein; HAE-C1INH, hereditary angioedema due to a deficiency in total (type I) or functional C1 inhibitor protein (type II).

autoactivation upon exposure to negatively charged surfaces, the exact endogenous identity of which remains elusive (2). KKS activation can also involve membrane-associated prolylcarboxypeptidase or a complex of PK, HK, and heat shock protein 90 (HSP90) on endothelial cells (2).

The initial generation of plasma kallikrein (PKa) promotes a rapid burst of localized KKS activation through the PKa-catalyzed conversion of FXII to its active form (FXIIa), which generates additional PKa (3). FXIIa can initiate the intrinsic coagulation pathway through the generation of FXIa, and inhibitors of FXIIa and FXIa have been proposed as novel anti-thrombotic agents, as they have the potential to be anti-thrombotic without increasing the risk of bleeding (4). PKa also cleaves HK to liberate bradykinin, a 9-amino acid peptide, and cleaved HK (HKa). Bradykinin-mediated edema occurs through the activation of the constitutively expressed B2 receptor or the inducible B1 receptor (5). Endogenous KKS activation is regulated through protease inhibitors, including α2-macroglobulin and C1 inhibitor (C1INH) (6).

A genetic deficiency in C1INH leads to dysregulated KKS activation, excess bradykinin production, and the debilitating disease of hereditary angioedema due to C1INH deficiency (HAE-C1INH) (3). HAE-C1INH is an autosomal dominant disease manifesting as intermittent edematous attacks at locations that include the gastrointestinal tract, facial tissue, the upper airway, oropharynx, urogenital region, and/or extremities. Patients with HAE-C1INH type I have mutations in the *SERPING1* gene that lead to a deficiency in the total amount of C1INH protein, whereas HAE-C1INH type II patients have a dysfunctional C1INH (7). The role of excess KKS activation in HAE-C1INH pathophysiology has been validated by the approval of therapies that include C1INH protein replacement products, a bradykinin B2 receptor antagonist (icatibant), and PKa inhibitors (ecallantide, lanadelumab, and berotralstat).

HKa has previously been shown to be a disease state biomarker of KKS activation and is elevated in plasma from HAE-C1INH patients and other disease states (8–18). We describe the development of a novel enzyme-linked immunosorbent assay (ELISA) to specifically detect HKa in human plasma and demonstrate the utility of the assay in comparing the *in vitro* potency of PKa inhibitors.

2 Methods

2.1 Materials

HK, HKa, PKa, FXII, and FXIIa were obtained from Enzyme Research Laboratories (South Bend, IN, USA). Low-molecular-weight kininogen (LK) was procured from Athens Research (Athens, GA, USA). Mouse monoclonal antibodies 11H05 and 13B12 were generated at BBI Solutions (Portland, ME, USA) following immunization with human HKa. PK-deficient and FXII-deficient plasma were obtained from George King Biomedical (Overland Park, KS, USA) using sodium citrate as an anticoagulant. HK-deficient human plasma using sodium citrate as an anticoagulant was obtained from Affinity Biologicals (Ancaster, ON, Canada). SCAT169 plastic evacuated blood collection tubes (5 ml volume) were prepared by Prolytix (Essex Junction, VT, USA) and contained 0.5 ml of a 10× concentrated mixture [100 mM benzamidine, 400 µg/ml polybrene, 2 mg/ml soybean trypsin inhibitor, 20 mM EDTA, 263 µM leupeptin, and 20 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) dissolved in acid citrate dextrose (100 mM sodium citrate, 67 mM citric acid, and 2% dextrose, pH 4.5)]. P100 Blood Collection System tubes were purchased from BD Biosciences (San Jose, CA, USA). Normal human plasma, collected with sodium citrate, SCAT169, or P100 tubes, was obtained from BioIVT (Westbury, NY, USA). Ellagic acid was obtained as a 0.003% solution (Pacific Hemostasis APTT-XL reagent) from Thermo Fisher Scientific (Waltham, MA, USA).

2.2 Plasma collection

Plasma was collected from healthy volunteers (HVs) or patients with HAE-C1INH with approval by the institutional review boards or ethics committees of the participating institutions (see Acknowledgments). Plasma was collected from HAE-C1INH patients at baseline (e.g., not during an attack) or within 6 h of the onset of an angioedema attack. HAE-C1INH plasma was collected and analyzed before the availability of recently approved prophylactic therapies (e.g., lanadelumab, berotralstat, or subcutaneous C1INH). All healthy volunteers and patients provided written informed consent to use the blood for the investigation of exploratory biomarkers of KKS activation. To minimize the *ex vivo* activation of the KKS during blood collection, plasma was collected from subjects with HAE-C1INH and healthy controls by means of a clean venipuncture with a butterfly needle/catheter kit (BD Biosciences, #367296). The first tube of blood was discarded, and blood was collected into polypropylene-evacuated tubes containing either 3.2% sodium citrate (BD Biosciences, San Jose, CA, USA) or a protease inhibitor/anticoagulant mixture (SCAT169 or P100 tubes). Blood samples were centrifuged within 1 h, and plasma was aliquoted and stored at −80°C until processing.

2.3 Phage display

Antibodies specific for HKa were discovered using a human antibody phage display library (19) by first performing a negative selection of the library with an input of approximately 1×10^{12} phage against biotinylated HK that was immobilized onto streptavidin-coated magnetic beads (Dynabeads^{TM} M-280, Thermo Fisher Scientific). The depleted library was then incubated with biotinylated HKa immobilized on streptavidin-coated magnetic beads. The beads were extensively washed with phosphate buffered saline (PBS) buffer and used to infect *Escherichia coli* for phage output amplification to complete a round of selection. Three rounds of selection were performed before screening individual phage colonies using an ELISA with

biotinylated HK and HKa immobilized on streptavidin-coated plates followed by detection with horseradish peroxidase (HRP)-conjugated anti-M13 antibody and absorbance detection through substrate hydrolysis of 3,3′,5,5′-tetramethylbenzidine (TMB). Recombinant fragment antigen-binding (Fab) fragments were expressed in *E. coli* and purified by protein A Sepharose (GE Healthcare, Piscataway, NJ, USA) (20). Recombinant immunoglobulin G (IgG1) human anti-HKa antibodies were transiently expressed in 293T cells and purified by protein A Sepharose chromatography. Fab specificity was determined by coating 96-well or 384-well plates with each purified Fab and measuring the binding to biotinylated HK, biotinylated HKa, or biotinylated LK.

2.4 Western blot

Plasma was analyzed by a western blot assay (WBA) after activation with various agents (e.g., ellagic acid, FXIIa, or PKa) or after the addition of a 1/10th volume of the 10× SCAT169 cocktail upon thawing of frozen plasma (45 µl) and diluted (1:20) in Tris-buffered saline. Samples were further diluted in NuPAGE® Sample Buffer (Life Technologies Corporation, Carlsbad, CA, USA) containing 50 mM of dithiothreitol and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a precast 4%–12% acrylamide gradient gel and the 2-(N-morpholino) ethanesulfonic acid sodium dodecyl sulfate buffer system (Life Technologies Corporation). Protein was transferred from the gel to a nitrocellulose membrane using the iBlot® system (Life Technologies Corporation). Membranes were blocked in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE, USA) containing 0.2% Tween 20 and incubated for 1 h at room temperature (RT) with a mouse monoclonal antibody that specifically binds the light chain of HK (Clone 11H05) diluted to 1 µg/ml in Odyssey blocking buffer. Goat anti-mouse IgG IRDye 680 was prepared at a 1:15,000 dilution (LI-COR Biosciences) and the membrane was incubated for 1 h at RT. After a PBS wash, membranes were analyzed using a LI-COR Odyssey CLx (LI-COR Biosciences). The percentage of HKa was calculated from the fluorescent intensities of bands attributed to the cleaved light chain of HKa compared with total HK in each sample.

2.5 HKa ELISA

For the initial ELISA, the Fab version of the HKa-specific antibody (M4-B4 Fab) was coated on 96-well plates (Costar, #9018) overnight in PBS before being washed and blocked with 2% bovine serum albumin (BSA) buffer. Samples, standards, and quality controls were diluted in LowCross-Buffer (Boca Scientific, Boca Raton, FL, USA) and added to the plate. After an incubation and wash, plate-bound HKa was detected by adding HRP-labeled sheep anti-HK polyclonal antibody (Affinity Biologicals, Ancaster, ON, Canada). After incubation with the detection antibody, the plate was washed, TMB substrate was added, the incubation was stopped with phosphoric acid, and the optical density was measured at 450 nm with subtraction at 630 nm. Further ELISA optimization involved coating the M4-B4 Fab or IgG on Nunc MaxiSorp plates overnight in PBS, before washing and blocking with BSA (protease/IgG-free) buffer. Samples, standards, and quality controls diluted in 0.1% BSA buffer were added to the plate and, after a subsequent incubation, plate-bound HKa was detected by adding mouse anti-HK monoclonal antibodies (either pooled 11H05 and 13B12 or just 11H05), which were selected based on their ability to form a sandwich pair with the M4-B4. The plate was washed and a secondary goat anti-mouse IgG HRP was added to the plate, followed by TMB substrate, stopping with phosphoric acid, and OD measurement at 450 nm with subtraction at 630 nm.

2.6 *Ex vivo* KKS activation assay in minimally diluted plasma

To assess the inhibition potency of PKa inhibitors, 2.5 µl of serially diluted inhibitors (synthetic compounds were diluted in DMSO and biologics were diluted in PBS) were dispensed into 96-well PCR plates (Thermo Fisher Scientific, #AB0600) followed by the addition of 45 µl of sodium citrate plasma (pooled from three donors). Samples were incubated for 5 min at RT before the addition of 2.5 µl of 100 nM human FXIIa solution followed by incubation in a shallow 37°C water bath for 30 min. The activation was terminated by a 150-fold dilution of plasma samples in ice-cold dilution buffer (1% BSA/0.01% casein in PBS).

2.7 Statistical methods

Western blot and ELISA data comparisons for HKa levels in plasma from healthy volunteers or patients with HAE-C1INH were analyzed using the Mann–Whitney *U*-test (SigmaPlot software, Grafiti LLC, Palo Alto, CA, USA), for which $p < 0.05$ was considered significant. Receiver operator characteristic (ROC) analysis was performed using Prism software (GraphPad, Boston, MA, USA). ELISA standard curves were fitted to a four-parameter logistic equation (Softmax, San Jose, CA, USA). Half-maximal inhibitory concentration (IC_{50}) values were obtained through a non-linear regression analysis of dose responses fitted to averaged values from at least three experiments of duplicate measurements through non-linear regression (Prism software) using a three-parameter hyperbolic competitive equation $\{Y = Bottom + [Top\text{-}Bottom]/[1 + (X/IC_{50})]\}$ to provide estimated IC_{50} values.

3 Results

A human antibody phage display library (19) was depleted against HK and panned against HKa, from which over 6,700 putative positive phage colonies were obtained. Phage clones positive for binding to immobilized HKa were selected, sub-cloned for soluble Fab fragment expression, and further screened

for HKa specificity by an ELISA. As target immobilization could mask epitope(s) present in HKa as opposed to HK, and as the goal was to develop an ELISA to detect soluble HKa in plasma, subsequent screening was performed using purified Fabs immobilized on 384-well or 96-well plates with the detection of either biotinylated HK, LK, or HKa binding via streptavidin-HRP. The specificity of 190 anti-kininogen binding Fabs is shown in Figure 1 where the Fab M4-B4 (aka M004-B04) exhibited the highest specificity for HKa vs. HK or LK (see Supplementary Figure S1 for the specificity of two other Fabs and Supplementary Figure S8 for the amino acid sequence of the M4-B4 Fab).

The ability of the M4-B4 antibody to detect HKa was investigated in human plasma treated with the KKS activator ellagic acid (21), purified FXIIa, or purified PKa (Figure 2). KKS activation was assessed by WBA (Figure 2A) using a mouse monoclonal antibody (11H05) that binds intact HK as well as the 56- and 46-kDa forms of the HKa light chain. With WBA, activated human plasma showed a reduction in the band corresponding to intact HK and increases in the two bands corresponding to light chain (Figure 2A). Activated plasma displayed an increased HKa ELISA signal (Figure 2B). Of note, our ELISA did not detect endogenous HKa in plasma from cynomolgus monkeys, mice, or rats after activation with ellagic acid (data not shown).

FXII- and PK-deficient plasma demonstrated the specificity of M4-B4 for HKa. As expected, HKa was not generated in FXII-deficient plasma treated with ellagic acid (lane 10 of Figure 2A and the solid gray bar in Figure 2B), but was produced after the addition of FXIIa (lane 11 of Figure 2A and the hatched bar in Figure 2B) or PKa (lane 9 of Figure 2A and the dotted bar in Figure 2B). Ellagic acid activates the KKS through the formation of an insoluble metal ion complex that serves as a charged surface to promote FXII autoactivation to FXIIa (22). It was therefore expected that ellagic acid would be unable to activate the KKS in FXII-deficient or PK-deficient plasma. PK-deficient plasma was only activated by the addition of PKa.

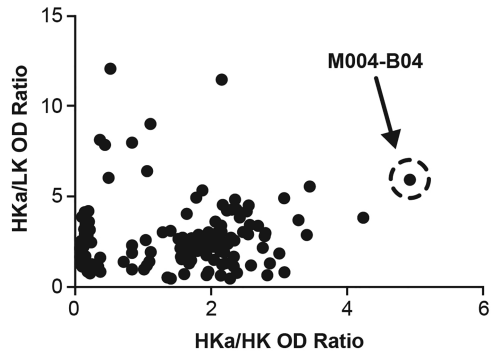

FIGURE 1
Discovery of an HKa-specific antibody by phage display selection and screening. Recombinant Fab fragments were passively immobilized onto 384-well plates before the addition of either biotinylated HKa, intact HK, or LK followed by detection using streptavidin-HRP and TMB as described in the Methods section. Fab, fragment antigen-binding; HK, high-molecular-weight kininogen; HKa, cleaved high-molecular-weight kininogen; HRP, horseradish peroxidase; LK, low-molecular-weight kininogen; OD, optical density; TMB, 3,3′,5,5′-tetramethylbenzidine.

Human plasma collected using EDTA as an anticoagulant was activated similarly to sodium citrate plasma, as shown by HKa generation using WBA (Figure 2A), supporting the previous observation that metal ions are not required for KKS activation (6). Interestingly, despite HKa formation in activated EDTA plasma by WBA (Figure 2B), HKa was not detected by an ELISA (Figure 2B), suggesting that M4-B4 binding to HKa may be metal ion-dependent, which was restored by the addition of zinc chloride at concentrations that exceeded the EDTA concentration in the blood collection tube (Supplementary Figure S2). Surface plasmon resonance analyses demonstrated that M4-B4 bound HKa with approximately a sixfold higher affinity in the presence of zinc chloride ($K_D = 1.06 \pm 0.25$ nM) than in its absence ($K_D = 6.08 \pm 0.26$ nM) (Supplementary Figure S3 and Supplementary Table S2). Zinc has been previously shown to induce conformational changes in HK (23, 24).

HK and LK are cleaved by tissue kallikrein 1 (KLK1) (25), as confirmed by WBA (Figure 3A). Although the form of HKa generated by KLK1 was detected by the ELISA, KLK1-cleaved LK was not detected (Figure 3B). KLK1-cleaved HK consists mainly of the 56-kDa light chain (lanes 4 and 5 of Figure 3A), whereas the purified HKa standard protein mainly consists of the 46-kDa light chain (see lane 2 of Figure 3A), which suggests that M4-B4 binds HKa at either light chain variant.

Standard curves prepared by spiking HKa into citrated plasma from HVs demonstrated a lower limit of quantitation of approximately 156 ng/ml at a dilution of 1:320 (Supplementary Figure S4). HKa levels in citrated and SCAT169 plasma from HVs and HAE-C1INH patients were compared using a WBA and an ELISA (Figure 4, Supplementary Table S1). HKa levels were higher ($p < 0.05$) in both plasma types from HAE-C1INH patients collected either during an attack or in a basal state of disease quiescence than from HVs, using either assay (Supplementary Table S3). Although the percentage of HKa by WBA was higher during an attack than under the basal disease state, it did not reach statistical significance ($p < 0.05$). In contrast, a statistical difference in HKa plasma levels between the basal and attack disease states was observed by an ELISA.

ROC analysis of WBA data demonstrated that HKa levels in citrated plasma can differentiate HAE-C1INH from HVs with an area under the curve (AUC) value of 0.977 for the comparison of basal with HVs or 1.0 for the comparison of attack with HVs (Supplementary Table S3, Supplementary Figure S5). Plasma HKa levels for HAE-C1INH patients in citrated plasma collected between attacks (i.e., basal samples) were less differentiated from attack samples (AUC = 0.625). Similarly, ROC analysis of WBA data demonstrated that HKa levels in SCAT169 plasma differentiates HAE-C1INH from HVs, with an AUC value of 0.915 for the comparison of basal with HVs or 0.967 for the comparison of attack with HVs (Supplementary Table S3, Supplementary Figure S5). Plasma HKa levels for HAE-C1INH patients in SCAT169 plasma collected between attacks were less differentiated from attack samples (AUC = 0.597).

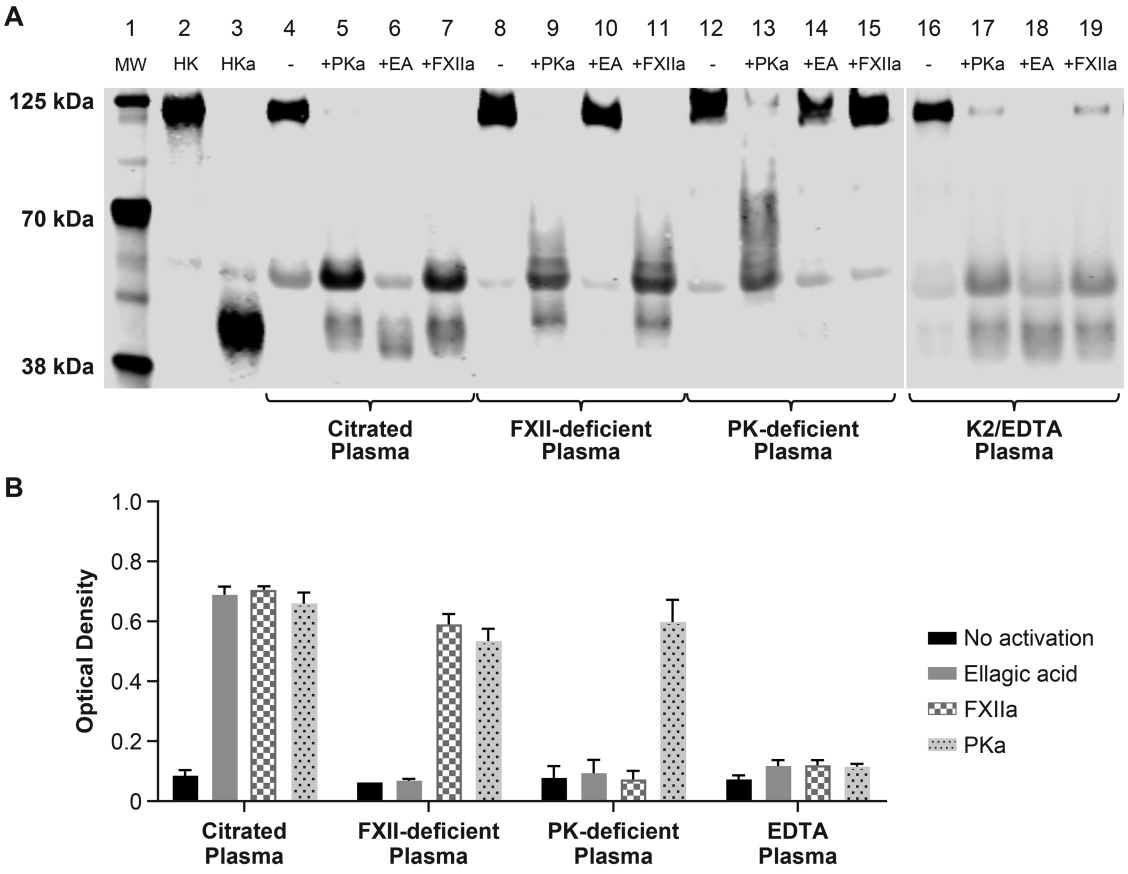

FIGURE 2
Characterization of M4-B4 reactivity in human plasma. Sodium citrate and EDTA plasma from healthy volunteers, FXII-deficient sodium citrate plasma, and PK-deficient sodium citrate plasma were either untreated or activated for 20 min at 37°C with either 100 nM PKa, ellagic acid reagent, or 10 nM FXIIa before analysis by western blotting **(A)** using an anti-HK monoclonal antibody (11H05) or by an ELISA **(B)** using M4-B4 as a capture antibody and a 1:1 mixture of 11H05 and 13B12 anti-HK monoclonal antibodies as detection antibodies. Lane composition are as follows: molecular-weight markers (MW) (lane 1), purified HK (lane 2), purified HKa (lane 3), citrate plasma (lane 4), citrate plasma + PKa (lane 5), citrate plasma + ellagic acid (lane 6), citrate plasma + FXIIa (lane 7), FXII-deficient plasma (lane 8), FXII-deficient plasma + PKa (lane 9), FXII-deficient plasma + ellagic acid (lane 10), FXII-deficient plasma + FXIIa (lane 11), prekallikrein-deficient plasma (lane 12), prekallikrein-deficient plasma + PKa (lane 13), prekallikrein-deficient plasma + ellagic acid (lane 14), prekallikrein-deficient plasma + FXIIa (lane 15), EDTA plasma (lane 16), EDTA-deficient plasma + PKa (lane 17), EDTA-deficient plasma + ellagic acid (lane 18), and EDTA-deficient plasma + FXIIa (lane 19). EA, ellagic acid; FXIIa, coagulation factor XIIa; HK, high-molecular-weight kininogen; HKa, cleaved high-molecular-weight kininogen; K2/EDTA, dipotassium ethylenediaminetetraacetic acid; LK, low-molecular-weight kininogen; MW, molecular weight; PK, prekallikrein; PKa, plasma kallikrein.

ROC analysis of HKa ELISA data in citrated plasma also differentiated HAE-C1INH from HVs, with an AUC value of 0.915 for the comparison of basal with HVs or 0.866 for the comparison of attack with HVs (Supplementary Table S3, Supplementary Figure S5). HAE-C1INH basal citrated plasma samples were better differentiated from attack samples (AUC = 0.709) by an ELISA than by a WBA. ROC analysis suggests that the ELISA with SCAT169 plasma may be best suited for differentiating HAE-C1INH from HVs, as shown by ROC analysis, with an AUC value of 0.999 for the comparison of basal with HV or 1.0 for the comparison of attack with HV. Finally, the ELISA with SCAT169 plasma appeared best suited for the differentiation of HAE-C1INH basal from attack samples (AUC = 0.818).

HKa levels assessed by an ELISA in plasma collected using P100 tubes (BD Biosciences), which contain protease inhibitors, were lower than in citrated plasma from the same 30 individual HVs (Supplementary Figure S6). When the plasma was subjected to five freeze/thaw cycles, there was an apparent reduction in the ELISA signal after four cycles in citrated plasma and in P100 plasma collected in 8-ml tubes that contained a serum separator, but the ELISA signal appeared more stable in freeze/thaw cycles in P100 plasma collected in 2-ml tubes (Supplementary Figure S7).

The complete inhibition of the HKa ELISA signal by the specific antibody inhibitor of PKa, lanadelumab (26, 27), with an IC_{50} of approximately 0.044 μM, or BD-105294, a novel small-molecule PKa inhibitor [the synthesis (28) of which is described in the supplementary methods] with an IC_{50} = 0.082 μM, indicates that the ELISA signal is PKa mediated (Figure 5). The observation that other reported PKa inhibitors were less effective in reducing HKa generation in activated plasma requires further investigation (Supplementary Table S4). However, confirmation that the HKa signal does indeed originate from PKa activity in

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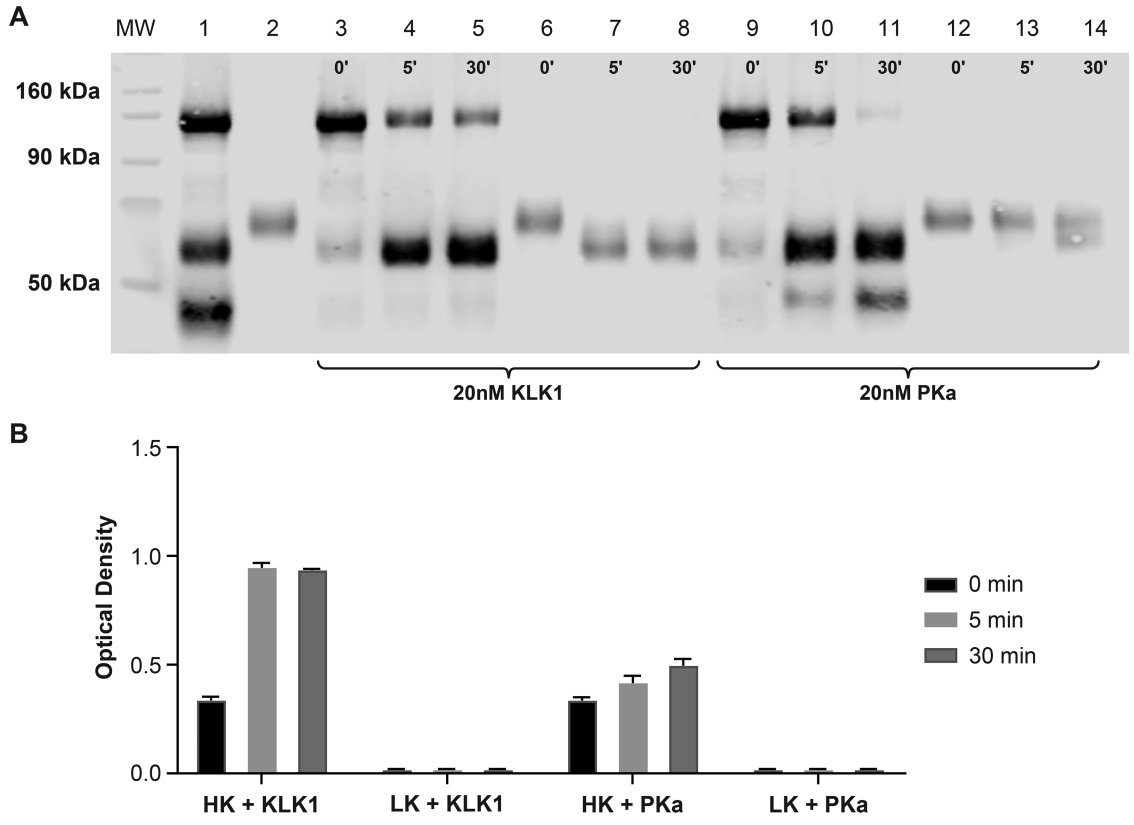

FIGURE 3
Comparative kininogen digestion by tissue vs. plasma kallikrein. Purified HK and LK were analyzed by western blotting **(A)** or an ELISA **(B)** using M4-B4 as a capture antibody and 11H05 and 13B12 as detection antibodies following incubation with either KLK1 or PKa. The western blot analysis was performed using a polyclonal sheep anti-kininogen antibody for detection that cross-reacted with LK as well as HK. The lane compositions are as follows: molecular-weight markers (MW), a mixture of purified intact HK and HKa (1.5 μM each) (lane 1); purified LK (1.5 μM) (lane 2); HK (1.5 μM) incubated with 20 nM KLK1 for 0, 5, and 30 min (lanes 3–5); LK (1.5 μM) incubated with 20 nM KLK1 for 0, 5, and 30 min (lanes 6–8); HK (1.5 μM) incubated with 20 nM PKa for 0, 5, and 30 min (lanes 9–11); and LK (1.5 μM) incubated with 20 nM PKa for 0, 5, and 30 min (lanes 12–14). HK, high-molecular-weight kininogen; HKa, cleaved high-molecular-weight kininogen; KLK1, tissue kallikrein 1; LK, low-molecular-weight kininogen; MW, molecular weight; PK, prekallikrein; PKa, plasma kallikrein.

activated plasma was obtained by multiple small-molecule PKa inhibitors screened at Takeda in the HKa ELISA and assessed for oral bioavailability in the rat (Supplementary Figure S9) and by EPI-KAL-2, a recombinant Kunitz domain PKa inhibitor previously discovered using phage display (29), which was also a potent inhibitor in the HKa ELISA with FXIIa activation (IC_{50} = 0.15 μM).

4 Discussion

The HKa-specific antibody M4-B4 was identified from a phage display library (19). The specificity of M4-B4 suggests that the antibody binds a neo-epitope(s) on HKa not present on HK or LK that is dependent on PKa or KLK1 activity. HK is a 626 amino acid glycoprotein that consists of six domains, and PKa or KLK1 cleave within domain four to release bradykinin or Lys-bradykinin, respectively, and generate HKa, which consists of two polypeptide chains linked by a disulfide (30). HKa has bioactivities (e.g., induction of endothelial cell apoptosis and inhibition of angiogenesis) not exhibited by HK (31), which is consistent with HKa having a distinct conformation from HK that is recognized by M4-B4. The increased binding affinity of M4-B4 for HKa in the presence of zinc suggests that the epitope may be near, or influenced by, the zinc-binding site on domain five of the light chain, which mediates the assembly of the KKS on cell surface receptors (32).

The importance of plasma collection methods and stabilization against proteolytic degradation has been described previously for the analysis of KKS activation (11). Contact of plasma with glass or other surfaces can result in extensive *ex vivo* KKS activation (6). Consequently, plasma obtained in this study relied on a blood collection protocol to minimize *ex vivo* KKS activation. *Ex vivo* KKS activation can be further minimized using an anticoagulant mixture that includes protease inhibitors (11, 13). In this study, *ex vivo* KKS activation, as measured by the concentration of HKa, was higher in citrated plasma from HVs and HAE-C1INH patients than in plasma collected in blood collection tubes that contained protease inhibitors (SCAT169 or P100 tubes).

The amount of HKa in citrated plasma by WBA during HAE-C1INH attacks was approximately 61.5% (Supplementary

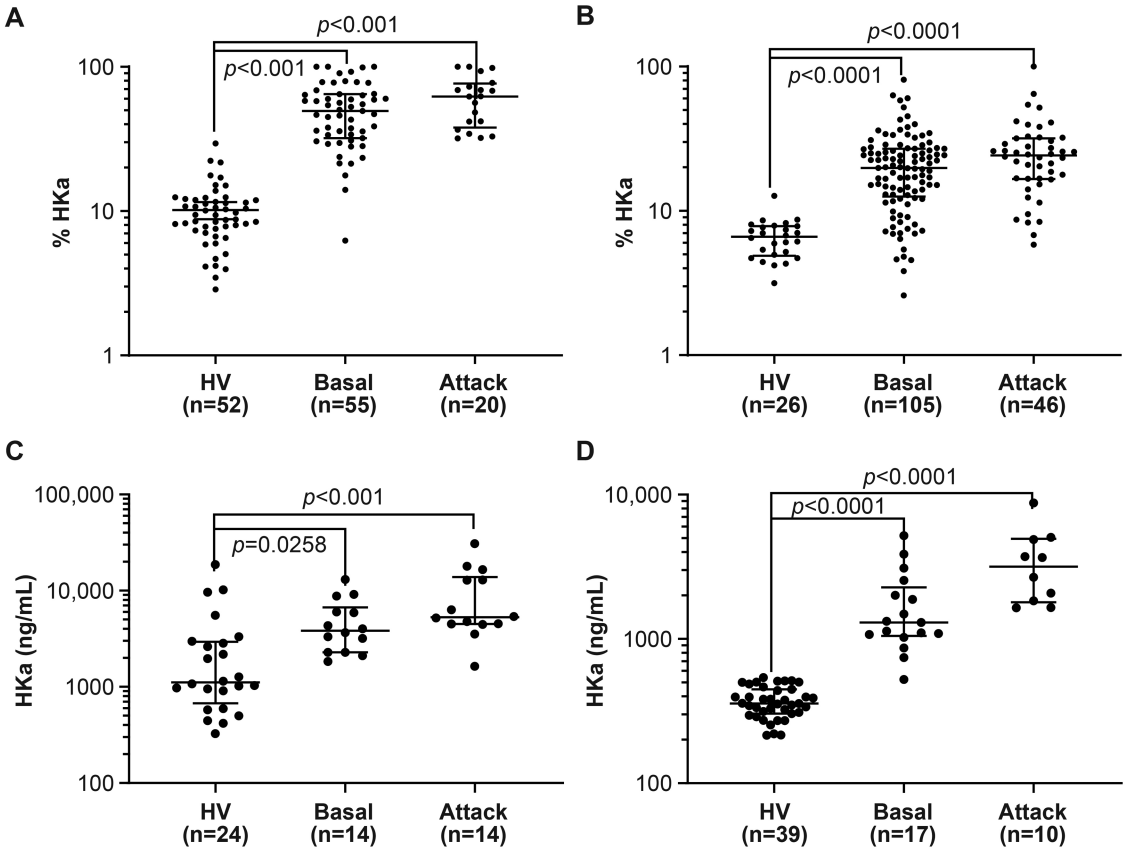

FIGURE 4
Comparison of HKa levels in healthy volunteers and HAE-C1INH patients by western blotting and an ELISA. The statistical analysis of the data in (A–D) is summarized in Supplementary Table S3. (A) Comparison of the percentage of HKa measured by western blotting in the citrated plasma of healthy volunteers with that from HAE-C1INH patients collected between (basal) and during an attack. (B) Comparison of the percentage of HKa measured by western blotting in the SCAT169 plasma of healthy volunteers with that from HAE-C1INH patients collected between (basal) and during an attack. (C) Comparison of HKa levels measured by an ELISA in the citrated plasma of healthy volunteers with those from HAE-C1INH patients collected between (basal) and during an attack. (D) Comparison of HKa levels measured by an ELISA in the SCAT169 plasma of healthy volunteers with those from HAE-C1INH patients collected between (basal) and during an attack. Group means and 95% confidence intervals are shown. Groups were compared with each other by one-way ANOVA. HAE-C1INH, hereditary angioedema due to a deficiency in total (type I) or functional C1 inhibitor protein (type II); HKa, cleaved high-molecular-weight kininogen; HV, healthy volunteer.

Table S1), which is similar to previous estimates (11, 33). An HKa ELISA in SCAT169 may provide estimates of the amount of HKa generated during an HAE-C1INH attack (4.4% or 32.7 nM), in basal HAE-C1INH conditions (2.2% or 16.2 nM), or in healthy controls (0.5% or 3.4 nM) (Supplementary Table S1). HKa concentrations should match the concentration of bradykinin generated during an attack, and the following evidence indicates that an approximately equivalent amount of PKa is generated during an HAE-C1INH attack: (1) the concentration of covalent PKa-α-macroglobulin complex (30–110 nM) during an attack (34) and (2) PKa estimates calculated from the extent of PK consumption during an attack (35, 36) and (3) from the maximum concentration (C_{max} = 83 nM) of ecallantide, which is a PKa inhibitor approved for treating an HAE-C1INH attack (37). Comparable concentrations of PKa and HKa suggest that when the KKS is activated at angioedema attack locations, the generated PKa cleaves HK with approximately equimolar stoichiometry (i.e., single turnover catalytic conditions).

Therefore, KKS activation occurring *in vivo* during an HAE-C1INH attack involves approximately equal concentrations of PKa protease and HK natural protein substrate and are expected to undergo fewer catalytic turnovers than *in vitro* reactions using a high concentrations of a synthetic peptide PKa substrate (e.g., 100 μM) that is at least 1000-fold higher than the concentration of PKa in undiluted activated plasma (~100 nM) or in purified protein assays (~1 nM). The higher catalytic turnovers expected with synthetic peptide substrates may contribute to the higher observed potency of certain reported PKa inhibitors measured using a fluorogenic synthetic peptide in minimally diluted plasma activated with FXIIa than with our HKa ELISA (see Supplementary Table S4). Furthermore, the use of 90% plasma in our HKa ELISA, as opposed to higher dilutions of plasma, may better predict endogenous PKa inhibition as the effects of interfering substances in the plasma (e.g., plasma protein binding) can be minimized at higher plasma dilutions and thereby artifactually increase the observed potency. The use of different KKS activators may also contribute to different observed

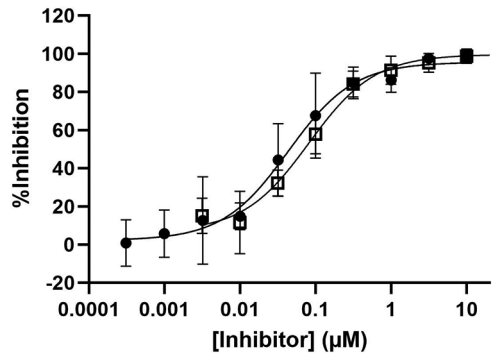

FIGURE 5
Inhibition of HKa generation in activated plasma by PKa inhibitors. Pooled sodium citrate human plasma samples were spiked with the antibody PKa inhibitor lanadelumab (closed circles) or a small-molecule PKa inhibitor BD-105294 (open squares). Plasma samples were activated for 30 min with 5 nM FXIIa. Post activation samples were analyzed at a 150-fold dilution. A dose-response relationship was fitted to averaged values from at least three experiments of duplicate measurements through non-linear regression (GraphPad Prism) using a three-parameter hyperbolic competitive equation {Y = Bottom + [Top-Bottom]/[1 + (X/IC_{50})]} to provide estimated IC_{50} values for lanadelumab [IC_{50} = 0.04 μM, with a 95% confidence interval (CI) from 0.030 to 0.064 μM] and BD-105294 (IC_{50} = 0.082 μM, with a 95% CI from 0.059 to 0.11 μM). FXIIa, coagulation factor XIIa; HKa, cleaved high-molecular-weight kininogen; IC_{50}, half maximal inhibitory concentration; PKa, plasma kallikrein.

potencies for PKa inhibitors; ellagic acid or dextran sulfate activates through inducing FXII autoactivation, the kinetics of which are not as immediate as directly adding FXIIa (38). Therefore, KKS activation induced by FXIIa addition may be expected to generate PKa more rapidly than other reported activators and thereby require higher inhibitor concentrations than KKS activators that act via FXII autoactivation. Consequently, our HKa ELISA may be a useful *in vitro* assay for helping to predict *in vivo* efficacy for PKa inhibitors in development for HAE-C1INH and other indications potentially mediated by PKa, such as bradykinin-mediated angioedema (39), diabetic macular edema (40), Alzheimer's disease (15), ischemic stroke (41), myocardial infarction (42), systemic lupus erythematosus (43), cancer (44), and sepsis (45).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the institutional review boards of Massachusetts General Hospital, Immunology Unit, Boston, MA, USA; the Bernstein Allergy Group, Cincinnati, OH, USA; Virant Diagnostics, Wheaton, MD, USA; the Washington University School of Medicine, St. Louis, MO, USA; the Icahn School of Medicine at Mount Sinai, New York, NY, USA; Ohio State University, Columbus, OH, USA; Albert Einstein College of Medicine, New York, NY, USA; the University of Washington, Spokane, WA, USA; the Alabama Allergy and Asthma Center, Birmingham, AL, USA; and BioIVT, Westbury, NY, USA. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DS: Writing – review & editing, Writing – original draft. RF: Writing – review & editing. MR-H: Writing – review & editing. JK: Writing – review & editing. NP: Writing – review & editing. JC: Writing – review & editing. KK: Writing – review & editing. GS: Writing – review & editing. CB: Writing – review & editing. SJ: Writing – review & editing. DY: Writing – review & editing.

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Conflict of interest

JC, KK, CB, and DY are employees of Takeda Development Center Americas, Inc., and hold stock/stock options in Takeda Pharmaceuticals Company Limited. SJ is an employee of Takeda Pharmaceuticals USA, Inc., and holds stock/stock options in Takeda Pharmaceuticals Company Limited. DS is an employee of Sexton Bio Consulting, LLC, and a former employee of Takeda Development Center Americas, Inc., and holds Takeda Pharmaceuticals Company Limited stock or stock options. NP, JK, and MR-H are former employees of Takeda Development Center Americas, Inc., and hold stock/stock options in Takeda Pharmaceuticals Company Limited. RF is a former employee of Shire, a Takeda company. GS is an employee of Charles River Laboratories. The authors declare that this study received funding from Takeda. The funder had the following involvement in the study: research and publication of this article.

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Publisher's note

Supplementary material

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Case Report: Identification of a novel mutation, c.1067T > A, in the *SERPING1* gene in a Chinese male with type 1 hereditary angioedema

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Hereditary angioedema (HAE) is a rare autosomal dominant genetic disorder characterized by recurrent, unpredictable episodes of angioedema that commonly involve the face, limbs, respiratory tract, and gastrointestinal tract. Clinical presentations vary substantially among individuals, increasing the likelihood of misdiagnosis or missed diagnosis. In severe cases, if not properly managed, laryngeal edema can result in asphyxiation or even death. Here, we report a Chinese male patient who experienced recurrent limb swelling and abdominal pain. Laboratory tests revealed low levels of complement C4 and C1 inhibitors, along with impaired C1 inhibitor function. Genomic DNA extracted from peripheral blood samples underwent PCR amplification and Sanger sequencing, which identified a *de novo* heterozygous mutation in the *SERPING1* gene at chr11:57379227, confirming a novel missense mutation NM_000062.c.1067T > A (p.V356E). Ultimately, the patient was diagnosed with HAE-C1INH-Type1 and successfully protected from recurrent attacks through subcutaneous administration of lanadelumab.

KEYWORDS

***SERPING1* gene, hereditary angioedema (HAE), mutation, case report, C1 inhibitor (C1INH)**

1 Introduction

Hereditary angioedema (HAE) is a rare autosomal dominant disorder affecting approximately 1/50,000 people globally (1). It is characterized by unpredictable and painful swelling, typically involving the face, oropharynx, abdomen, extremities, and genitals. Swelling episodes often result in significant functional impairment, reduced quality of life, and potentially fatal outcomes in case of laryngeal attacks (1, 2). HAE is classified into two categories: HAE due to C1-esterase inhibitor deficiency (HAE-C1INH) and HAE with normal C1INH. HAE-C1INH is further divided into types 1 and 2, based on the deficiency or dysfunction of circulating C1INH protein caused by inherited or spontaneous mutations in the *SERPING1* gene. These mutations lead to uncontrolled activation of factor XII and plasma kallikrein, resulting in excessive

bradykinin production and recurrent episodes of subcutaneous or submucosal swelling (3–5). While more than 700 *SERPING1* variants have been identified worldwide (6), only about 60 have been reported in the Chinese population (7). The *SERPING1* gene, the known pathogenic gene for HAE, is located on chromosome 11 (q11_q13.1) and consists of eight exons and seven introns. Previous studies indicate that approximately 75% of HAE patients (types 1 or 2) exhibit an autosomal dominant inheritance pattern, resulting in a 50% chance of passing the disease to their offspring (3). In addition, approximately 20%–25% of patients represent *de novo* cases within a family (8). Research has underscored the importance of parental genetic testing for all patients, regardless of whether the parents are affected, and highlighted the implications of gonosomal mosaicism for genetic counseling (9). In this report, we present a novel *SERPING1* gene mutation that caused type 1 HAE.

2 Case report

The index patient (Figure 1), a 34-year-old man, began experiencing localized edema of the limbs, skin, and buttocks 8 years ago. Each episode varied in severity and, regardless of whether the patient received medical treatment, every swelling episode resolved completely on its own within 2–3 days. Subsequently, these symptoms recurred irregularly. Over the previous 6 months, the episodes became more frequent (occurring more than three times in a month), often accompanied by abdominal pain, with some attacks triggered by fatigue. The index patient had no prior history of surgery and special medication. A family history inquiry revealed that his mother had passed away due to "laryngeal edema," his elder sister experienced buttock swelling after prolonged sitting, and his uncle suffered episodic abdominal pain and unilateral upper-limb swelling, thus, both had similar histories of edema. At presentation, his vital signs were within normal limits. On physical examination, the index patient exhibited non-pitting edema in the right hand (Figure 2). Laboratory tests showed that complete blood count, D-dimer, PCT, CRP, HP, 25(OH)D, immunoglobulin A/G/M, C3, C1q, rheumatoid factors, ANA + ENA, ANCA qualitative, total IgE, and specific IgE (the specific IgE against *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*, *Alternaria alternata*, *Ambrosia artemisiifolia*, *Artemisia sieversiana*, *Humulus scandens*, *Platanus acerifolia*, cat hair, dog hair, protein, milk, peanuts, fish, wheat, and soybeans) were all within normal range. Tests for HBsAg, HBeAb, and HBcAb were positive (the patient denied any past history of hepatitis and refused to undergo quantitative HBV-DNA testing).

Serum C4 levels, C1INH concentrations, and functional assays were assessed for this family (Table 1). The index patient's C4 level was 0.02 g/L (reference range: 0.1–0.4 g/L), C1INH concentration was 0.07 g/L (reference range: 0.21–0.39 g/L), and C1INH functional activity was 4.3% (reference range: ≥68.0%). In addition, his 5-year-old asymptomatic daughter and his uncle both exhibited reduced C4 levels, with their C1INH concentrations and functional activities measuring below 50% of the normal lower limit, consistent with a diagnosis of type 1 HAE. Genomic DNA was extracted from peripheral blood

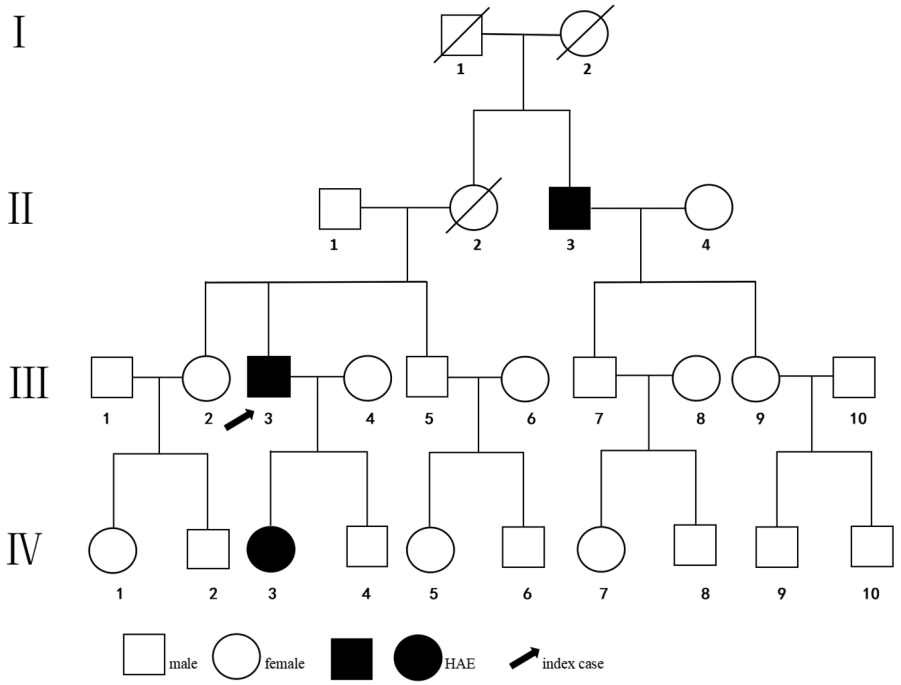

FIGURE 1
Pedigree of the family with HAE. Circles indicate females, squares indicate males, black-filled symbols indicate affected individuals, the arrow indicates the index patient, and a slash indicates a deceased individual.

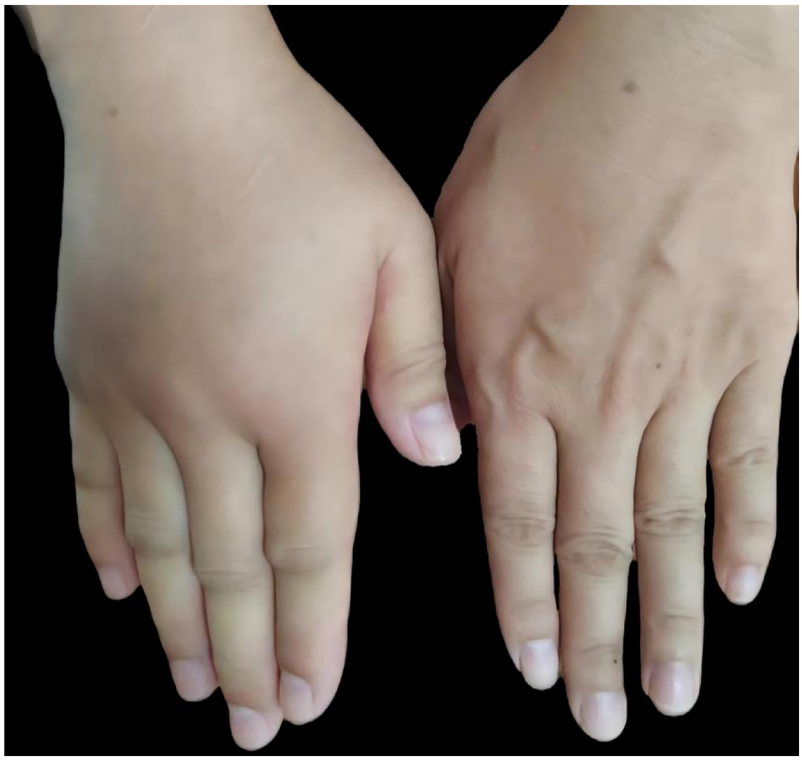

FIGURE 2
Clinical image. Non-pitting edema is visible in the index patient's right hand.

TABLE 1 The C4 and C1INH serum level results.

Subject	Laboratory test (normal ranges)		
	C4 (0.10–0.40 g/L)	C1INH (0.21–0.39 g/L)	C1INH function (≥68.0%)
II1	0.19	0.27	87.8
II3	0.09	0.05	2.5
II4	0.31	0.36	>93
III1	0.29	0.23	91.2
III2	0.18	0.34	79.7
III3	0.02	0.07	4.3
III4	0.23	0.34	93.8
III5	0.33	0.29	82.9
III6	0.38	0.33	>93.0
III7	0.24	0.25	84.9
III8	0.34	0.31	>93.0
III9	0.38	0.33	72.6
III10	0.25	0.28	79.3
IV1	0.24	0.33	>93.0
IV2	0.37	0.38	89.5
IV3	0.08	0.08	21.5
IV4	0.33	0.24	86.7
IV5	0.27	0.35	>93.0
IV6	0.31	0.36	>93.0
IV7	0.33	0.29	71.3
IV8	0.17	0.23	89.5
IV9	0.38	0.20	>93.0
IV10	0.19	0.26	89.4

samples, and the coding region of the *SERPING1* gene (NM_000062) was analyzed using PCR amplification and Sanger sequencing. A *de novo* heterozygous missense mutation, NM_000062: c.1067T > A (p.V356E), was identified in the *SERPING1* gene at chr11:57379227. Sequence analysis confirmed a heterozygous missense variant in exon 7 of the *SERPING1* gene, resulting in an amino acid substitution at position 356 to change from valine to glutamic acid. The same *SERPING1* gene mutation was subsequently identified in the patient's daughter (IV3) and uncle (II3) (Figure 3). Due to the family members' refusal to provide blood samples for genetic testing, we were unable to verify the sequencing of the suspected pathogenic mutations in individuals with normal serum C4 and C1INH concentrations and function.

According to VarSome (https://varsome.com), the *SERPING1* gene mutation c.1067T > A occurs in a functional domain (PM1_Moderate). The REVEL software predicts a score of 0.930 for this mutation, supporting evidence for pathogenicity (PP3_Strong). This variant is not listed in the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), HGMD (http://www.hgmd.cf.ac.uk/), or the Leiden Open Variation Database (LOVD, https://databases.lovd.nl/shared/variants/SERPING1), and no carrier frequency data were available for East Asian populations in the gnomAD_exome (http://gnomad.broadinstitute.org) (PM2_Supporting). Based on the American College of Medical

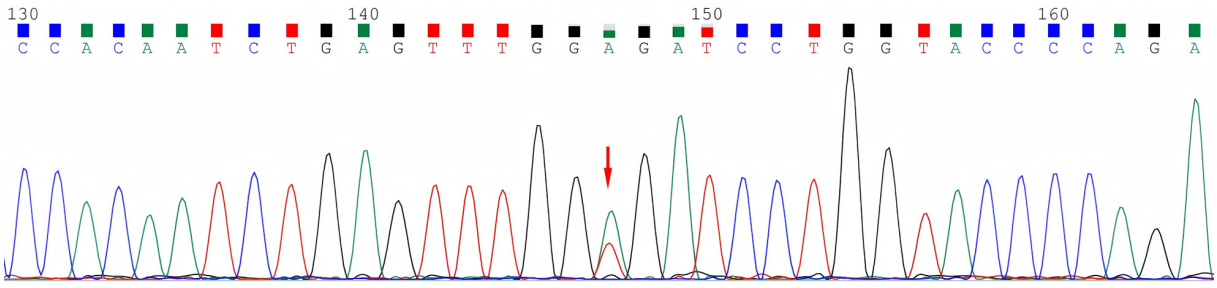

FIGURE 3
Sequencing map. Heterozygous missense mutation of the *SERPING1* gene (NM_000062) c.1067T > A in the patients.

Genetics and Genomics (ACMG) standards and guidelines for variant classification, this variant can be categorized as "likely pathogenic" (PP3_Strong + PM1_Moderate + PM2_Supporting). While we predicted this mutation to be pathogenic using *in silico* modeling tools, supported these predictions by measuring serum C4 and C1INH concentrations and functions within this family, and considered the clinical characteristics of the patients, the lack of additional family genetic sequencing to exclude asymptomatic carriers and perform segregation studies imposes certain limitations on our study.

3 Discussion

We have identified a previously unreported mutation, c.1067T > A, in the *SERPING1* gene, with a REVEL score of 0.930, suggesting that c.1067T > A may impact protein function. This newly discovered mutation is likely the underlying cause of the disease in this family. We recommend genetic testing for HAE patients and their relatives to enable the early identification of mutation carriers, particularly those who are asymptomatic or exhibit atypical symptoms, ensuring timely and targeted prevention and treatment. Such testing can enhance patient quality of life, lower mortality rates, and provide molecular-level diagnostic insights for precise diagnosis and genetic counseling.

In China, the average age of onset for HAE is approximately 21.25 years, with approximately 75% of patients experiencing their first episode between the ages of 10 and 30. However, the interval between onset and definitive diagnosis is approximately 12.64 years (10). Several factors may contribute to the generally later onset of HAE observed in Chinese patients. These include limited disease awareness and diagnostic capabilities, which may inflate the recorded age of onset, and genetic or environmental variations that could influence clinical phenotypes. Further research and epidemiological studies are needed to clarify these underlying factors. HAE presents with a wide range of clinical manifestations, from asymptomatic cases to life-threatening episodes, with the severity, frequency, and affected areas varying significantly, even within the same family. Edema can occur in any part of the body, most commonly affecting the limbs, face, genitals, respiratory tract, and gastrointestinal mucosa. Upper airway edema is particularly concerning, as laryngeal involvement can progress rapidly, leading to respiratory distress or asphyxiation; without prompt treatment, this can be fatal. Gastrointestinal involvement can cause severe abdominal pain, nausea, and vomiting, which are often mistaken for acute abdomen and may result in unnecessary surgical interventions. Although HAE edema episodes typically resolve on their own, 56.86% of Chinese HAE patients experience laryngeal edema, and 11.39% die from asphyxia caused by laryngeal swelling (10, 11). Consequently, early diagnosis and appropriate treatment are essential for improving outcomes and enhancing the overall quality of life for individuals affected by this serious and potentially disabling disease. According to current guidelines (3), the primary goal of HAE management is to normalize patients' lives and achieve total disease control (zero episodes). Treatment approaches are generally categorized into two types: on-demand therapy [icatibant, ecalantide, recombinant human C1 inhibitor (rhC1INH), and plasma-derived C1 inhibitor (pd-C1INH)] and prophylactic therapy (lanadelumab, berotralstat, and pd-C1INH). Unfortunately, ecalantide and berotralstat are not yet approved for marketing in China.

Due to the patient's frequent episodes of edema over the previous 6 months, accompanied by abdominal pain, we initiated long-term prophylactic treatment with subcutaneous lanadelumab (300 mg every 2 weeks) on 20 April 2024. Over nearly 10 months of prophylactic treatment with lanadelumab, the patient has experienced only six mild edema episodes, occurring on 25 May (left hand), 23 June (right foot), 26 August (left foot with pain), 31 October (right shoulder joint), and 3 December 2024 (left hand) and 27 January 2025 (right hand), each resolving spontaneously without requiring on-demand therapy. The most recent episode was suspected to be related to compression of the right hand and fully resolved within 1 day. In addition, the patient's angioedema control test (AECT) scores consistently remained above 11 during this 10-month period, reaching 13 on 10 March 2025. These clinical outcomes indicate effective disease management, as the mild and brief breakthrough episodes did not meaningfully impact the patient's quality of life. In accordance with guideline recommendations (3) and based on the patient's current level of disease control, we are continuing prophylactic treatment at 300 mg every 2 weeks.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by Ethics Committee of Henan Provincial People's Hospital [Approval No. 2022 Ethical New Technology (51)]. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

WD: Data curation, Formal analysis, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. KY: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. QZ: Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. XL: Methodology, Validation, Writing – review & editing, Formal analysis. WZ: Methodology, Validation, Writing – review & editing. WG: Methodology, Validation, Writing – review & editing. ZM: Methodology, Validation, Writing – review & editing. SW: Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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Hereditary angioedema plasma proteomics following specific plasma kallikrein inhibition with lanadelumab

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Introduction: Plasma proteomics analyses were performed to identify novel disease state biomarkers of hereditary angioedema due to C1 inhibitor deficiency (HAE-C1INH) and investigate the biological consequences of specific plasma kallikrein inhibition with lanadelumab.

Methods: Affinity proteomic analyses were performed using plasma from healthy controls (n=30) and patients with HAE-C1INH before (baseline, n=125) and after 6 months of treatment with lanadelumab (300 mg every 2 weeks, n=112) using the SomaScan platform.

Results: Relative plasma levels for several proteins differed significantly between controls and patients with HAE-C1INH, and between matched baseline and post-treatment samples from patients with HAE-C1INH. As expected, C1 inhibitor and complement C4 were significantly lower (P<1.10e-39 false discovery rate [fdr], P<6.6e-25 fdr, respectively) in HAE-C1INH baseline plasma versus controls. Cleaved high-molecular-weight kininogen, a biomarker of excess kallikrein-kinin system (KKS) activation, was higher in HAE-C1INH baseline plasma versus controls (P<6.7e-6 fdr) and was reduced in HAE-C1INH plasma after lanadelumab treatment. Of 1041 identified proteins that differed significantly (P<0.05) from controls and HAE-C1INH baseline plasma, 120 proteins were no longer different between controls and patients with HAE-C1INH after 6 months of lanadelumab treatment. Canonical pathway and local network analyses of HAE-C1INH plasma proteomics suggest dysregulation in KKS, coagulation, cell adhesion, and connective tissue degradation that approach that of healthy controls following treatment with lanadelumab.

Conclusion: Proteomic analyses of plasma from patients with HAE-C1INH before and after treatment with lanadelumab compared with healthy controls confirmed known HAE-C1INH biomarkers and identified additional potential biomarkers of plasma kallikrein dysregulation for further investigation.

KEYWORDS

kallikrein-kinin system, lanadelumab, bradykinin, antibody inhibitor of protease, hereditary angioedema

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Introduction

Hereditary angioedema due to C1INH deficiency (HAE-C1INH) is an autosomal dominant genetic disease mediated by a dysregulated plasma kallikrein-kinin system (KKS), which generates excess bradykinin in the vascular compartment; pathophysiology that causes episodic attacks of angioedema (1). KKS activation occurs upon FXII activation to FXIIa, which converts prekallikrein to active plasma kallikrein (PKa), that activates additional FXII and cleaves high-molecular-weight kininogen (HK) to generate cleaved HK (HKa) and bradykinin (1, 2).

Despite the well described pathophysiological role of the KKS and the multitude of approved treatments for HAE-C1INH, the search for novel biomarkers (3) remains in order to elucidate further biological consequences of excess KKS activation, develop improved therapies, and reduce diagnosis time for patients, which for many patients can take 4–9 years on average (4), if the identified biomarkers can be developed as diagnostic assays.

Lanadelumab (Takhzyro, TAK-743, SHP643, DX-2930) is a specific antibody inhibitor of PKa approved to prevent attacks in patients with HAE (5). We performed plasma proteomics with samples from HAE-C1INH patients before and after treatment with lanadelumab to assess the impact of specific PKa inhibition on the plasma proteome.

Methods

Plasma was collected from patients with HAE-C1INH enrolled in the phase III HELP study for lanadelumab as well as from patients who only enrolled into the open-label extension portion of the HELP study (ClinicalTrials.gov ID: NCT02586805 and NCT02741596, respectively). Plasma was collected from age- and gender-matched healthy controls (n = 30) by BioIVT (Westbury, NY). All participants provided written informed consent for blood samples to be used for the investigation of exploratory biomarkers of contact system activation. To minimize ex vivo activation of the contact pathway system during blood collection, plasma was collected from patients with HAE-C1INH and healthy controls by means of a clean venipuncture with a butterfly needle/catheter kit (BD Biosciences part number 367296, San Jose, CA, USA) and removal of the tourniquet upon blood flow to decrease stasis. The first tube of blood was discarded and blood was collected into polypropylene evacuated tubes containing 3.2% sodium citrate (BD Biosciences). Blood samples were centrifuged within 1 hour, and plasma was aliquoted and stored at –80°C until processing.

Plasma proteomic analyses were performed using a multiplex approach that compares relative levels of >7000 proteins (SomaScan, Somalogic, Denver, CO, USA) (6) on the following 3 groups: 1) healthy control plasma (n = 30); 2) HAE-C1INH plasma before lanadelumab treatment (baseline, n = 125); and 3) HAE-C1INH plasma after 26 weeks of lanadelumab treatment (300 mg Q2W, after treatment, n = 114). Of the 114 HAE-C1INH patients for which baseline plasma was available, matched post-treatment samples were available for 112 patients.

Results

We compared plasma proteomics of HAE-C1INH plasma at baseline (i.e., before treatment with lanadelumab) to that of healthy control plasma in a volcano plot of P-value versus fold change (Figure 1). Out of the proteins detected, 1041 proteins were statistically different between HAE-C1INH and healthy controls (Supplementary Table 1).

Of the 1041 proteins, C4 and C1INH were lower in HAE-C1INH baseline plasma than healthy control plasma (Figure 2), which was expected since low levels of both proteins are used in the diagnosis of HAE-C1INH (7). HAE-C1INH may be associated with C1INH plasma levels that are <50% of a healthy control standard at 1 U/mL (~0.24 g/L) and C4 plasma concentrations below that of healthy controls (8, 9).

We also compared plasma proteomics between healthy controls, baseline HAE-C1INH, and HAE-C1INH after 26 weeks of lanadelumab treatment. Out of the 1041 proteins that were different between healthy controls and HAE-C1INH baseline, 120 proteins were observed to be no longer different from healthy controls after HAE-C1INH patients were treated with lanadelumab for 26 weeks. The list of the 120 proteins is provided in Supplementary Table 1 and includes HKa, thrombin, tissue kallikrein 14, interleukin-21, α-2-macroglobulin (A2M), and apolipoprotein B (Supplementary Figure 1).

The first one of these 120 potential biomarkers to highlight is HKa, a protein previously shown to be elevated in plasma of patients with HAE-C1INH and a pharmacodynamic biomarker of

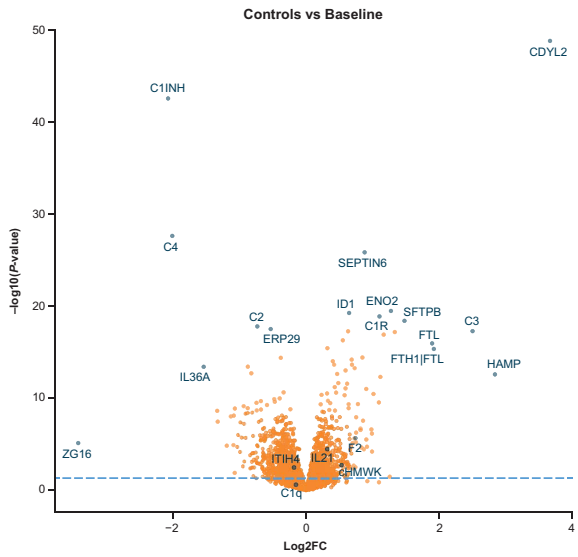

FIGURE 1
Volcano plot comparison of protein levels from SomaScan analyses in plasma from patients with HAE-C1INH that was collected at baseline to levels present in plasma from age- and gender-matched healthy controls. The x-axis is the fold change (log2FC) in signal for each protein in HAE-C1INH plasma as compared to healthy control plasma. The y-axis is the calculated P-value (Log10) for the difference in signal for each protein between HAE-C1INH patients and healthy controls plasma. HAE-C1INH, hereditary angioedema due to C1 inhibitor deficiency.

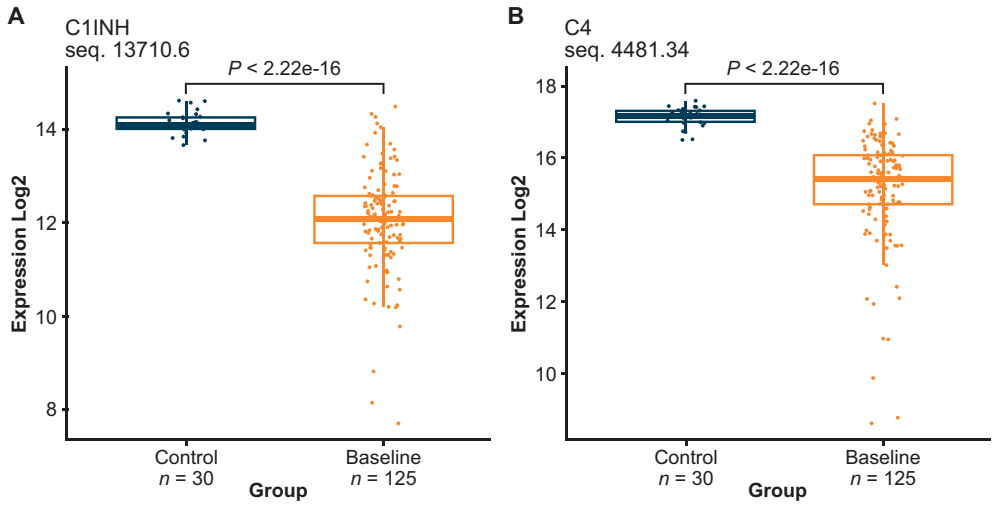

FIGURE 2
C1INH **(A)** and complement 4 (C4) **(B)** plasma levels were both decreased in HAE-C1INH samples collected at baseline as compared to healthy control plasma. HAE-C1INH, hereditary angioedema due to C1 inhibitor deficiency.

lanadelumab bioactivity (10–12). Figure 3 shows the HKa signal measured using SOMAmer sequence 19631-13, which shows that HKa levels were elevated in HAE-C1INH baseline plasma, but were no longer different from healthy control plasma levels after HAE-C1INH patients received lanadelumab for 26 weeks. HKa levels measured using sequence 19631-13 positively correlated to the %HKa measured by Western blot analyses performed during the clinical study with lanadelumab (13). The SomaScan panel contains 3 other SOMAmers against kininogen (15343-337, 7784-1, and 4918-21). The profile for 15343-337 appeared similar to 19631-13, whereas the profile of 7784-1 suggests preferential binding to intact HK, and the profile of 4918-21 suggests that it may bind both HK and HKa (Supplementary Figures 2, 3). Furthermore, the observed reduction in HKa signal using SOMAmers 19631-13 and 15343-337 in paired HAE-C1INH patient samples collected at baseline and after 26 weeks of lanadelumab treatment is consistent with the pharmacodynamic activity previously reported for this PKa inhibitor (Supplementary Figure 4) (14).

We next investigated whether additional potential biomarkers from the 120 proteins discovered previously would show measurable protein differences with commercially available ELISA kits. To test this, additional plasma samples (from different HAE-

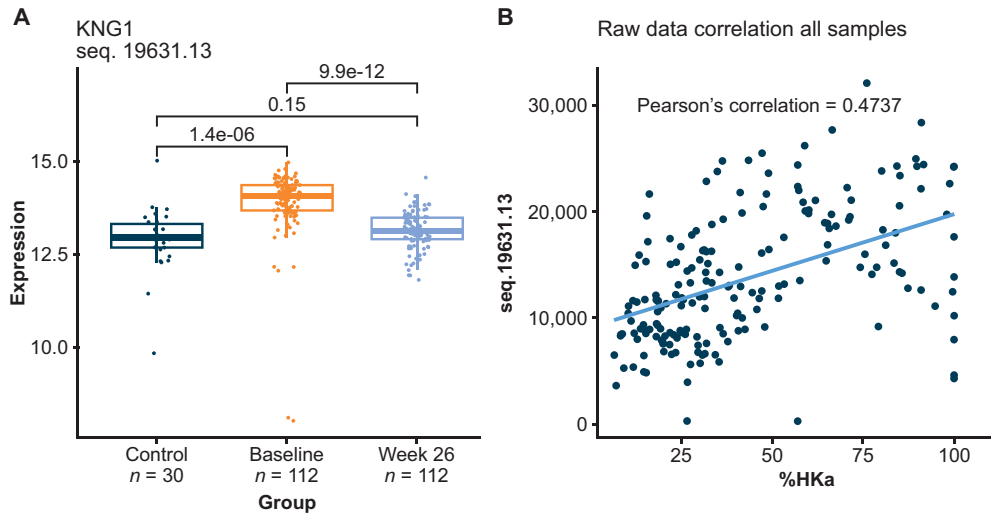

FIGURE 3
HKa relative levels measured using SOMAmer 19631-13 increased in plasma from HAE-C1INH patients prior to receiving lanadelumab (baseline), relative to healthy controls (Control) and HKa decreased following lanadelumab treatment **(A)**. Correlation between HKa levels as measured using SOMAmer 19631-13 and Western blot analyses of HK **(B)**. SOMAmer 19631-13 was reported by the manufacturer to be raised against kininostatin (domain 5 of HK) with binding observed to HKa (although with~10-folder weaker affinity than for kininostatin) and no binding observed with intact HK or intact LK. HAE-C1INH, hereditary angioedema due to C1 inhibitor deficiency; HK, high-molecular-weight kininogen; HKa, cleaved HK; LK, low-molecular-weight kininogen.

C1INH patients from the same clinical study with lanadelumab and different healthy controls) were analyzed using commercially available ELISA kits (RayBiotech, Peachtree Corners, GA) for interleukin-21, A2M, and apolipoprotein B. We observed no differences between healthy controls and HAE-C1INH plasma for these 3 proteins (data not shown). Differences between results obtained using affinity proteomic methods, including SomaScan, and immunoassays have been previously reported and potentially attributed to differences in the epitopes or protein complexes targeted between the different assays (15). Furthermore, targeted immunoassays often disagree with each other, so disagreement with a proteomic platform does not definitively imply which result is superior. Consequently, the lack of concordance between methods does not demonstrate that the results from the affinity proteomic analyses are necessarily incorrect and require further validation (e.g., using assays based on the specific SOMAmers used for each protein).

We next performed pathway enrichment analysis on these 120 altered proteins using Metacore (Clarivate). As shown in Table 1, the KKS is the most significant enriched pathway, which is consistent with the fact that this is a study on patients with HAE-C1INH and the effects of their treatment. Other enriched pathways include blood coagulation, cell adhesion, and connective tissue degradation. These 120 proteins were then analyzed using a known knowledge network approach called Causal-ASsociational NETwork (CASNET), which refers to consistently active subnetworks of known protein-protein interactions (16). Finding active subnetworks in diseases can be a way of generating biological insights, including novel feedback loops, using data with differentially expressed genes or in our case different protein expression levels from proteomic analyses (Supplementary Figure 5). Proteins identified by CASnet analyses out of the list of 120 proteins include proteases (thrombin, tissue kallikreins, cathepsin K, plasminogen) protease inhibitors (inter-alpha trypsin inhibitor heavy chain 4, A2M), apolipoproteins, and complement system proteins (Supplementary Table 2, Supplementary Figure 5).

Discussion

Biomarker discovery in HAE-C1INH remains an active area of research that can provide novel insights into pathophysiology and may identify novel biomarkers to potentially improve diagnosis (3, 17–20). We used an affinity proteomic platform capable of measuring relative amounts of more than 7000 different human proteins to compare the plasma proteome of patients with HAE-C1INH to that of healthy controls. In addition, we investigated the effect of PKa inhibition using a highly specific antibody inhibitor (lanadelumab) on the plasma proteome of patients with HAE-C1INH.

SomaScan proteomics includes the known diagnostic biomarkers of HAE-C1INH, C1INH and C4, both of which were lower than healthy controls as expected. In addition, SomaScan proteomics measures HKa, a previously identified disease state and pharmacodynamic biomarker of KKS activation (10–12). C1INH, C4, and HKa levels in HAE-C1INH plasma measured using SomaScan proteomics indicate that this technology could be considered for investigations of HAE-C1INH plasma biomarkers and possibly as a pharmacodynamic assay for the investigation of novel therapies targeting the KKS.

By comparing the HAE-C1INH plasma proteome before and after 26 weeks of treatment with lanadelumab to that of healthy controls, we were able to investigate the effect of specific PKa inhibition on the 1041 proteins with levels that differed between HAE-C1INH plasma baseline and healthy controls. These 1041 proteins are listed in the Supplementary Table 1 and may find use in further studies into the biological consequences of excess KKS activation in HAE-C1INH and other diseases potentially mediated by the KKS, including comorbidities associated with HAE-C1INH (21–23). From this analysis, we identified 120 out of the 1041 proteins that were no longer different from that of healthy controls after 26 weeks of lanadelumab treatment.

A2M, one of these 120 proteins, is a broad-spectrum covalent protein inhibitor of proteases, including PKa, which has multiple functions, including binding and regulating pro-inflammatory cytokines and hormones (24). A2M has been investigated as a biomarker for a number of different diseases, including diabetes mellitus (25, 26). We observed that A2M was elevated in HAE-C1INH plasma relative to that of healthy controls and returned to approximate healthy control levels after 26 weeks of treatment with lanadelumab (Supplementary Figure 1). Further studies could elucidate whether the SOMAmer against A2M also binds the A2M-PKa covalent complex, which has been shown to be elevated during an attack in plasma from patients with HAE-C1INH (27).

Complement protein C3 is another one of 120 altered proteins that has been implicated in the pathophysiology of many diseases

TABLE 1 Pathway analysis using the 120 proteins.

Metabase process network pathway[13]	r	R	n	N	Z-score	*P*-value	q-value
Kallikrein-kinin system	13	68	187	7113	8.538585	0	3e-06
Blood coagulation	5	68	93	7113	4.409437	0.00186	0.138223
Cell adhesion: Amyloid proteins	7	68	197	7113	3.799111	0.00260	0.138223
Cell adhesion: Platelet-endothelium-leukocyte interactions	6	68	174	7113	3.420398	0.00613	0.243986
Proteolysis: Connective tissue degradation	4	68	118	7113	2.73963	0.02597	0.707345

Where r: intersection of proteomic experiment with ontology term in Metabase; R: size of user's experiment; n: size of map/process; N: size of "background list" - total number of network objects in ontology; Z-score: enrichment Z-score (the more Z-score, the more significant enrichment); *P*-value: enrichment *P*-value from hypergeometric test; q-value: false-discovery rate–adjusted *P*-value from hypergeometric test.

including diabetic macular edema (28). C3 is a central protein component of the complement system (29). The observation here of increased C3 cleavage fragments (C3b, C3a, and C3d) in baseline HAE-C1INH plasma as compared with that of healthy controls, followed by a reduction upon lanadelumab treatment is consistent with previous reports of C3 activation by PKa (Supplementary Figure 6) (30).

Apolipoprotein B, a protein involved in cholesterol deposition in the arterial wall and a marker of cardiovascular risk (31), was among the 120 altered proteins that were elevated in HAE-C1INH baseline plasma and reduced after 26 weeks of treatment with lanadelumab to levels comparable with that of healthy controls. It was previously reported that apolipoprotein B is cleaved by PKa (32). Consequently, it could be useful in future studies to determine whether the SOMAmer against apolipoprotein B exhibits differential binding to intact protein compared with the PKa-cleaved protein. Further investigation into a role for PKa in cholesterol metabolism is supported by the recent observation that prekallikrein binds the low-density lipoprotein receptor and could be a therapeutic target to decrease cholesterol (33).

Another protein among the 120 altered proteins worthy of further investigation is interleukin-21, which is a pleiotropic cytokine involved in T helper 17 cell expansion that was previously identified as being elevated in patients with HAE (34). Inflammation and elevated cytokine levels have been suggested as potentially contributing to attack onset triggers (35).

The pathways identified from comparing pre-dose HAE-C1INH plasma proteomics to that of healthy controls are supported by previous observations. For example, biomarkers of blood coagulation and fibrinolysis have previously been shown to be associated with HAE-C1INH, especially upon attack onset (36). Even though patients with HAE-C1INH may not have an increased risk of thrombosis (37), recent preclinical and clinical studies indicate that high plasma levels of C1 INH reduce the risk of venous thromboembolism (38). Pathway analyses using this proteomic data also identified differences in cell adhesion pathways based on plasma proteomics in HAE-C1INH that appeared to approach levels in healthy controls after treatment with lanadelumab. Elevated levels of adhesion proteins such as VE-cadherin have also been previously shown in plasma from HAE-C1INH patients (39, 40).

In summary, we used plasma proteomic analyses to identify potential HAE disease state biomarkers for further examination with additional patient samples and orthogonal methods for confirmation. Combining proteomic analyses with matched samples from patients before and after treatment with a highly specific antibody therapeutic can help identify active subnetworks in the disease that are mediated by the target.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Western Institutional Review Board (WIRB), Puyallup, WA 98374-2115, USA; IRB Services, Aurora, Ontario, L4G 0A5, Canada; Program for the Protection of Human Subjects, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; Penn State Milton S. Hershey Medical, Center/Penn State College of Medicine, Human Subjects Protection Office/Institutional Review Board, Hershey, PA 17033, USA; Human Research Protection Office, Washington University School of Medicine, St. Louis, MO 63110, USA; Human Subjects Committee, University of Kansas Medical Center, City, KS 66160, USA; Cleveland Clinic Foundation, Cleveland, OH 44195, USA; University of Michigan Medical School Institutional Review Board (IRBMED), Ann Arbor, MI 48109-2800, USA; Duke University Health System Institutional Review Board, Durham, NC 27705, USA; Children's Hospital Institutional Review Board, Wauwatosa, WI 53226, USA; Health Research Authority, Manchester, M1 3DZ, UK; Ethik-Kommission des Landes Berlin, 10707 Berlin, Germany; Ethik-Kommission der Landesärztekammer Rheinland-Pfalz, 55116 Mainz, Germany; Comitato Etico Interaziendale Milano Area A, Segreteria Centrale, c/o Ospedale L. Sacco COMITATO ETICO, 20157 Milano, Italy; Ethikkommission der Landesärztekammer Hessen, 60488 Frankfurt am Main, Germany; Ethik-Kommission des Fachbereichs Medizin der Johann Wolfgang Goethe-Universität, 60590 Frankfurt am Main, Germany; University of Alberta Research Ethics, 308 Campus Tower, Edmonton, AB T6G 1K8, Canada; Triumpharma Institutional Review Board, Amman 11941, Jordan; BioIVT, Westbury, NY, USA. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DS: Writing – original draft, Writing – review & editing. AK: Writing – review & editing, Writing – original draft. SJ: Writing – review & editing, Writing – original draft. DY: Writing – review & editing, Writing – original draft. AM: Writing – review & editing, Writing – original draft. EA: Writing – review & editing, Writing – original draft. BL: Writing – review & editing, Writing – original draft.

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Conflict of interest

AK and EA are employees of Clarivate PLC. DS is an employee of Sexton Bio Consulting, LLC, former employee of Takeda Development Center Americas, Inc., and holds stock and/or share options in Takeda Pharmaceutical Company Limited. AM is a former employee of Takeda Development Center Americas, Inc., and holds stock and/or share options in Takeda Pharmaceutical Company Limited. SJ is an employee of Takeda Pharmaceuticals USA Inc., and holds stock and/or share options in Takeda Pharmaceutical Company Limited. DY and BL are employees of Takeda Development Center Americas, Inc., and hold stock and/or share options in Takeda Pharmaceutical Company Limited.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1471168/full#supplementary-material

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Case Report: Two cases of hereditary angioedema in a Chinese family

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Background: Hereditary angioedema (HAE) is a life-threatening condition characterized by repeated asymmetric cutaneous and mucosal edema. It is a rare autosomal dominant genetic disease with a mortality rate of 8.6%. Family survey of HAE in China is seldom reported since it is still under recognized.
Case report: We reported two cases of HAE and a family survey conducted in Hebei Province, China. The proband was a woman who had edema for over 7 years. She was diagnosed with type I HAE in her 50s after a life-threatening asphyxia attack. Her elder brother was initially diagnosed with mild symptoms.
Conclusion: Two diagnosed and three suspected patients were identified in our family survey. Family surveys are important method for identifying asymptomatic patients and preventing attacks. It is valuable for rescuing people from sudden death, particularly from asphyxia.

KEYWORDS

family survey, C1-inhibitor, case report, hereditary angioedema (HAE), bradykinin

Introduction

Hereditary angioedema (HAE) is a rare, autosomal dominant, genetic disease. According to a worldwide epidemiological investigation, it has a morbidity rate of 0.13–1.6 in 100,000 (1). In China, the prevalence of HAE in patients with decreased complement 4 levels has been reported to be 2.43 cases per 10,000 (2). One of nine studies that reported the lifespan of patients with undiagnosed HAE type 1/2 who died of asphyxiation was shorter than that of patients with undiagnosed HAE type 1/2 who died of other causes (40.8 years vs. 72.0 years) (3). Therefore, early recognition and diagnosis are important for families with HAE to identify asymptomatic patients before the condition aggravates. Family surveys are the most commonly used method as recommended by the World Allergy Organization (4). Notably, a family survey that has been conducted in China since 1980 found over 400 patients from 120 families with HAE, which implies significant underdiagnosis nationally. The median age of onset in Chinese patients was in the teens (50.5%) and twenties (31.8%) (5). Herein, we report two cases of HAE and a family survey conducted in Hebei Province, China. The proband had a first attack at 54 years of age, which was later than.typical for HAE. All participants provided written informed consent for blood testing and the publication of their clinical information and test results. This study was approved by the Institutional Review Board of the Tianjin Union Medical Center.

Case report

A 61-year-old woman (proband, ZHY) initially experienced angioedema during her 54th year of life. The patient had no significant history of illness. She experienced angioedema approximately six times per year, with each episode resolving spontaneously within five days. The sites of edema included the hands, feet, cheeks, lips, eyelids, neck, and legs. The most common sites were the hands and feet. The attacks were consistently triggered by physical factors (pressure, minor injury, heavy lifting, or prolonged sitting) and were not accompanied by itching or pain. Hand ultrasonography did not indicate any disease at the time of edema. Her father and older brother had similar symptoms of edema. She had experienced neck edema and asphyxia once in her 55th year of life. Dexamethasone was administered in the emergency department; however, the symptoms remained unresolved. The patient achieved a complete remission after danazol treatment. Due to her repeated edema and positive family history, emergency physicians prescribed laboratory tests for diagnosis. The investigations showed extremely low levels of C1-INH and C4 (Table 1). Quality of Life (QOL) score was seven points out of 36 (Table 2). Hereditary angioedema of type I was diagnosed at this time based on markedly reduced C1-INH level and function (confirmed twice) with low C4 according to its guidelines (4). The differential diagnosis was acquired angioedema with C1 inhibitor deficiency (AAE-C1-INH) due to the relatively late onset of symptoms. AAE-C1-INH showed symptoms similar to those of HAE, characterized by a later-onset age of over 40 years, negative family history, and lower C1q levels. A positive family history and normal C1q level (Table 1) further confirmed the diagnosis. In the years following the diagnosis, angioedema recurred and was relieved without any preventive treatment. One case of life-threatening edema was treated with a 30 mg icatibant acetate injection in her 61st year and was relieved within two hours. The timeline of the key events was shown in Figure 1. The patient underwent continuous follow-up.

Family survey

Family investigations were initiated immediately after the patient visited the outpatient department for treatment. Ten family members from three generations were investigated, including their relationships with the proband, medical history, detailed angioedema history, and treatment. C1-INH, fC1-INH (a functional C1 inhibitor), and C4 were tested using two different methods to avoid errors. QOL was designed related to HAE Three generations of information from parents, grandparents, and great-grandparents were provided according to the memory of the proband. Only one member (NO.1) had edema symptoms, whereas the others were asymptomatic. The family tree was shown in Figure 2.

Four individuals from three generations exhibited angioedema symptoms, excluding the proband. Two of them [the father and the niece (brother's daughter) of the proband] died of asphyxia without an HAE diagnosis. A second cousin also had angioedema symptoms; however, the details were unclear because of a lack of close connection. The proband's elder brother (ZYM) had extremely low C1-INH, fC1-INH, and C4 levels similar to those of his sister (Table 1). The patient initially experienced angioedema in the 22nd year, which resolved spontaneously. Symptoms occurred twice a year and were relieved within five days. The patient had never experienced any life-threatening angioedema. The most common sites were the hands, feet, upper limbs, and genitals. The main causative factor was heavy weight lifting without any accompanying symptoms. He did not recognize it as a disease; therefore, he never visited the hospital for diagnosis or treatment. A score of zero indicated no QOL

TABLE 1 Experimental results of two diagnosed patients. Dried blood spot sample was tested using MS. Serum sample was tested using NMA, IMA, and ELISA.

Name	Item	Value			
		MS	NMA	IMA	ELISA
ZHY (No.2)	fC1-INH	<7.0 (Ref: ≥ 58.9%)			0.0 (Ref: ≥ 68%)
	C1-INH	19.79 (Ref:81.46–291.29μg/ml)	0.03 (Ref:0.21–0.39 g/L)		
	C4	24.92 (Ref:72.85–372.95μg/ml)		0.01 (Ref: 0.1–0.4 g/L)	
	C1q			7.3 （Ref: 5.0–8.6 mg/dl）	
ZYM (No.1)	fC1-INH	<7.0 (Ref: ≥ 58.9%)			0.0 (Ref: ≥ 68%)
	C1-INH	24.20 (Ref:81.46–291.29 μg/ml)	0.04 (Ref:0.21–0.39 g/L)		
	C4	44.72 (Ref:72.85–372.95 μg/ml)		0.06 (Ref: 0.1–0.4 g/L)	

MS, mass spectrometry; NMA, nephelometric assay; IMA, immunoturbidimetry assay; ELISA, enzyme-linked immunosorbent assay; Ref, reference range; C1-INH, C1 esterase inhibitor; fC1-INH, C1 esterase inhibitor function.

TABLE 2 QOL scale used to evaluate the degree of influence that hereditary angioedema has on a patient's daily life. A total of 12 questions were asked. A score of up to 36 points was calculated based on the score of each answer (0-never, 1-light, 2-moderate and 3-severe).

Questions	0, Never	1, Light	2, Moderate	3, Severe
Does your skin feel uncomfortable?				
Do you feel sad, embarrassed, or depressed because of your skin problems?				
Do your skin problems prevent you from shopping or housework?				
Do you choose special clothes or shoes because of your skin problems?				
Do your skin problems prevent you from social communication, outings, or entertainment?				
Do your skin problems prevent you from sports?				
Do your skin problems prevent you from work or study?				
Do your skin problems have any influence on the relationship between you and your spouse, friends, or relatives?				
Do your skin problems bring inconvenience in your daily life?				
Do your skin problems prevent you from sleep?				
Do your skin problems occupy a lot of your time or attention?				
Do your skin problems become an economic burden?				

QOL, quality of life, HAE, hereditary angioedema.

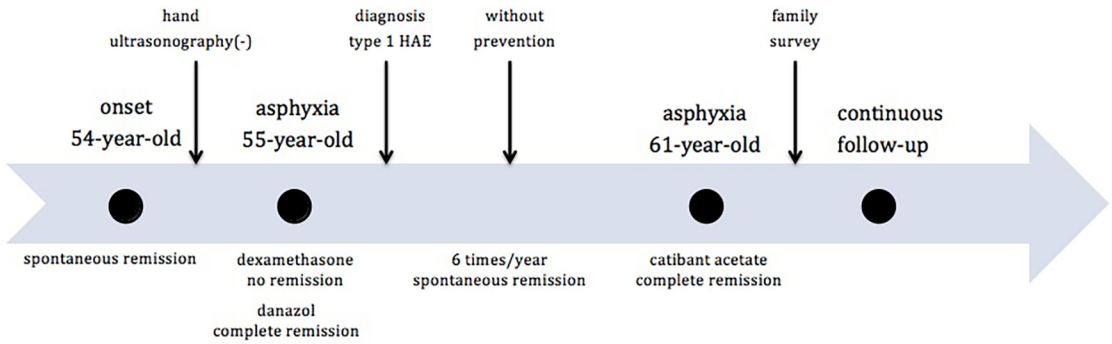

FIGURE 1
Timeline of proband's episodes.

impact. All other living tested relatives were found to have normal C1-INH, fC1-INH and C4 levels.

Discussion

HAE is a life-threatening disease characterized by repeated asymmetric cutaneous and mucosal edema. It also causes extreme abdominal pain, accompanied by nausea and vomiting. The estimated risk of death due to asphyxiation is 8.6% (1). Patients initially visit the dermatology department, allergy department, or emergency room. HAE is often unrecognized and misdiagnosed in clinical settings because of low awareness. Depression, anxiety, stress, and alexithymia are the most common emotional symptoms.

HAE is grouped into three types based on the C1-INH levels. Patients with type I subtype have low levels of C4, C1-INH, and fC1-INH. Those with type II subtype have low levels of C4 and fC1-INH but normal levels of C1-INH. Patients with HAE-nC1-INH subtype have normal levels of C4, C1-INH, and fC1-INH. Types I and II are caused by a functional C1 inhibitor deficiency, which leads to an increase in bradykinin-mediated vascular permeability (6). Mutations in SERPING1 may be the mechanism underlying these two types. The transmission pattern of the SERPING1 variant favors the transmission of wild-type alleles in males, especially when the father is a carrier (7). This presents a significant challenge in the diagnosis of HAE-nC1-INH. Family history, plasminogen (8) levels, factor XII (9), kininogen-1, myoferlin, heparan sulfate-glucosamine 3-sulfotransferase 6, and angiopoietin-1 (10) gene missense variants may help in the diagnosis. The World Allergy Organization (WAO)/European Academy of Allergy and Clinical Immunology (EAACI) guidelines for HAE recommend testing fC1-INH, C1-INH, and C4 levels twice for diagnosis, and also testing for known mutations of HAE-nC1-INH (4).

Early on-demand treatment has also been considered. Patients should be well-educated in HAE and trained in self-treatment. The first-line treatment is an intravenous plasma-derived C1 inhibitor (11)(Berinert, Cinryze, or Ruconest), a kallikrein inhibitor (12) (ecallantide), or a bradykinin B2 receptor antagonist (13) (icatibant). When these therapies are not available, SDP (solvent detergent-treated plasma) or FFP (fresh frozen plasma) is used as

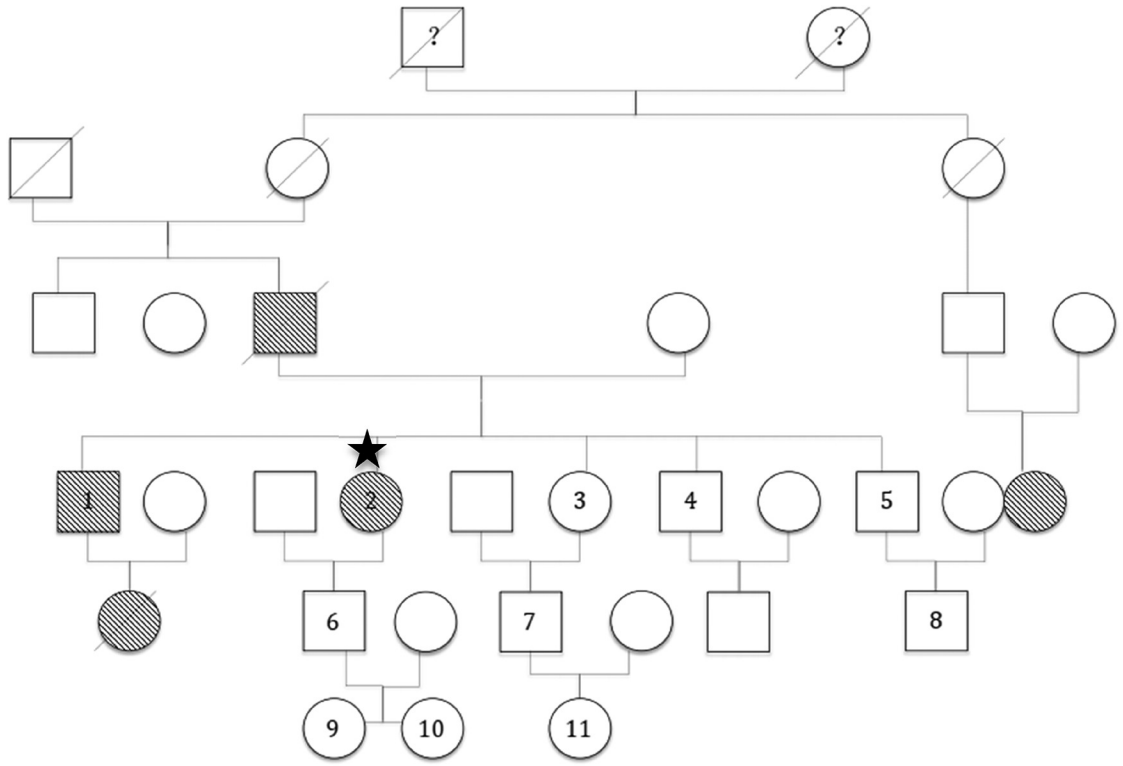

FIGURE 2
Family tree. □: male ○: female: ★ proband. 1–11: Subjects No.1 to No.11 in the proband's family. 1: ZYM. 2: ZHY. ?: It is unknown whether the patient had angioedema symptoms ▨ or ⊘: male or female, respective, who had angioedema symptoms. ▨ or ⊘: male or female, respectively, who died of suspected hereditary angioedema. ⊡ or ⊘: male or female, respectively, who died of another disease.

an alternative treatment (14). If the edema progresses in the airways, intubation or surgical airway intervention should be considered. Prophylaxis is an important component in disease management. Short-term prophylactic treatments, such as intravenous pdC1-INH (plasma-derived C1 inhibitor) or FFP, should be administered before medical, surgical, or dental procedures (15). Long-term prophylactic treatment is used to improve patients' quality of life by completely controlling HAE. The recommended medicines are pdC1-IN (16), lanadelumab (17) (anti-active plasma kallikrein monoclonal antibody), berotralstat (18) (plasma kallikrein inhibitor), androgens (19), and antifibrinolytics (20). The CRISPR/Cas9 gene editing technology may be an effective method for further treatment (21).

In our patient, the proband was not initially on prophylaxis after diagnosis according to the guideline recommendation. At that time (in 2017), prevention and treatment resources of HAE were extremely insufficient in China. Available treatments were limited to freshly frozen plasma or danazol. First-line therapies were not always available in all hospitals and are not known by all emergency physicians. Short-term prophylaxis was initiated five days before triggering with danazol or tranexamic acid. Long-term prophylaxia was rarely applied because of side effects. With the continuous development of new drugs, she was injected with icatibant acetate for a life-threatening attack that occurred at 61 years of age, which provided complete relief. She was intended to receive lanadelumab for a long-term prophylaxia. Notably, a recent survey identified the experiences of long-term lanadelumab prophylaxis in China (22), which led to a 97.8% reduction in the attack rate. Because her brother had mild symptoms and no significant life burden, he refused any preventive methods. We advised him to avoid relevant triggers and emphasized on emergency hospitals. They all prepared one acute attack medication at home.

Family surveys of HAE in China have seldom been reported because it is still under recognized. The most important finding in our case was the late-onset disease in the proband. Although most patients with HAE show symptoms in young adulthood, occasional cases of later manifestations have been reported. The family's story highlights that the absence of symptoms in youth does not preclude HAE, especially if a parent is affected.

Conclusion

Herein, we reported a case of family screening for HAE in China. Family screening is important to identify patients with mild of no symptoms. It is valuable on saving lives by identifying hidden cases. In this case study, screening of the family of the initial patient revealed a second patient diagnosed with type I HAE and three suspected patients. Experts who focus on rare

diseases may present the clinical manifestations and experimental information disclosed in this report in Chinese.

Limitations

Unfortunately, two of the suspected patients died; therefore, we could not perform any diagnostic tests. Further investigations could be carried out if the proband had a close familial connection with a third suspected patient with angioedema symptoms. We attempted to persuade the two diagnosed patients to undergo further gene detection for HAE; however, they refused. If genetic data were available, it would provide a better understanding of the mutations-identity in the suspected patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Institutional Review Board in Tianjin Union Medical Center. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YG: Data curation, Investigation, Project administration, Writing – original draft. MQ: Conceptualization, Funding acquisition, Investigation, Project administration, Writing – review & editing. JD: Data curation, Investigation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Case reports of subcutaneous pdC1INH in pregnancy and lactation: expanding treatment options for hereditary angioedema in Portugal

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Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent episodes of subcutaneous and/or submucosal angioedema. Pregnancy and breastfeeding may be associated with an increased frequency of attacks. Plasma-derived C1 inhibitor (pdC1INH) is the recommended first-line treatment for long-term prophylaxis (LTP) in these special populations. The pdC1INH currently available in Portugal is one intravenous (IV) formulation not approved for LTP, as are the other IV and subcutaneous (SC) formulations. This report documents the first cases of SC pdC1INH use during pregnancy and breastfeeding in Portugal. It describes two cases of 37-year-old women with HAE type 1 treated with SC pdC1INH as LTP during pregnancy and lactation. Both patients had been previously treated with tranexamic acid. In the first case, the patient was started on IV pdC1INH at 8 weeks' gestation due to clinical deterioration. Due to difficult IV access and inability to space out administrations, SC pdC1INH at a dose of 4,000 U (~43.5 U/kg) every 72 h was started at 21 weeks' gestation. Administration intervals were progressively increased to 96 and later 120 h. LTP was continued throughout lactation. In the second case, LTP was not administered during pregnancy. However, after delivery, the patient experienced a worsening of angioedema episodes during breastfeeding, which persisted despite tranexamic acid treatment. SC pdC1INH was started six months postpartum at a dose of 2,000 U (~45 U/kg) twice weekly. The administration interval was later increased to 120 h. Both patients remained free of angioedema episodes and reported no systemic adverse events. The safety of SC pdC1INH was consistent with reports in the literature. Overall, these positive results support the future use of SC pdC1INH in a broader population of pregnant and lactating women in clinical practice.

KEYWORDS

hereditary angioedema, lactation, long-term prophylaxis, plasma-derived C1 inhibitor, pregnancy

Introduction

Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent episodes of localized cutaneous and submucosal angioedema (1). The most commonly affected areas include the skin (especially the extremities, face, and genitals) and the gastrointestinal tract. Other sites may be affected, and laryngeal attacks are of particular

concern due to their potential to cause fatal airway obstruction (1, 2). The recurrent, unpredictable and potentially life-threatening nature of HAE attacks has a significant impact on patients' quality of life (3), resulting in physical, emotional, and psychological distress.

HAE can be classified into HAE due to C1 inhibitor (C1INH) deficiency (HAE-C1INH) and HAE with normal C1 inhibitor (HAE-nC1INH). Patients with HAE-C1INH may have low plasma levels of C1INH protein (HAE type 1) or normal to elevated plasma levels of dysfunctional C1INH protein (HAE type 2) (4).

Regarding treatment, the primary goals in HAE are to achieve complete disease control and enable patients to lead normal, unrestricted lives (1, 5). Often, this can only be achieved with the use of long-term prophylaxis (LTP). In Portugal, the following drugs are commercially available for LTP: lanadelumab and berotralstat as first-line therapies; danazol and antifibrinolytics (e.g., tranexamic acid), which are classified as second-line therapies according to international guidelines; and an intravenous (IV) plasma-derived C1INH (pdC1INH), which is only approved for on-demand treatment and short-term prophylaxis (STP) (1, 6).

Pregnancy may increase, decrease, or have no effect on the frequency and severity of HAE attacks (1). However, an increase in disease activity during pregnancy appears to be the most common scenario (7, 8). Notably, the course of HAE in a previous pregnancy does not reliably predict how the disease will evolve in subsequent pregnancies (7). The course of the disease during gestation is also unpredictable, with some studies reporting a worsening of angioedema attacks during the first trimester, and others reporting a higher frequency of attacks in the last trimesters (9–11).

During eutocic delivery, the risk of experiencing an angioedema attack is low, and STP is not routinely recommended (12). However, STP is recommended in cases of dystocic delivery, such as cesarean sections or instrumented vaginal deliveries (7).

Breastfeeding may be associated with an increased frequency of angioedema attacks in women with HAE, possibly related to elevated serum prolactin levels (10).

During pregnancy and lactation, therapeutic options for the management of HAE are limited. Attenuated androgens are absolutely contraindicated during pregnancy as they may lead to virilization of the female fetus (13). In addition, danazol should also be avoided during lactation, as it can potentially decrease milk production (14). The safety of lanadelumab and berotralstat during pregnancy and lactation has not been established (1, 15). Antifibrinolytics may be used during pregnancy despite lack of proven efficacy and potential increase in thrombotic risk (1, 7). Tranexamic acid appears to be safe during lactation (16). PdC1INH has been used during pregnancy and lactation with proven safety and efficacy and is the recommended LTP in these special populations (1).

In Portugal, only IV pdC1INH is currently available for on-demand prophylaxis and STP. Subcutaneous (SC) pdC1INH has not been approved for commercialization by INFARMED and can only be used by special authorization.

The aim of this report was to describe the first cases of women with HAE treated with SC pdC1INH for LTP during pregnancy and lactation in Portugal.

Case presentation

This report describes the cases of two women with HAE followed at the Department of Allergy and Clinical Immunology of two Portuguese centers, who were treated with SC pdC1INH during pregnancy and lactation. The first patient started treatment in March 2024 during pregnancy, and the second patient started treatment in June 2024 during lactation.

Case 1

Patient information, clinical findings, and diagnostic assessment

The first case is a 37-year-old woman with HAE type 1 (Table 1). Her diagnosis was established at the age of 20 by C4 and C1INH measurement and later confirmed by genetic testing, which identified the p.Ala275Thr variant in exon 5 of the SERPING1 gene.

At the age of 19, shortly after starting an estrogen-containing contraceptive pill, the woman experienced her first angioedema attack, which affected the abdomen and feet. During her first pregnancy at the age of 32, she experienced a moderate worsening of the disease, especially during the first trimester, with weekly abdominal HAE attacks. She was treated with on-demand IV pdC1INH, but declined the proposed option of LTP with the same drug.

At the age of 36, she was on LTP with tranexamic acid (1,000–1,500 mg/day) with only partial HAE control. She experienced angioedema attacks approximately every 2 months, primarily peripheral and typically induced by stress. This treatment was discontinued when she decided to become pregnant. After the first few weeks of pregnancy, there was a marked increase in HAE attacks, which significantly affected the patient's quality of life. In the 8th week of pregnancy, a joint decision was made to start IV pdC1INH at a dose of 1,500 U (~16 U/kg) twice a week.

At 15 weeks' gestation, as the patient remained attack-free but had difficult IV access, the frequency of IV pdC1INH administration was reduced to once a week. However, due to the recurrence of HAE attacks, a special authorization for the use of SC pdC1INH was requested from INFARMED.

Therapeutic intervention and outcome

After INFARMED's approval, the patient started LTP with SC pdC1INH at a dose of 4,000 U (~43.5 U/kg) every 72 h at 21 weeks' gestation. After three hospital administrations and patient and family education, the treatment was successfully transitioned to home administration.

TABLE 1 Summary of treatment timeline and outcomes.

Cases	Age (in years)	SC pdC1INH initiation date	SC pdC1INH initiation dose	SC pdC1INH dose adjustments	Adverse events	Outcomes
Case 1	37	21st week of gestation	43.5 U/kg every 72 h	Dosing intervals increased to 96 h at the 23rd week of gestation and to 120 h at the 29th week of gestation.	Mild pain at the injection site	No recurrence of angioedema attacks following treatment initiation
Case 2	37	6 months postpartum	45 U/kg twice weekly	Dosing intervals increased to 120 h at 8 months postpartum	Mild pain at the injection site	No recurrence of angioedema attacks following treatment initiation

At 24 weeks' gestation, the administration interval was increased to 96 h, and at 29 weeks' gestation, it was further increased to every 120 h (i.e., every five days). The patient remained attack free throughout this period and reported no side effects other than mild pain at the injection site.

A healthy baby boy was delivered at 38 weeks gestation. STP with IV pdC1INH 1,500 U was administered prior to cesarean section and the delivery was uneventful. After delivery and throughout lactation, LTP was continued with SC pdC1INH 4,000 U (~43.5 U/kg) every five days. The patient remained free of angioedema attacks and with good disease control, as evidenced by an Angioedema Control Test (AECT) score of 15, an Angioedema Quality of Life Questionnaire (AE-QoL) score of 20, and an Angioedema Activity Score (AAS) of zero at five weeks postpartum, all collected in paper forms during medical appointments, as part of the routine clinical assessment.

Case 2

Patient information, clinical findings, and diagnostic assessment

The second case is another 37-year-old woman with HAE type 1 (Table 1). She experienced her first episode of angioedema at the age of 15 following a dental procedure, with swelling predominantly in the limbs and abdomen. The diagnosis was established at the age of 32 through the detection of C1-INH protein deficiency. Genetic testing further identified the SERPING1 c.1480C > T mutation. The patient was started on LTP with tranexamic acid, which resulted in a reduction in the frequency and severity of angioedema attacks.

During her first pregnancy at the age of 32, the disease remained under control without the need for LTP and delivery was uneventful. However, during breastfeeding, the frequency of angioedema attacks increased and LTP with tranexamic acid was initiated with a good clinical response.

During her second pregnancy, at the age of 36, the disease was managed exclusively with on-demand treatment. In the second trimester, the patient experienced an increase in the frequency of episodes, with abdominal angioedema occurring once or twice a week. These episodes resolved with medical leave and rest. Delivery occurred at 38 weeks' gestation under STP with IV pdC1INH and was uneventful.

During breastfeeding, the patient experienced an increase in the frequency of abdominal and peripheral angioedema attacks, prompting the initiation of LTP with tranexamic acid at a dose of 2,000 mg daily. Despite prophylaxis, she continued to experience weekly angioedema episodes lasting an average of two days each, with a significant impact on her quality of life. Special authorization for the use of SC pdC1-INH was requested and granted.

Therapeutic intervention and outcome

At six months postpartum, treatment with SC pdC1INH was initiated at a dose of 2,000 U (~45 U/kg) administered twice weekly. After five in-hospital administrations and training, the patient transitioned to home administration. The only adverse event reported was tolerable pain in the injection site.

Following the introduction of SC pdC1INH LTP, the patient remained free of angioedema attacks. Two months later, based on the good clinical response as evidenced by an AECT score of 16, an AE-QoL score of zero, and an AAS of zero, combined with a reduction in breastfeeding frequency, the SC pdC1INH administration interval was increased to every 120 h (i.e., every five days).

Currently, both women continue to breastfeed, with treatment duration adjusted according to clinical progression.

Discussion

The efficacy and safety of SC pdC1INH as LTP at the doses of 40 and 60 U/kg administered twice weekly was previously demonstrated in the COMPACT (Clinical Study for Optimal Management of Preventing Angioedema with Low-Volume Subcutaneous C1-Inhibitor Replacement Therapy) open-label extension study (17). In this study, four women were exposed to SC pdC1INH during the first pregnancy trimester without complications, and all delivered healthy babies (17). Additionally, a few published case reports have further confirmed the effectiveness and safety of SC pdC1INH as HAE prophylaxis during pregnancy and breastfeeding (18, 19).

In this report, the first patient experienced an increase in angioedema attacks during the first pregnancy trimester. Prophylaxis with IV pdC1INH successfully achieved complete disease control. However, the need for twice-weekly hospital visits and the difficulty in IV access hampered treatment adherence. It should be noted that several IV pdC1INH preparations are available, but Berinert© is not approved for LTP

(6) and Cinryze©, although approved for LTP, is not available in Portugal (20).

The second patient experienced an increase in the frequency of angioedema attacks during the second pregnancy trimester, but the symptoms were controlled with rest. However, during breastfeeding, she experienced weekly angioedema attacks before the introduction of SC pdC1INH.

The administered doses, 43.5 UI/kg in the first case and 45 UI/KG in the second, did not exactly match the recommended dosage of 60 UI/kg due to limitations related to the fixed-dose format of the syringes used for subcutaneous administration (1).

In both cases, stopping breastfeeding itself may have reduced the number of angioedema episodes in the postpartum period (7). Nevertheless, breastfeeding is widely recommended due to its significant benefits for the newborn (1).

Since the initiation of SC pdC1INH, both patients have remained completely asymptomatic, consistent with findings from a similar case report (18). The complete clinical response allowed the administration interval to be extended to every five days while maintaining effective symptom control. Apart from mild injection site pain, no relevant side effects were reported.

Although SC pdC1INH has been shown to be effective and safe during pregnancy and lactation, its use in Portugal is limited by the requirement to obtain it from abroad through a special authorization, resulting in delays in access to this life-changing treatment.

To the authors' knowledge, these are the first two cases of SC pdC1INH use in Portugal. The authors emphasize the importance of individualized treatment and highlight the potential of SC pdC1INH as a therapeutic option for other pregnant and lactating women in the country.

Patient perspective

Case 1: "When I started using subcutaneous Berinert®, the changes in my life were significant: I gained more independence (no need to go to the hospital for intravenous medication) and more security (my disease is under control with effective medication and no side effects). I feel that my life has returned to normal, which has had a very positive impact on both my physical and mental well-being."

Case 2: "Berinert® has brought me a great improvement in my quality of life. Today, I feel more confident, more active, and freer."

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ALP: Investigation, Writing – original draft, Writing – review & editing. NS: Conceptualization, Writing – review & editing. EDdC: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

NS has received advisory board and/or speaker fees from Abbvie, Novartis, CSL Behring, Leo Pharma and Sanofi and congress support from Medinfar, Biocryst, Bial, Roxall and Mylan. EDdC has received advisory board and/or lecture fees from Takeda, Kalvista, CSL Behring and Biocryst and congress support from Takeda and CSL Behring. ALP has received congress support from Biocryst, Takeda and CSL Behring.

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One-year real-life outcomes of lanadelumab therapy in Romanian patients with hereditary angioedema due to C1-inhibitor deficiency

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Introduction: In the majority of patients with hereditary angioedema (HAE) due to C1-inhibitor deficiency (HAE-C1INH), effective long-term prophylactic (LTP) treatment can achieve complete disease control. Lanadelumab is one of the first-line option recommended for this purpose. Our study aimed to evaluate changes in disease control, quality of life, and attack frequency among Romanian HAE-C1INH patients, during the first year of treatment with lanadelumab.

Methods: This non-interventional prospective study included the Romanian HAE-C1INH patients enrolled in the first year of the national lanadelumab treatment program. Angioedema Control Test, (AECT), Angioedema Quality of Life Questionnaire (AE-QoL) and attacks frequency were recorded at baseline and at 3, 6, 9 and 12 months.

Results: Twenty-four patients (14 women [58.3%], 10 men [41.7%]) initiated lanadelumab therapy, with a mean age of 40.7 years. Most had HAE-C1INH type I (22 patients, 91.7%), and one patient was under 18 years of age. Ten patients switched from LTP with intravenous plasma-derived C1-INH, while 14 were previously managed with on-demand therapy only. Baseline scores were: AECT 4.5 [interquartile range (IQR) 2.0], AE-QoL 66.1 [standard deviation (SD) 18.3], and a mean attack frequency of 10.0 (IQR 4.0) (over the preceding three months). Improvements were observed at each follow-up point, with respective scores at 3, 6, 9 and 12 months as follows: three months: AECT 12.0 (IQR 5.8) / AE-QoL 35.3 (SD 23.2)/ attacks 3.4 (IQR 5.0); six months: AECT 12.3 (IQR 5.3) / AE-QoL 35.4 (SD 25.4)/ attacks 2.8 (IQR 4.8); nine months: AECT 12.6 (IQR 5.8) / AE-QoL 34.1 (SD 23.2)/ attacks 2.2 (IQR 3.8) and 12 months: AECT 12.9 (IQR 5.5) / AE-QoL 32.1 (SD 21.6) / attacks 1.4 (IQR 2.0). Seven patients became symptom-free after the first dose, and four more achieved this status within the first three months.

Discussion: LTP with lanadelumab provided effective disease control and significantly improved quality of life in patients with HAE-C1INH over the course of one year. Regular evaluations at relatively short intervals by the availability and ease of administration of validated questionnaires serve as a useful tool for clinicians in the comprehensive assessment of HAE patients and offer a valuable means of monitoring treatment effectiveness.

KEYWORDS

hereditary angioedema, C1-inhibitor, treatment, outcome, lanadelumab

Introduction

Hereditary angioedema (HAE) is a rare, genetic disorder, attributed in most cases to the *SERPING 1* gene mutations, resulting in functional (HAE-C1INH type1) or quantitative (HAE-C1INH type2) deficiency of the C1 esterase inhibitor (C1INH) protein (1, 2).

The disease follows an autosomal dominant inheritance pattern, with *de novo* mutations accounting for approximately 25% of cases (2, 3).

The prevalence of the disease is estimated at approximately 1 in 50,000 individuals, with no significant differences observed across sex or ethnic groups (2, 4).

Clinical symptoms consist in recurrent painful swellings of the subcutaneous and/or submucosal tissues, mediated by the vasoactive peptide, bradykinin (5). The swelling can affect any body part, with the skin, gastrointestinal tract, and upper airways being the most commonly involved sites (2, 4).

If untreated, the attacks last 2–5 days (2, 3, 6, 7) and in case of upper airway edema it can be life-threatening due to risk of asphyxiation (2, 8).

Similar clinical pictures are found in the HAE forms with normal C1-INH level (HAE-nC1INH), which result from distinct genetic mutations. These includes variants in factor XII *(HAE-F12)* (9), plasminogen (HAE-*PLG*), angiopoietin (HAE-*ANGPT1*), kininogen (HAE-*KNG1*), myoferlin (HAE-*MYOF)* (2, 10–15) (HAE-HS3ST6) (16), (HAE-CPN) (17), or (HAE-DAB2IPE) (18) genes. Nevertheless, a substantial number of HAE-nC1INH cases remain genetically unexplained (HAE-UNK) (19).

Although certain trigger factors have been identified, HAE attacks are often unpredictable and can be intensely painful, significantly affecting patients' daily functioning, including work and school productivity (1, 2, 20–27).

In a survey conducted among Romanian HAE-C1INH patients, the mean number of missed work or school days was 9.3 over the 12-month evaluation period (28). As such, treatment primarily aims to enhance the patient's quality of life and prevent the occurrence of attacks, ultimately seeking to achieve complete disease control (19). In most cases, this goal can be attained through the regular administration of specific medications, referred to as long-term prophylaxis (LTP).

At present, three medications are approved as first-line therapy for LTP: the plasma derived C1INH, the kallikrein inhibitor monoclonal antibody—lanadelumab and the small molecule plasma kallikrein inhibitor berotralstat. Attenuated androgens are considered second-line treatment drugs in HAE -LTP and the antifibrinolytics, such as tranexamic acid, are no longer recommended (19).

In accordance with EAACI/WAO guideline recommendations, the initiation of LTP should be guided by an assessment of disease activity, the impact on the patient's quality of life, the availability of healthcare resources, insufficient response to on-demand therapy, and the patient's informed preferences (19).

These parameters can be assessed using patient-reported outcomes (PROs), such as the Angioedema Control Test (AECT), the Angioedema Quality of Life Questionnaire (AE-QoL), and the attack diary. These tools are also valuable for evaluating the safety and efficacy of chronic treatments. In this manner, a personalized and comprehensive treatment plan can be developed for each patient with HAE.

As an angioedema attack can appear during the LTP, the on-demand therapy (pdC1INH, rhC1INH icatibant and ecallantide) should be available (19, 29), or, if these are not accessible, fresh frozen plasma can be administered (19, 29).

Our study aimed to assess the changes of quality of life, disease control and attacks frequency in the Romanian HAE-C1INH patients during the first year of treatment with the kallikrein inhibitor monoclonal antibody—lanadelumab, based on the above-mentioned PRO's.

Material and methods

This was a non-interventional study of the HAE-C1INH patients from Romania included in the first year of lanadelumab treatment program, conducted by the Romanian HAE Center of Reference and Excellence. Patients were announced about the availability of this drug via an on-line meeting when the inclusion criteria were also presented: age of ≥12 years, confirmed diagnosis of HAE-C1INH type1 or 2, and for the last 3 months period: 1. the frequency and severity of the attacks (≥2 attacks/last 3 months based on patient diary),and/or 2. an inadequate control of the disease on used treatment (AECT score <10), and/or 3. the impact of the disease on quality of life (based on AE-QoL questionnaire, score ≥39, moderate or severe effect), and/or 4. an important burden of the used treatment and 5. the patients preferences.

Those who did not attend this meeting were informed at the annual visit (face-to-face visit or phone-call visit).

At pre-treatment visit (T0) the mentioned questionnaires were completed electronically, after a phone-call discussion or during the annual visit at the Center. Patients included in the treatment program were evaluated using the same tools, at every three months (T1, T2, T3 and T4).

Participation in the treatment program was independent of the patients' consent to participate in this survey.

Both the AECT and the AE-QoL are available in Romanian. While not specific to HAE, these tools have been validated and are frequently used in the context of this disorder, both in clinical trials and routine clinical practice, due to their brevity and ease of scoring.

AECT is a four-item patient-related tool used in subjects with recurrent angioedema. The first question refers to the attack's frequency and the second one to their consequences on the patient's daily life. The unpredictability of the attacks is evaluated by the third question and, the last one assesses the used treatment efficiency (30, 31). Two versions for AECT are accessible: for a recall period of four weeks and three months respectively. In our study the last one was used.

AE-QoL contains 17 items grouped in four domains: functioning, fatigue/mood, fears/shame, and nutrition. In this case the questions are referred to the previous four weeks (2, 32).

In both cases answers are predetermined using a five-point verbal rating scale, and for all responses the score ranges from 0 to 4 points (33).

In the case of AECT the total score is 16 points, which reflects a complete control of the disease, the main goal of the treatment in HAE. A result of ≥10 points show a well-controlled disease and if it is <10 points, indicates an inadequately controlled status. The grades used for this questionnaire were the following: very often = 0 points, often = 1 point, sometimes = 2 points, seldom = 3 points, and not at all = 4 points Increasing the score by three points or more at two consecutive visits, can be considered as an important improvement for the patient (34).

The AE-QoL score is obtained by converting the sum of points for every question to a linear 0–100 scale with higher values pointing to a higher QoL impairment. Intervals of total scores of 0–23, 24–38, and 39 or more describe patients with "no effect," "small effect," and "moderate to large effect" of swelling episodes on their quality of life, respectively (14). In the case of this questionnaire, the minimal clinically important difference was established to be six points (35).

For this questionnaire the following grades were used: never = 0 point, seldom = 1 point, sometimes = 2 points, often = 3 points and very often = 4 points.

The four individual domain scores were computed by summing the points assigned to the specific questions to each dimension, as follows: functioning—questions 1, 2, 3 and 4, fatigue/mood questions 6, 7, 8, 9 and 10, fears/shame questions 12, 13, 14, 15, 16 and 17 and nutrition questions 5 and 11.

Attacks frequency and severity were established using the attacks diary. For every swelling episode the same data were recorded: date of the attack, location, severity (mild/moderate or severe depending on the capability to perform daily activities) and the used treatment. Patient utilized either the paper-and-pen diary or the electronic one.

Information regarding socio-demographic data (age, sex), HAE type, and used treatment, were collected from the Romanian HAE Registry.

The study was conducted in compliance with the requirements set forth in the Declaration of Helsinki and was approved in December 2022 by the IRB of the George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania (Decision no. UMFST-nr 1914/02.11.2022 -REG-74-F03-Ed.02).

Data analysis

Descriptive statistics were calculated to summarize patient demographics and therapeutic management of HAE.

The normality of numerical variables was checked using the Kolmogorov–Smirnov test of normality.

Mean AECT and AE-QoL scores were calculated at three months intervals, and the Friedman test with pairwise post-test comparison were applied to compare values at each successive interval. Comparison between mean scores in men vs. women, and between patients on LTP vs. OD treatment were performed using Mann–Whitney or independent samples *t* test.

Two-sided *p*-values were calculated, and the threshold for statistical significance was set at 0.05. Statistical analyses were performed using the Statistical Package for the Social Sciences Software (version 22.0, SPSS, Chicago, IL).

Results

Study population

At the time of the study, 123 patients aged 12 years and older with HAE-C1INH were registered in the Romanian HAE Registry. Of these, 30 patients participated in an online informational meeting, and 93 received details about the lanadelumab treatment program during their annual clinical visit. A total of 24 patients, 14 women (58.3%) and 10 men (41.7%) were subsequently enrolled in the first year of the lanadelumab treatment program.

Numerical details about the study population, eligible patients, and final study sample are represented in Figure 1.

Socio-demographic data

The mean age was 40.70 years (range 15–66 years), including one adolescent patient and one patient >65 years old. Twenty-two (91.66%) patients had HAE-C1INH type 1 and two (8.34%) HAE-C1INH type 2.

Ten patients used long term prophylaxis therapy with the intravenous form of pdC1INH administered twice per week in dose of 1,000 UI, and 14 only on-demand treatment with icatibant or pdC1-INH.

Co-morbidities were present in eight patients (33.34%). Cardiovascular disorders were most common, observed in four patients (including hypertension, ischemic heart disease, and dyslipidemia). Autoimmune conditions were identified in two patients (thyroiditis and psoriasis), while single cases of gastrointestinal disease (chronic gastritis), allergy (grass pollen rhinitis), and endocrine disorder (polycystic ovary syndrome) were also reported.

The sociodemographic data (sex, age, HAE type), specific treatment used at T0 evaluation and comorbidities are presented in Table 1.

Lanadelumab treatment

Lanadelumab was administered in a dose of 300 mg every 2 weeks. In case of 6 months symptom-free interval the dose was reduced to 300 mg at 4 weeks. This was the case in seven patients (29.16%). In five (20.83%) cases, due to patients' preferences (fear to have an attack), the interval of administration was increased progressively with four days, up to 20/21 days (based on German experience, 36). Seven patients (29.16%) continued having attacks and they remained at the dose of 300 mg every two weeks. One patient increased the interval of

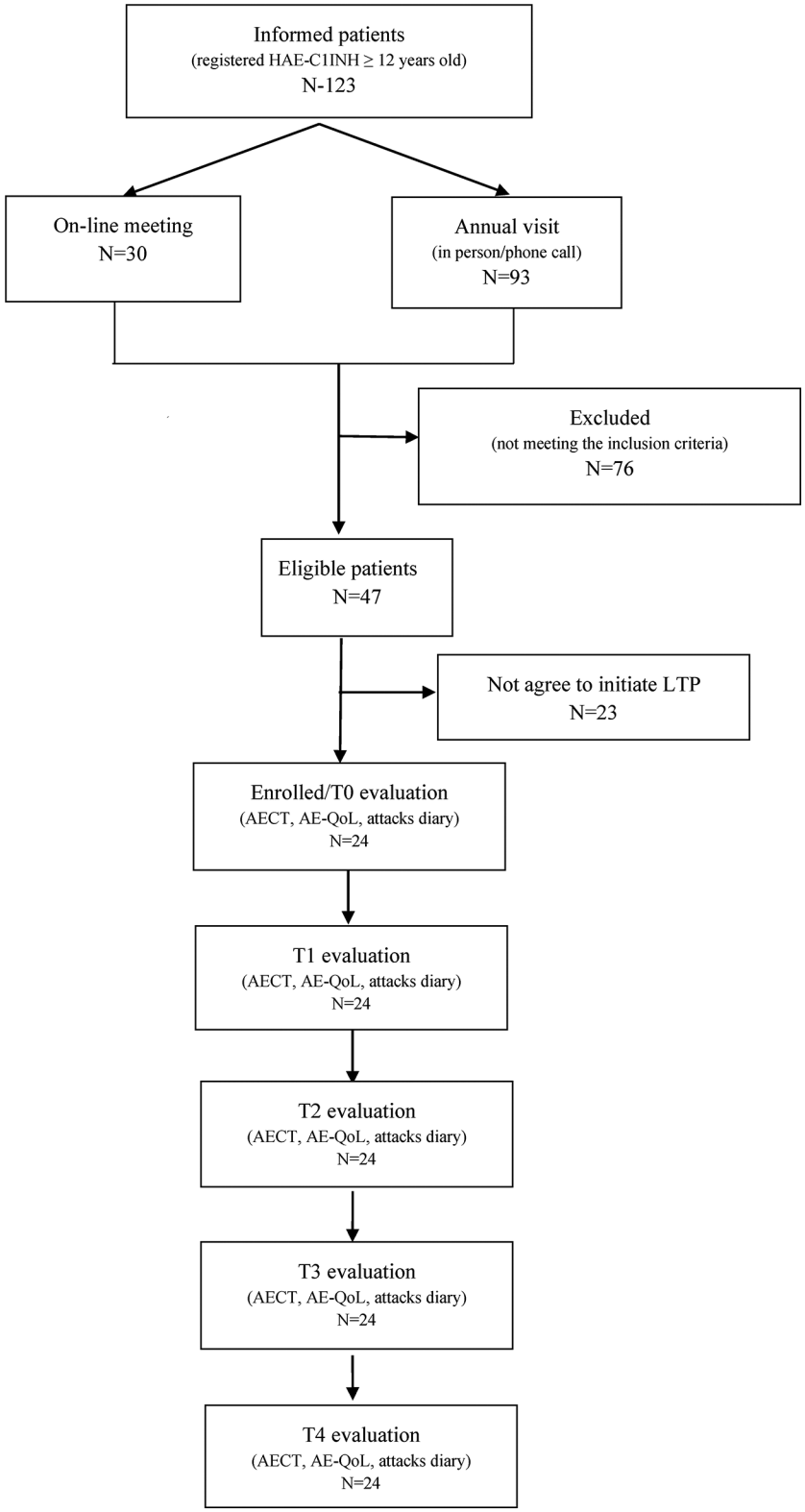

FIGURE 1
Study flow chart.

TABLE 1 Socio-demographic data (sex, age, HAE type), specific treatment and co-morbidities at T0 evaluation.

Patient characteristics	Value
Sex	
Female	14 (58.3%)
Men	10 (41.7%)
Age (mean)	40.7
15–18	1 (4.2%)
19–45	14 (58.3%)
46–64	8 (33.3%)
≥65	1 (4.2%)
HAE type	
1	22 (91.66%)
2	2 (8.34%)
LTP	
Yes	10 (41.66)
No	14 (58.33)
Co-morbidities	
Yes	8 (33.34%)
No	16 (66.67%)

LTP, long-term prophylaxis.

administration to six weeks, and the symptom free status was maintained at the 12 month and 24-month evaluation too.

In all cases, the first two doses of lanadelumab were administered by medical personnel, and then by self-administration as home-treatment. Adverse effects were evaluated following the initial two doses and subsequently at each follow-up visit (T1, T2, T3, and T4).

In patients receiving LTP with pdC1INH, the switch to lanadelumab was made as soon as possible after the last dose of pdC1-INH.

Attacks frequency

The mean attack frequency was 10.0 [interquartile range (IQR) 4.0] at T0 visit, and decreased to 3,4 (IQR: 5.0) at T1assessment ($p < 0.0001$). Swelling episodes continued to reduce, being 2.8 (IQR: 4.8) at T2 ($p = 0.104$), 2.2 (IQR: 3.8) at T3 ($p = 0.0273$) and 1.4 (IQR: 2.0) at T4 evaluation ($p = 0.0049$) (Figure 2).

Patient-related outcome measures

AECT score

At the pre-treatment evaluation, the mean AECT score was 4.5 (IQR: 2.0). With the exception of one patient, who had a controlled disease at baseline (AECT score = 10) and was already on LTP, all patients had scores below 10. In this particular case, the patient was switched to lanadelumab due to the burden associated with frequent intravenous injections.

Following treatment initiation, the AECT scores showed a progressive improvement: the mean score at T1 was 12.0 (IQR: 5.8), at T2 it was 12.3 (IQR: 5.3), at T3 it increased to 12.6 (IQR: 5.8), and at T4 reached 12.9 (IQR: 5.5) (Figure 3).

AE-QoL assessment

The mean AE-QoL total score at baseline, prior to lanadelumab initiation, was 66.1 [standard deviation (SD) 18.7], indicating a substantial impairment in quality of life. At the T1 assessment, this score significantly decreased to a mean of 35.3 (SD: 23.7). The improvement was sustained over time, with mean scores of

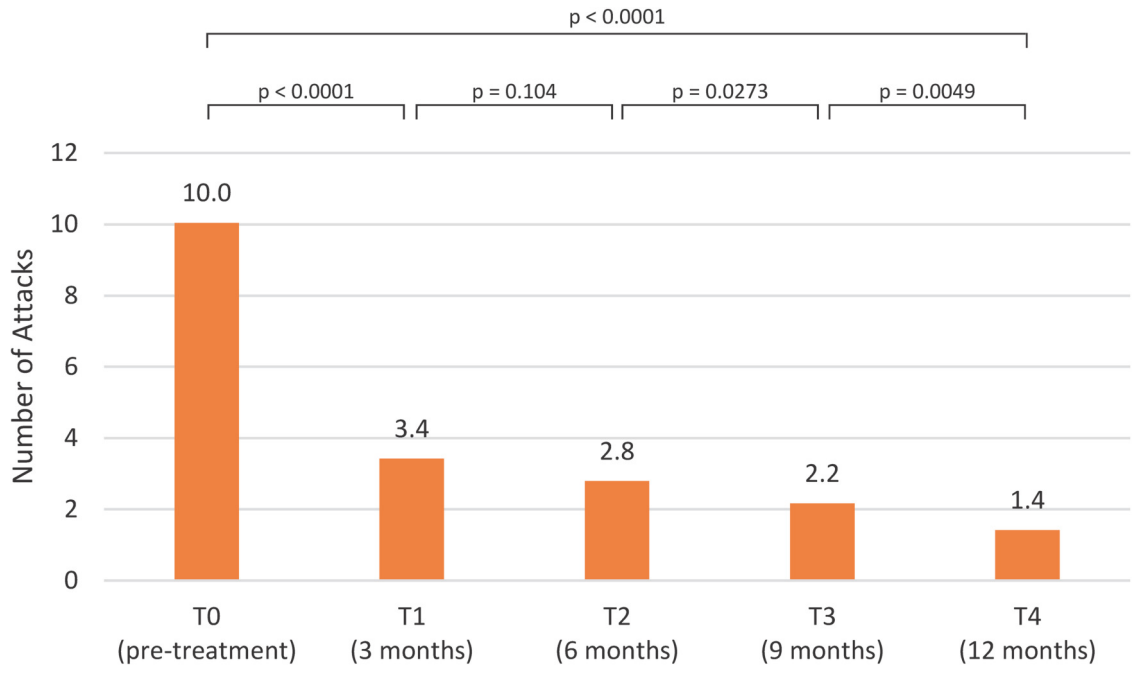

FIGURE 2
Attacks frequency pre-treatment and post-treatment visits.

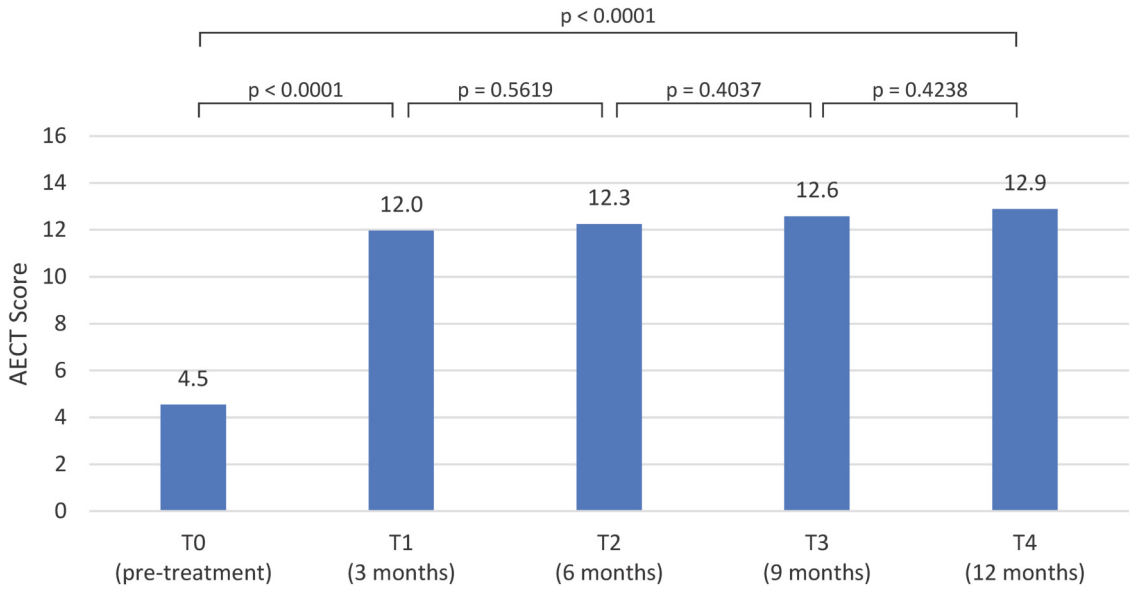

FIGURE 3
Mean AECT scores pre-treatment and post-treatment visits.

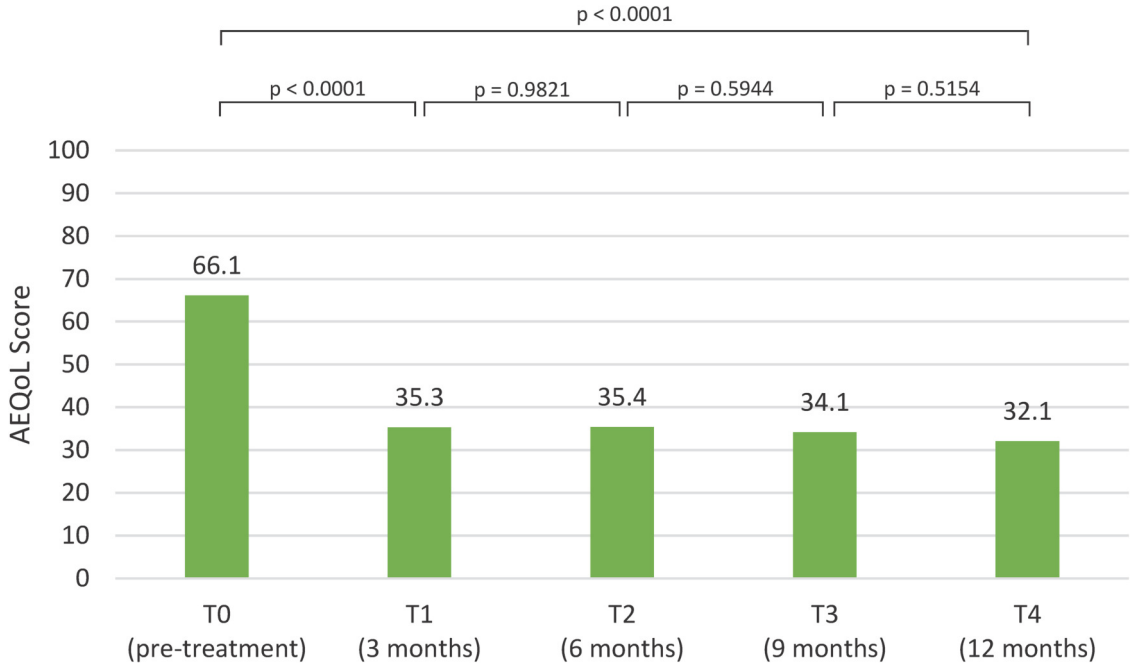

FIGURE 4
AE-QoL scores pre-treatment and post-treatment visits.

35.4 (SD: 25.9) at T2, 34.1 (SD 33.0) at T3, and 32.1 (SD: 22) at the T4 evaluation (Figure 4).

Regarding the AE-QoL domain scores, comparison of rank sum differences between baseline (T0) and the first follow-up (T1) demonstrated a statistically significant improvement ($p < 0.0001$) in the total score: 56, as well as across all domains: functioning 49.3, fatigue/mood 48.5, fears/shame 47.5, and nutrition 40. At later time points, slight deterioration was observed: in the total score (−5.5) and the fatigue/mood score (−2.0) between T1 and T2, and in the functioning score (−1.15) from T3 to T4. The nutrition domain showed a more pronounced decrease from T1 to T2 (−12.5). The fear/shame domain showed an improvement trend at all follow-up evaluations.

A summary of the AE-QoL total and domain-specific scores across all time points is presented in Table 2.

When comparing quality-of-life impairment between women and men, the following results were observed: at the T0 evaluation, the mean total AE-QoL score was 73.79 in women vs. 55.40 in men. Domain-specific mean scores at T0 were: functioning—76.57/58.20, fatigue/mood—67.86/47.50, fears/shame—83.07/62.60, and nutrition—57.43/65.20 (women/men). By the T4 visit, these scores had improved, with the mean total score decreasing to 34.29 in women and 29.00 in men. Corresponding domain scores at T4 were: functioning—19.29/23.20, fatigue/mood—43.57/26.00, fears/shame—42.50/37.60, and nutrition—24.71/35.20 (women/men). A full summary of these results, including data from T2 and T3 assessments, is provided in Table 3.

Comparison of AECT, AE-QoL and attacks frequency regarding previously used treatment

At the T0 evaluation, patients previously managed with on-demand treatment showed mean values of 3.7 (AECT), 68.9 (AE-QoL), and 11.1 (attack frequency), compared to 5.9, 61.6, and 8.2 respectively, in patients already receiving long-term prophylaxis (LTP). At the T1 visit, these values improved to 12.1/11.7 (AECT), 30.8/38.7 (AE-QoL), and 3.1/3.9 (attack frequency) in the on-demand and LTP groups, respectively. By the T4 assessment, values further improved to 13.2/12.3 (AECT), 28.9/37.4 (AE-QoL), and 1.4/1.4 (attack frequency). A detailed summary of outcomes across all time points, including T2 and T3 evaluations, is provided in Table 4.

TABLE 2 AE-QoL total and domain scores.

AE-QoL score	Friedman test	Pairwise comparison	Rank sum difference	*P*-value
Total	Fr = 53.769 $p < 0.0001$	T0–T1	56	<0.0001
		T1–T2	−5.5	>0.05
		T2–T3	12	>0.05
		T3–T4	3.5	>0.05
Functioning domain	Fr = 59.074 $p < 0.0001$	T0–T1	49.33	<0.001
		T1–T2	2.54	>0.05
		T2–T3	0.13	>0.05
		T3–T4	−1.15	>0.05
Fatigue/Mood domain	Fr = 39.012 $p < 0.0001$	T0–T1	48.5	<0.001
		T1–T2	−2	>0.05
		T2–T3	1	>0.05
		T3–T4	7.5	>0.05
Fears/Shame domain	Fr = 42.922 $p < 0.0001$	T0–T1	47.5	<0.001
		T1–T2	4	>0.05
		T2–T3	3	>0.05
		T3–T4	4.5	>0.05
Nutrition domain	Fr = 25.947 $p < 0.0001$	T0–T1	40	<0.001
		T1–T2	−12.5	>0.05
		T2–T3	10.5	>0.05
		T3–T4	4	>0.05

TABLE 3 AE-QoL comparison by sex (F-female, M-male).

AE-QoL	T0	*p*-Value	T1	*p*-Value	T2	*p*-Value	T3	*p*-Value	T4	*p*-Value
Total										
F	73.79	0.0303	41.93	0.1138	41.64	0.1688	38.21	0.4464	34.29	0.558
M	55.40		26.10		26.60		28.40		29.00	
Functioning domain										
F	76.57	0.0375	27.07	0.0936	23.21	0.6377	23.64	0.7461	19.29	0.7017
M	58.20		10.70		14.60		12.60		23.20	
Fatigue/mood domain										
F	67.86	0.1875	44.43	0.1349	48.43	0.0531	44.71	0.4121	43.57	0.2186
M	47.50		28.50		22.50		31.50		26.00	
Fears/shame domain										
F	83.07	0.0059	53.43	0.2412	51.14	0.2533	49.07	0.3955	42.50	0.8147
M	62.60		39.50		36.10		37.60		37.60	
Nutrition domain										
F	57.43	0.5178	33.29	0.6381	37.71	0.6382	33.21	0.9298	24.71	0.5571
M	65.20		25.20		32.60		31.40		35.20	

TABLE 4 Comparison of AECT, AE-QoL and attacks frequency regarding treatment.

Time of measurement	Treatment	AECT	*p* value	AE-QoL	*p* value	No. of Attacks	*p* value
T0 (pre-treatment)	LTP	5.9	0.039	61.6	0.3665	8.2	0.488
	OD	3.7		68.9		11.1	
T1 (3 months)	LTP	11.7	0.5706	38.7	0.3775	3.9	0.1429
	OD	12.1		30.8		3.1	
T2 (6 months)	LTP	12.4	0.881	38.6	0.6518	2.9	0.4155
	OD	12.1		33.5		2.7	
T3 (9 months)	LTP	13.1	0.8814	32.4	0.7943	2.4	0.7172
	OD	12.3		35.1		2.1	
T4 (12 months)	LTP	12.3	0.21	37.4	0.3671	1.4	0.4851
	OD	13.2		28.9		1.4	

LTP, long term preventive therapy; OD, on demand treatment; AECT, angioedema control test; AE-QoL, angioedema quality of life.

Discussions

The present study describes real-life clinical data regarding the benefits of the LTP with lanadelumab in Romanian HAE-C1INH patients. Being a relative new drug, with limited data on its long-term effectiveness and safety, our results can serve as additional information to the data published so far.

The evaluated patients were predominantly adults, females, with HAE type 1, and most of them used only OD therapy. Lanadelumab treatment was assessed during one year period using three parameters: AECT, AE-QoL and attacks frequency. These tools were completed every three months assuring a close monitoring of these patients.

Adherence to lanadelumab treatment was excellent, with no missed doses (each dose was recorded and verified) through the evaluation period. Although all participants reported a local, injection site reaction (redness, swelling, pain) as adverse event, with the exception of one patient who experienced moderate pain during the first two administrations, all side effects were mild in intensity. No issues with tolerability were observed throughout the study duration.

At pre-treatment evaluation (T0), patient showed a high frequency of attacks (mean score: 10.0), significant impairment of quality of life (total score: 66.1), and uncontrolled disease (AECT score: 4.5).

Comparable data were obtained in a survey conducted by our Center using the Hereditary Angioedema Quality of Life Questionnaire (HAE-QoL), completed by 94 patients with HAE-C1INH during the second year of the COVID-19 pandemic. The survey recorded a total HAE-QoL score of 78 (range: 25–135) and a mean attack rate of 13.8, assessed in a subgroup of 30 patients (36). Additional evidence of suboptimal disease control was provided by a separate survey conducted by our Center in March 2022, involving 56 HAE-C1INH patients, which showed mean AECT scores of 6.9 and 7.0 for the 3-month and 1-month recall versions, respectively (30).

These data indicate an important burden of the disease and an insufficient control with the treatments currently used in HAE patients from Romania.

Initiation of LTP with lanadelumab resulted in statistically significant improvements ($p < 0.0001$) across all three evaluated parameters at the T1 assessment: AECT score (12.0), AE-QoL score (35.1), and attack frequency (3.4). These improvements were sustained at T2, T3, and T4 visits, with no statistically significant differences observed between these time points for AECT and AE-QoL (Figures 2–4).

Regarding attacks frequency, the initial reduction observed between T0 and T1 was both statistically significant ($p < 0.0001$) and clinically meaningful, representing a substantial decrease of approximately 66%. Although this reduction was notable, it was somewhat lower than those reported in the Hereditary angioEdema Long-term Prophylaxis Study (HELP, NCT02586805) (37) which demonstrated an 87.0% reduction and its open-label extension part (HELP OLE, NCT02741596) (38) which reported reductions of 82.0% in non-rollover patients and 92.4% in rollover patients.

In contrast, subsequent intervals demonstrated smaller reductions. From T1 to T2, the decrease was not statistically significant ($p = 0.104$) and corresponded to a 17.7% reduction. However, statistically significant decreases were again observed between T2 and T3 ($p = 0.0273$) and between T3 and T4 ($p = 0.0049$), with reductions of 24.1% and 36.4%, respectively. This can be explained by the fact that in some patients the number of attacks decreased progressively over time, with several individuals becoming asymptomatic by the T3 or T4 evaluations.

Our findings demonstrate the rapid onset of lanadelumab's effectiveness shortly after treatment initiation, and its sustained efficacy over time. Similar results have been reported by other groups in Germany (39–41) Poland (42), the United States (43), and Canada (44), confirming both early (within the first month) and long-term (6-, 12-, and 48-month) effectiveness.

Based on our findings, routine follow-up every three months appears unnecessary when lanadelumab is initiated as a LTP therapy. We propose a structured follow-up schedule: an initial assessment at 3 months, a second visit at 6 months to evaluate the possibility of dose reduction to 300 mg/month in asymptomatic patients, and subsequent annual follow-up.

In our study, improvement was observed regardless of whether patients were receiving only OD treatment or were on LTP with intravenous pdC1-INH at a dose of $2 \times 1{,}000$ IU per week. The degree of amelioration was similar between the two groups; however, a statistically significant difference ($p < 0.05$) was noted at the T0 assessment in AECT scores (3.7 vs. 5.9), but not in AE-QoL scores (38.9 vs. 61.6) or attack rates (11.1 vs. 8.2)

(Table 4). Similar findings were reported in research conducted by Buttgereit et al. (39) who observed AECT score improvement in HAE patients treated with lanadelumab, without statistically significant pre-treatment differences between those receiving only OD therapy and those on LTP with pdC1-INH. Collectively, these results suggest that lanadelumab may be more effective than intravenous pdC1-INH for long-term prophylaxis. This finding is further supported by data from the PATCH study (44), an indirect treatment comparison demonstrating that patients treated with lanadelumab experienced less than half the number of attacks compared to those receiving intravenous pdC1-INH.

A reduction in attack frequency was also reported by Sánchez-Machín et al., who observed a similar trend in four patients with HAE-C1-INH. In their study, patients previously receiving long-term prophylaxis with pdC1-INH (three patients) or Berotralstat combined with danazol (one patient) experienced a decrease in attack frequency after switching to lanadelumab treatment (45).

Complete disease control, defined as an AECT score of 16, was achieved in five patients at the T1 assessment; by the T4 visit, one additional patient had reached this level, bringing the total to six, one from the prior LTP group and five from the OD treatment group. All six patients became symptom-free starting at T1. At the T4 visit, an additional 15 patients had AECT scores between 10 and 15, indicating controlled disease. In two cases, an AECT score of 10 was observed only at the T2 visit, with values below 10 at T1, T3, and T4. One patient consistently presented with an AECT score below 10 at all visits and did not achieve the minimal clinically important difference (MCID) of three points between assessments. Interestingly, this patient's subjective report contradicted the AECT findings; she reported feeling significantly better with lanadelumab treatment, citing reduced attack frequency (from 15 at T0 to 9 at T4), decreased severity, and, most notably a change in attack location, with monthly episodes of upper airway edema decreasing to one episode every three months. In this same patient, a single episode of respiratory tract swelling occurred after a dental extraction performed without pre-procedural prophylaxis.

Upper airway edema occurred in two additional patients during lanadelumab treatment and was successfully managed with self-administered icatibant. Prior to initiating LTP with lanadelumab, 19 patients (79.2%) had a history of upper airway edema. These findings align with those reported by Magerl et al. (44), who demonstrated that lanadelumab reduced the incidence of potentially life-threatening laryngeal attacks fivefold compared to LTP with pdC1-INH.

In our cohort, lanadelumab achieved a symptom-free status in seven patients (29.2%) after the first dose, and in an additional four patients within the first three months of treatment (without swelling episodes after the first dose of lanadelumab and subsequent injections within the first three months of treatment respectively). By the T4 assessment, 14 patients (58.3%), equally distributed between males and females remained attack-free, suggesting comparable efficacy across sexes. Although HAE is generally more severe in females, as also reflected in our baseline data (T0), statistically significant differences ($p < 0.05$) were observed in total AE-QoL scores (73.79 vs. 55.40 in females vs. males), as well as in the functional domain (76.57 vs. 58.20) and the fear/shame domain (83.07 vs. 62.60). Notably, these sex-related disparities were no longer present starting at the T1 assessment and remained absent at T4 (Table 3).

Quality of life improved significantly in patients treated with lanadelumab, as demonstrated by a statistically significant decrease in AE-QoL total scores between T0 and T1 ($p < 0.0001$). Although improvement continued at subsequent time points, it was modest, and remained within this range throughout the study period (Figure 4).

A similar pattern was observed across domain-specific AE-QoL scores: significant improvements were recorded at T1 for all domains ($p < 0.0001$), but no statistically significant changes were observed at T2, T3, or T4 (Table 2).

Notably, some domains showed slight deterioration at specific intervals. A minor decline in functioning was observed between T3 and T4, while both the fatigue/mood domain and the total score decreased slightly from T1 to T2. The most pronounced reduction occurred in the nutrition domain between T1 and T2. In contrast, the fear/shame domain showed continuous improvement across all visits, with no decline observed These findings suggest that each domain contributed relatively equally to the persistence of moderate impairment.

Further investigation is warranted to identify the underlying factors responsible for the sustained moderate level of quality-of-life impairment despite clinical improvements.

Regarding treatment effect classification, a "no effect" AE-QoL category (score 0–23) was observed in eight patients (33.4%) at T1 and nine (37.5%) at T4. A "small effect" category (score 24–38) was recorded in seven patients (29.2%) at T1 and six (25%) at T4.

Study limitations and further studies

A strength of this study lies in the use of validated tools adapted for the Romanian language. However, a limitation is that these instruments are not specific to hereditary angioedema (HAE). Additionally, the simultaneous administration of two questionnaires may introduce potential bias, particularly given the different recall periods: the AECT assesses disease control over the previous three months, while the AE-QoL reflects quality of life over the past four weeks. Another possible source of bias is the online administration of the questionnaires in many cases, which may have influenced participant responses.

As more patients are enrolled in the lanadelumab treatment program, we anticipate that further studies will be conducted to expand the evidence base regarding the long-term efficacy and safety of this therapy.

Conclusions

Our study demonstrated that lanadelumab is an effective and well-tolerated treatment for patients with hereditary angioedema (HAE) in Romania. This therapy provided adequate disease control and significantly improved the patients "quality of life

within a relatively short time after initiation, with sustained benefits observed over the one-year treatment period. Regular evaluations at relatively short intervals by the availability and ease of administration of validated questionnaires serve as a useful tool for clinicians in the comprehensive assessment of HAE patients and offer a valuable means of monitoring treatment effectiveness."

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Ethics Committee of George Emil Palade Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

NB: Investigation, Conceptualization, Project administration, Data curation, Writing – original draft, Resources. VN: Writing – review & editing, Visualization, Methodology, Supervision, Formal analysis, Software, Data curation. DD: Validation, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Risk factors for angiotensin converting enzyme inhibitor angioedema in a South African population

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Introduction: Angiotensin converting enzyme inhibitors (ACEI) have proven mortality and morbidity benefit in hypertension, ischemic heart disease, heart failure, and renal disease and are among the most prescribed medications globally. ACEI angioedema (AE-ACEI) is a potentially life-threatening adverse drug reaction that is reported to occur more frequently in African American populations. However, the clinical profile of AE-ACEI is poorly characterized in diverse African populations.

Methods: A case-controlled cohort study with enrolment of AE-ACEI cases and drug-tolerant controls in Cape Town, South Africa. Univariable and multivariable analysis was performed. Controls were defined as patients tolerating ACEI for a minimum of two years. Cases were defined as patients who had angioedema while using an ACEI, patients with a history of angioedema while not on an ACEI were excluded. Cases and controls were recruited from the same demographic areas, including both hospitals and clinics. Information regarding demographics and clinical history was captured via both in person interviews and folder review.

Results: A total of 237 AE-ACEI cases, and 466 ACEI tolerant controls were enrolled from seven sites in Cape Town. Features of IgE-mediated immediate drug hypersensitivity were present in 24 cases, which excluded them from analysis. The median age was 58 years (IQR: 47; 67) and 57% were female. AE-ACEI cases more frequently had Black genetic ancestry compared to controls [53% (81/154), vs. 29% (146/407), $p < 0.001$]. AE-ACEI occurred within 30 days of initiating ACEI therapy in only 31.1% (70/225), with median treatment time to AE-ACEI of 6.9 years (IQR: 2.9; 13). The ACEI tolerant controls were using ACEI for median 9.5 years (IQR: 5; 15.5). All AE-ACEI cases developed swelling above the shoulders, involving the lips and tongue in 72% (165/213) and 50% (107/213) cases respectively. Hospitalisation for AE-ACEI was required in 82% (175/213), however only two patients were intubated, and there were no mortalities. In multivariable analysis traditional risk factors of age, gender, immunosuppression and atopy did not differ between cases and controls. Black genetic ancestry [aOR 15.4 (95% CI 2.94–283), p value = 0.01] and calcium channel blocker use [aOR 1.77 (95% CI 1.17–2.72), p value = 0.008] were significant risk factors for developing AE-ACEI. Cardiac failure, chronic kidney disease, and statin use reduced the risk of AE-ACEI in this model.

Conclusion: In this South African population, Black genetic ancestry and calcium channel blocker use were the major risk factors for AE-ACEI. The majority of AE-ACEI occurred after several years of treatment, with most cases involving the lip and/or tongue. Long-term follow-up, genetic, and further mechanistic studies are warranted in additional diverse African populations.

KEYWORDS

angioedema, angiotensin converting enzyme inhibitor, ACEI angioedema, Africa, drug latency

Introduction

Angiotensin converting enzyme inhibitors (ACEI) are a class of antihypertension medications that inhibit the Angiotensin Converting Enzyme (ACE; also known as kinase II). ACE is responsible for the conversion of angiotensin I to angiotensin II, as well as degradation of the vasoactive peptide bradykinin. ACEI are one of the most widely used medications globally as a result of their affordability, and many large studies have demonstrated their efficacy in the treatment of hypertension (1), cardiac failure (2, 3), ischemic heart disease (4), and chronic renal disease (5). ACEI also show improved efficacy, when compared to angiotensin receptor blockers (ARBs), in preventing hypertension related cardiovascular events, and all-cause mortality (6). Baptiste et al. found that when comparing ACEI to ARBs in hypertensive patients that in the Black African cohort ARB use was associated with a higher risk of cardiovascular related death when compared to ACEI [HR 1.2 (95% CI: 1.02–1.4)]. The two major adverse drug reactions with ACEI are cough and angioedema.

Angioedema is defined as localised swelling in subcutaneous and submucosal tissues. The frequency of AE-ACEI varies from 0.2% to 1% (7), but ubiquitous use of these medications means that AE-ACEI is the most common single cause of angioedema globally, including in South Africa (8–11). AE-ACEI most commonly affects the head and neck (12–14), with mortality rates reported as high as 11%, and intubation rates at 22% internationally (9, 12, 15). Several studies have investigated epidemiological and clinical risk factors for AE-ACEI (see Table 1) with consistent identified risk factors including: African American ancestry (with a 3–5 times increased AE-ACEI angioedema frequency) (10, 13, 16–18, 20, 23, 26, 40), female gender (17, 20, 21), older age (12, 16, 20–22), use of immunosuppression (25, 29, 30), and seasonal allergies (16, 21, 36). Additional identified risk factors (with some conflicting findings) include a variety of concomitant medications (18, 20–22, 25, 30, 41, 42), current smoking status (16, 23–25), and obesity (16, 22). The recent initiation of ACEI and first 30 days of treatment with ACEI have previously been identified as the highest risk period for AE-ACEI (17, 18, 26, 29, 37–39). Most of these epidemiological studies of AE-ACEI are from High Income Countries (HICs) and predominantly European populations, despite the reported increased risk in African Americans. Thus, the aim of this study was to examine the clinical profile and assess risk factors for AE-ACEI in an African setting.

Methods

This is a case control study comparing patients who tolerate ACEI vs. patients who have developed AE-ACEI. We aim to describe this cohort of patients and identify significant risk factors for AE-ACEI in this community. AE-ACEI cases were defined as participants who developed angioedema while on an ACEI, with no history of angioedema while not using an ACEI. ACEI tolerant controls were defined as participants who had safely tolerated an ACEI for at least two years, with no history of angioedema. In this study, AE-ACEI cases were identified both prospectively (through referral) and retrospectively (through folder review). Cases and matched controls were recruited from seven sites: District 6 Clinic, Victoria Wynberg Hospital, Mitchell's Plain District Hospital, Heideveld Emergency Centre, Green Point Clinic, The University of Cape Town Lung Institute Allergy and Immunology Clinic, and Groote Schuur Hospital (GSH) (allergy division, emergency unit, hypertension clinic, and general medical wards). All the above facilities are primary or secondary level centers that refer to GSH and the demographics of this cohort match the demographics of the Western Cape Province (see Supplement for more information about each facility, a detailed description of recruitment at each site as well as consort diagrams, Supplementary Figures 1, 2). The Western Cape Provincial Health Data Centre (PHDC) team assisted with accessing HECTIS admission data (43). All cases and controls were interviewed in person, and all data was assessed by an Allergist/Allergy Medical Officer. Information regarding angioedema history, medical history, and demographics was collected. This study is part of the Angioedema Biomarkers in Africa project, which has been approved by the University of Cape Town Human Research Ethics Committee (HREC 057/2020).

We acknowledge that neither self-reported race nor Fitpatrick skin tone accurately captures genetic ancestry. Therefore, we have decided to group patients based on our available genetic ancestry data into White ancestry, Admixed ancestry, and Black ancestry based on location and grouping in the Principle Component Analysis plot (Supplementary Figure 3) from our previous genome wide association study in this cohort. We did not have genotype data for 118/679 (17.4%) of patients.

Proportions and frequencies were used to describe categorical variables. Normally distributed variables, tested using the Shapiro Wilk test of normality, were described using mean and standard deviation or else median and interquartile range was used. The

TABLE 1 Risk factors for ACEI angioedema from existing published studies.

Intrinsic patient risk factors	Environmental factors
African race as risk factor (10, 13, 16–18) and not as risk factor (12, 19) described	Habits
Age greater than or equal to 65 years (12, 16, 20–22)	Smoking (16, 23–25)
Female gender (17, 20, 21)	Medication
Male gender (22)	Use of Calcium Channel blockers (21, 22, 24)
Increased BMI (16, 22)	Use of antihistamines (21)
Comorbid illness	Use of NSAIDs (20, 22, 26)
C1 inhibitor deficiency or defect (27, 28)	Use of immunosuppressants (25, 29, 30)
History of allergic rhinitis (16, 21)	Use of diuretic (18, 22)
Seasonal allergies (10, 21, 25)	Use of systemic corticosteroids (21)
DM increased risk (12, 16) and protection described (10, 21)	Higher ACEI doses (18)
Hypertension (12, 16)	Use of statins (22)
Rheumatoid Arthritis (21)	Use of anti-diabetic drugs (22)
COPD (21)	Other
History of drug induced rash (10)	Trauma (30–33)
CKD (25)	Recent hospitalisation (18)
Hyperlipidaemia (16)	Pollen (34, 35)
Autoimmune disease (16)	Spring season (36)
Rheumatoid arthritis (21)	
Known drug allergy (20)	
Solid organ transplant (25, 30)	
Malignancy (22)	
Anaemia (22)	
Atopic dermatitis (16)	
Other factors	
Highest rate of AE-ACEI within 30 days of starting ACEI (17, 18, 26, 29, 37–39)	
Recent reinitiation of ACEI (18)	
Unilateral angioedema (12)	
Absence of urticaria or itch (12)	
Angioedema of the lips (12)	
Polypharmacy (22, 24)	
No history of other ACEI use (23)	
ACEI cough (23)	

Yellow cells indicate conflicting data.
ACEI, angiotensin converting enzyme inhibitor; AE-ACEI, angiotensin converting enzyme inhibitor angioedema; BMI, body mass index; CKD, Chronic Kidney Disease; COPD, Chronic Obstructive Pulmonary Disease; DM, diabetes mellitus; NSAIDS, non-steroidal anti-inflammatory drugs.

mean difference test in continuous variables that were normally distributed between groups was done using the analysis of variance (ANOVA) or else the Kruskal Wallis test was used. Proportion difference test for all categorical variables that had at least 5 observations across all groups was done using the Chi square test of independence, or else the Fisher's exact test was applied. All statistical tests were done at 5% level of significance, and Stata 15.1 software was used for the analyses (44). Multivariable analysis was performed for this cohort with AE-ACEI as the outcome. Patients were stratified as "hospitalised" (including patients in general medical wards, intensive care or high care units, and the emergency center) or "not hospitalised" (patients from outpatient departments.). The following covariates were included: age, gender, skin tone, atopy, Human immunodeficiency virus (HIV), hypertension, elevated cholesterol, cardiac failure, and chronic kidney disease. Key selected medications included: immunosuppression, NSAIDs, Statins, and Calcium channel blockers. Cardiac disease was removed from the model as there was evidence of collinearity with cardiac failure. Multicollinearity was assessed using The Variance Inflation Factor (VIF) (Supplementary Table 2), and all results were less than 5. Adjusted odds ratios (aOR) with respective 95% confidence intervals (CI) were reported. There was minimal missing data in chosen co-variates (see Supplementary Table 6). We performed Multivariable analysis for this cohort with AE-ACEI within 30 days of initiating treatment as the outcome, but no covariates reached significance (Supplementary Table 3).

Results

A total of 237 AE-ACEI cases and 466 ACEI tolerant controls were enrolled. Between June 2021 and December 2024, 49 cases of acute AE-ACEI were referred to the GSH Allergy team, while 188 participants with a history of prior AE-ACEI were retrospectively enrolled via folder review and interview. In the AE-ACEI angioedema cases, 24 participants were excluded, as they had evidence of immediate drug hypersensitivity (urticaria $n = 10$; pruritis $n = 13$; anaphylaxis $n = 1$) as determined by two allergists (Supplementary Figures 1, 2). Most of our cohort were treated with the ACEI enalapril [99.7%, (699/701)]. In the ACEI tolerant controls the duration on an ACEI at the time of enrollment was median 9.5 years (IQR: 5; 15.5 years).

In the overall cohort, the median age was 58 years (IQR: 47; 67), 57.5% were female, and 94.8% (532/561) were classified as Admixed or Black ancestry (see Table 2). The cases and controls were similar in terms of age and gender, but significantly more AE-ACEI cases were classified as Black ancestry when compared to controls [53% (81/154), vs. 29%% (119/407), $p < 0.001$]. With regards to comorbid illness, 97.5% of patients had hypertension. HIV was more prevalent in the AE-ACEI cases [16.9% (36/213) vs. 11.4% (53/466), $p = 0.043$] but immunosuppressive treatment did not differ between cases and controls. Compared to cases the controls had significantly higher rates of hypercholesterolaemia [51.9% (242/466) vs. 39.0% (83/213), $p = 0.001$], cardiac disease [21.9% (102/466) vs. 12.2% (26/213), $p = 0.006$], or had previously tested positive for COVID-19 [16.3% (76/466) vs. 12.2% (26/213), $p = 0.001$]. Atopy was reported in 32.3% (69/213) of cases and 27.6% (126/466) of controls but the results did not reach significance ($p = 0.201$). The cases had significantly higher rates of asthma [42.0% (29/69) vs. 20.9% (27/129), $p = 0.001$], while atopic dermatitis was more common in the controls [31% (40/129) vs. 8.6% (8/69), $p = 0.023$]. Calcium channel blockers use was significantly greater in the cases [135/213 (63%) vs. 232/466 (50%), $p < 0.001$] while simvastatin use was significantly more common in the controls [267/466 (57%) vs. 88/213 (41%), $p < 0.001$]. In multivariable analysis Black ancestry (aOR 15.3; 95% CI 2.94–

TABLE 2 Univariate comparison of self-reported clinical variables between ACEI angioedema cases and ACEI tolerant controls and prevalence of previously reported risk factors.

Variable	All, *n* = 679	ACEI angioedema cases, *n* = 213	Controls, *n* = 466	*P* values
Age, years med (IQR)	58 (47;67)	57 (47;68)	59 (46;67)	0.7
Female, n(%)	390 (57.5)	132 (62.0)	258 (55.4)	0.092
Ancestry from genotype, n(%), *n* = 561				
White	29 (5.2)	1 (0.6)	28 (6.9)	**<0.001**
Admixed	332 (59)	72 (47)	260 (64)	
Black	200 (36)	81 (53)	119 (29)	
Genotype not available	118 (17.4)	59 (27.6)	59 (12.6)	
Location of recruitment				
Out patient department	425 (62.6)	122 (57.3)	304 (65.2)	**0.0281**
Emergency room	221 (32.5)	81 (38)	140 (30)	
General inpatient	31 (4.6)	8 (3.8)	23 (4.9)	
High care or intensive care unit	2 (0.3)	2 (0.9)	0 (0)	
Comorbidities[1], n(%)				
Hypertension	662 (97.5)	210 (98.6)	452 (97)	0.20
Hypercholesterolemia	325 (47.9)	83 (39)	242 (51.9)	**0.002**
Diabetes	237 (34.9)	67 (31.5)	170 (36.5)	0.202
Atopy	195 (28.7)	69 (32.3)	126 (27.6)	0.201
Cardiac disease[2]	128 (18.9)	27 (12.2)	101 (21.9)	**0.006**
Cardiac failure	119 (17.5)	20 (9.4)	99 (21.2)	**<0.001**
HIV	89 (13.2)	36 (16.9)	53 (11.4)	**0.043**
Vascular disease[3]	53 (8)	15 (7)	38 (8.2)	0.615
Chronic kidney disease	51 (7.5)	10 (4.7)	41 (8.8)	0.060
Prevalence of previously reported risk factors for AE-ACEI, n(%)				
Fitz Patrick V-VI skin tone	254 (38.3)	108 (50.7)	146 (31.3)	**<0.001**
Over 65 years old	219 (32.3)	63 (29.6)	156 (33.5)	0.313
Allergic rhinitis	117 (17.2)	37 (17.4)	80 (17.2)	0.948
On immunosuppression	34 (5)	8 (3.8)	26 (5.6)	0.312
NSAID use	162 (23.9)	43 (20)	119 (26)	0.129
Diuretic use	465 (68)	154 (72.0)	311 (67.0)	0.148
Statin use	355 (52.3)	88 (41.0)	267 (57.0)	**<0.001**
Calcium channel blocker use	367 (54.1)	135 (63)	232 (50.0)	**0.001**
Oral steroids	7 (1.1)	4 (1.9)	3 (0.6)	0.089
Oral antihistamines	68 (10.6)	19 (8.9)	49 (10.5)	0.900
>3 medications (polypharmacy)	565 (83.2)	181 (85.0)	384 (82.4)	0.405
Increased stress		47 (22.1)		
Acute illness		20 (9.4)		
New chronic illness		18 (8.5)		
Use of over the counter medications		16 (17.0)		

ACEI, angiotensin converting enzyme inhibitor; AR, allergic rhinitis; CKD, chronic kidney disease; HIV, Human immunodeficiency virus; IQR, interquartile range, LABA, long acting beta agonist, NSAID, non-steroidal anti-inflammatory; SABA, short acting beta agonist.

Bold *p* values indicate statistical significance.

[1]Only comorbidities >5% included in table. Others include: abnormal uterine bleeding *n* = 1; acute renal injury *n* = 1; allergic conjunctivitis *n* = 1; anxiety *n* = 3; anemia *n* = 1; arthritis *n* = 60; atopic dermatitis *n* = 7; B12 deficiency *n* = 1; bronchitis *n* = 1; cancer *n* = 10; cellulitis *n* = 1; chronic constipation *n* = 3; chronic gastroenteritis *n* = 1; chronic pain *n* = 10; clubfoot *n* = 1; COPD *n* = 9; Crohn's disease *n* = 1; current TB *n* = 6; depression *n* = 7; dermatitis *n* = 2; dyslipidemia *n* = 3; epilepsy *n* = 14; fibromyalgia *n* = 1; glaucoma *n* = 2; GORD *n* = 36; gout *n* = 36; headache *n* = 2; hernia *n* = 3; hip replacement *n* = 1; IgA disease *n* = 2; jaundice *n* = 1; meningitis *n* = 1; mixed connective tissue disease *n* = 1; muscle spasms *n* = 1; myasthenia gravis *n* = 1; obesity *n* = 2; overactive bladder *n* = 1; Penicillin allergy *n* = 1; peptic ulcer disease *n* = 3; polio *n* = 1; porphyria *n* = 2; post herpetic neuralgia *n* = 1; PPE *n* = 1; previous TB *n* = 80; prolapsed lumbar disc *n* = 1; prostate disease *n* = 2; psoriasis *n* = 2; pulmonary embolism *n* = 2; retinopathy *n* = 1; rheumatic hear disease *n* = 1; rheumatoid arthritis *n* = 8; schistosomiasis *n* = 1; schizoaffective disorder *n* = 1; silicosis *n* = 1; sinusitis *n* = 1; systemic lupus erythramatosis *n* = 10; spinal injury *n* = 1; gastrointestinal infection *n* = 1; tinea *n* = 2; thyroid disease *n* = 19; trisomy 21 *n* = 1; ulcerative colitis *n* = 1, urinary tract infection *n* = 1.

[2]Cardiac disease: valvular heart disease and ischaemic heart disease.

[3]Vascular disease: stroke and peripheral vascular disease.

283, $p = 0.01$), calcium channel blocker usage (aOR 1.77; 95% CI: 1.17–2.72, $p = 0.008$) were significant risk factors for developing AE-ACEI (Table 3). Chronic kidney disease, cardiac failure, and statin use were protective in this model.

In our cohort the duration of ACEI treatment before developing angioedema median of 6.9 years (IQR: 2.9; 13.0 years) with 31.5% (67/213) of patients developing angioedema within 30 days of ACEI initiation. The median duration of AE-ACEI was 48 h (IQR: 24; 72 h). Multivariable analysis for developing AE-ACEI within 30 days of initiating treatment found no significant covariates (Supplementary Table 3).

All AE-ACEI cases had swelling above the shoulders (see Figure 1) with the lip [72.3% (154/213)] and tongue [50.2% (107/213)] being the most common sites of swelling. Overall, 22

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TABLE 3 Unadjusted and adjusted analysis of clinical risk factors for ACEI angioedema.

Characteristic	Unadjusted			Adjusted		
	OR	95% CI OR	*p*-value	aOR	95% CI aOR	*p*-value
Age	1.00	0.99–1.01	0.70	1.01	1.01–1	0.11
Male	0.75	0.54–1.05	0.092	0.95	0.62–1.45	0.8
Admixed ancestry	7.75	1.61–139	0.046	6.16	1.22–112	0.081
Black ancestry	19.10	3.94–343	0.004	15.30	2.94–283	**0.01**
Atopy	1.23	0.87–1.75	0.2	1.44	0.92–2.25	0.11
HIV	1.60	1.01–2.52	0.045	1.08	0.58–1.98	0.8
Hypertension	2.17	0.70–9.48	0.20	0.3	0.05–1.87	0.2
Elevated cholesterol	0.60	0.43–0.83	0.002	1.44	0.78–2.70	0.20
Cardiac Failure	0.39	0.23–0.63	<0.001	0.43	0.22–0.82	**0.014**
Chronic Kidney Disease	0.51	0.24–1.00	0.064	0.24	0.05–0.72	**0.024**
Immunosuppressive drug use	0.66	0.28–1.42	0.3	0.94	0.29–2.62	>0.90
NSAID use	0.74	0.49–1.09	0.13	1.23	0.69–2.15	0.50
Statin use	0.52	0.38–0.73	<0.001	0.36	0.19–0.65	**<0.001**
Calcium channel blocker use	1.75	1.25–2.44	0.001	1.77	1.17–2.72	**0.008**
Hospitalised	1.39	0.99–1.93	0.053	1.66	1.07–2.58	**0.024**

aOR, adjusted odds ratio; HIV, human immunodeficiency virus; NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio.
Bold *p* values indicate statistical significance.

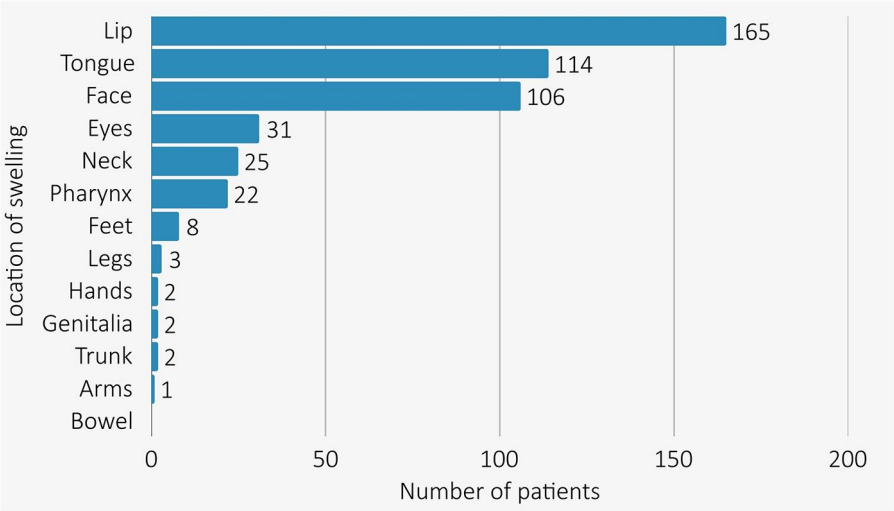

FIGURE 1
Bar graph describing distribution of AE-ACEI location in cases.

(10.3%) patients developed pharyngeal angioedema (10/22 with concomitant tongue and lip swelling), with two patients requiring intubation for airway protection (one emergency orotracheal intubation, and one emergency cricothyroidotomy both after reinitiation of an ACEI in patients with known AE-ACEI). There were no angioedema related deaths. Hospitalisation was required in 82.2% (175/213) of AE-ACEI cases. Information about treatment of AE-ACEI was available for 41.8% of cases (89/213). Therapy with fresh frozen plasma was given in 11 cases (11/89, 12.4%) while 73% (65/89) were treated with antihistamines, 64% (57/89) with corticosteroids, and 12.4% (12/89) required adrenaline for airway protection. One patient received Icatibant peri-intubation after discussion with our Angioedema Hotline.

Discussion

AE-ACEI is a common and potentially life-threatening complication of ACEI use, and the global rate of acute ACEI requiring care in emergency centers is rising (45, 46). To date there are no available tests or biomarkers to predict which patients are at risk of developing AE-ACEI. Currently, clinical risk factors remain the best clues to identify at-risk groups—however, these risk factors may be population-specific with limited data from diverse African countries. This large South African cohort of AE-ACEI and drug-tolerant controls were examined for traditional and novel risk factors. AE-ACEI cases more commonly had Black genetic ancestry, a higher prevalence of HIV, and lower rates of concomitant metabolic and non-hypertensive cardiovascular

diseases. Notably, the majority of AE-ACEI occurred following prolonged periods of treatment, and there was increased occurrence during the spring/summer seasons. Interestingly, we also found that all patients had angioedema of the face and neck. Despite high rates of hospitalisation there were low rates of intubation (0.9%) for airway protection, and no deaths. This is contradictory to international data where 22% (37) to 32% (13) of patients with AE-ACEI required intubation, and 11% demised (37). Unlike international groups, we did not find that older age, female gender, allergic rhinitis, or immunosuppressive therapy were associated with AE-ACEI. However, similar to international data, we found that calcium channel blockers were a significant risk factor (21, 22, 24).

In 1996, Brown et al. (18) were the first to describe an increased risk for AE-ACEI in African Americans with a relative risk of 4.5 compared to white Americans. They also found that African patients were more likely to have severe hypertensive disease as they required higher doses of ACEI, as well as having more severe AE-ACEI with higher rates of hospitalisation and intubation for airway protection. Later studies in the USA (10, 13, 16, 17, 20, 23, 41) and a systematic review (40) found that black and Hispanic patients had a significantly higher risk of AE-ACEI compared with patients with paler skin tone (41). However, other authors from the USA have found that African race were not associated with AE-ACEI (12, 19). Studies completed in Sweden, the United Kingdom, and Thailand either did not describe racial demographics (21), or had no patients with darker skin tones (24, 39) (see Supplementary Table 5 for a summary of these studies). Our data from an African Middle Income Country (MIC) setting does find a significantly higher prevalence of Black genetic ancestry in AE-ACEI cases with a high aOR of 15.3 ($p = 0.01$ (Table 3). This result aligns with African American data and suggests that examining African populations for genetic risk factors for AE-ACEI is warranted. We have recently published a GWAS from this cohort and we replicated findings for the single nucleotide polymorphisms (SNP) rs500766 on chromosome 10 (previously linked to AE-ACEI). We also found SNPs located close to the genes *PRKCQ* (protein kinase C theta), *RAD51B* (RAD51 Paralog B), and *RIMS1* (regulating synaptic membrane exocytosis 1), which have previously been linked with drug-induced angioedema. Additionally, SNPs near the *CSMD1* (CUB and sushi multiple domains 1) gene, which has been linked to ACEI cough (47), were identified. We have highlighted that further work across diverse African populations is justified, as given African genomic diversity extrapolation to all darker skinned populations would be flawed (48).

AE-ACEI can occur immediately upon drug exposure, or years into therapy, and its occurrence cannot be predicted. Some authors have found that AE-ACEI is most common within the first 30 days of starting treatment, reporting that 48.6–53.0% of AE-ACEI cases occur within that window (17, 18, 39), while others have found much lower AE-ACEI rates of 10.2% within the first month of treatment (15, 39, 41). Our cohort had a long drug latency, with only 31.1% developing AE-ACEI within the first 30 days of drug initiation, which is within range of global rates. In AE-ACEI this prolonged drug latency raises the question of whether there is an additional factor/second hit event in the late presenters which i) either effects other enzymes that metabolise bradykinin, such as neprilysin, or aminopeptidase P, ii) may represent another factor that leads to increased flux through the bradykinin pathway, potentially even at the tissue-level, or iii) represents the onset of a chronic urticaria/angioedema variant independent of AE-ACEI. A recent publication by Bocquet et al. found that, on review of reported AE-ACEI angioedema cases that almost 50% of patients were excluded, and that these patients likely had mast cell mediated angioedema rather than AE-ACEI (49).

All of our AE-ACEI cases presented with swelling above the shoulders, most commonly affecting the lips and tongue, aligning with global findings (14, 36). Previous studies have identified tongue swelling in AE-ACEI as a marker associated with poor clinical outcomes and increased likelihood of intensive care admissions (13, 15). Other forms of bradykinin mediated angioedema—such as hereditary angioedema and acquired angioedema—can affect multiple body sites, including the oropharynx, abdominal viscera, limbs, and genitalia (50). If AE-ACEI is also bradykinin mediated, one would expect it to involve a broader range of anatomical sites. The density of mast cells in tissue is higher in the face than other anatomical areas (51); so a possible explanation is that ACEI treatment is unmasking mast cell mediated angioedema (i.e., ACEI are acting as a cofactor). This is supported by recent findings of high rates of mast cell mediated angioedema in patients who developed recurrent angioedema after exposure to ACEI. Douillard et al. found that 41% of patients with a suspected ACEI/ARB angioedema still had recurrent angioedema without urticaria more than 6 months after stopping the drug, indicating a diagnosis of mast cell mediated angioedema rather than bradykinin mediated angioedema in these patients (52). These findings are important and underline the need for future investigations into the pathomechanisms of AE-ACEI, and potentially cross-talk between mast-cells and kallikrein-kinin pathways.

This study is the largest study to date describing AE-ACEI in an African MIC. Our limitations include prospective case finding, which was reliant on referrals from local sites; as well as retrospective case finding which is dependent on existing ICD10 coding and local databases. We encountered difficulties in contacting retrospective cases to schedule in-person interviews because of a highly migrant population, and incorrect or outdated contact details. Our cohort had higher rates of hypertension and dyslipidaemia than reported in South Africa (53), which likely reflects our recruitment from hospitals and local clinics, rather than the general population. However, ACEI are most commonly prescribed for hypertension, so high hypertension rates (and associated metabolic conditions) in this cohort are not an unexpected finding. The case-controlled nature of the study cannot exclude potential for unintentional selection bias; however, cases and controls were recruited from the same hospitals and drainage populations. It is also important to note that as this study is nested in the government funded state sector, almost all our cohort were using Enalapril, which is the ACEI on government tender. We did have some variables with high rates of missing data—especially with regards to treatment of AE-ACEI—as participants either did not know or could not recall the treatment that was received at the time of the event.

In summary, we have shown that Black genetic ancestry, calcium channel blocker use, and HIV co-infection are important risk factors for AE-ACEI—and that previously identified risk factors including older age, female sex, and allergic rhinitis were not risk factors in this cohort. We have also identified a long drug latency compared to international cohorts. These findings highlight the need for further epidemiological and clinical studies on AE-ACEI in diverse ethnic backgrounds and LMIC settings to provide a true global clinical understanding of AE-ACEI.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of Cape Town Human Research Ethics Committee (HREC 057/2020). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LM: Formal analysis, Software, Writing – review & editing. MR: Data curation, Methodology, Writing – review & editing. MD: Data curation, Writing – review & editing. CM: Data curation, Writing – review & editing. AE: Project administration, Writing – review & editing. EJ: Data curation, Project administration, Writing – review & editing. SP: Data curation, Investigation, Methodology, Project administration, Writing – review & editing. JP: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/falgy.2025.1664354/full#supplementary-material

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Implementation of genetic diagnosis and personalized management of hereditary angioedema in a Chinese regional center: a community case study of three families

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Background: Hereditary angioedema (HAE) remains significantly underdiagnosed and misdiagnosed in China, with laryngeal involvement leading to high mortality rates, creating an urgent need for exploring feasible diagnostic and management approaches in resource-limited settings.
Objectives: To establish and evaluate a community-oriented comprehensive HAE diagnosis and management program at a regional center in central China; characterize the clinical and biochemical phenotypes of three unrelated families; identify *SERPING1* variants; implement personalized treatment and family cascade screening; and evaluate key program elements as a proof-of-concept model.
Methods: From September 2022 to August 2025, we established a systematic workflow for suspected HAE cases at Henan Provincial People's Hospital, integrating clinical assessment, biochemical testing, and genetic analysis. Three unrelated families (45 subjects total) were enrolled. The program included standardized clinical assessment and real-time biochemical screening (C4, C1 inhibitor concentration/function), targeted *SERPING1* sequencing and variant classification American College of Medical Genetics and Genomics (ACMG), family cascade screening and genetic counseling, stratified personalized treatment (on-demand icatibant and lanadelumab prophylaxis), electronic follow-up Angioedema Control Test (AECT)/Angioedema Quality of Life Questionnaire (AE-QoL), and quality management [Standard Operating Procedures (SOPs) and provincial External Quality Assessment (EQA) planning)].
Results: Family 1: A heterozygous missense variant c.1034G > A (p.Gly345Glu) was detected in exon 7 of *SERPING1* in the proband, absent in 13 unaffected family members. Laboratory tests showed decreased serum C4 and C1INH levels with reduced functional activity, consistent with HAE type 1. Family 2: Three affected members carried the same heterozygous missense variant c.1396C > T (p.Arg466Cys) in exon 8 of *SERPING1*, absent in 8 unaffected members. Despite elevated C1INH antigen levels, functional activity was markedly reduced, establishing HAE type 2 diagnosis. Family 3: Three affected members carried the same heterozygous missense variant c.1483G > A (p.Val495Ile) in exon 8 of *SERPING1*, absent in 17 unaffected members.

Laboratory tests showed decreased serum C4 and C1INH levels with reduced functional activity, consistent with HAE type 1. Personalized treatment strategies achieved good disease control: mild cases with on-demand icatibant; severe phenotypes with lanadelumab prophylaxis. During 12–25 months of follow-up, the four symptomatic patients showed markedly reduced attack frequency with no life-threatening events.

Discussion & conclusion: This community program represents a proof-of-concept demonstrating what is possible in establishing specialized HAE services in resource-limited settings. Key facilitating factors included cascade screening and genetic counseling, standardized testing pathways and variant classification, flexible prophylactic strategies adapted to economic conditions, and electronic quality and outcome monitoring. This program has expanded the domestic *SERPING1* variant spectrum and provides preliminary insights and references for the future development of HAE services.

KEYWORDS

hereditary angioedema, c1 inhibitor, *SERPING1*, community case study, genetic diagnosis, China

Introduction

Hereditary angioedema (HAE) is a rare, life-threatening autosomal dominant genetic disease characterized by recurrent, unpredictable episodes of angioedema, with an estimated population prevalence of approximately 1/50,000 (1), individuals without ethnic or gender predilection, HAE remains significantly underdiagnosed, though epidemiological data remains lacking in China.

The pathophysiology of HAE primarily involves dysregulation of the contact activation system due to deficiency or dysfunction of C1 inhibitor (C1INH), a serine protease inhibitor encoded by the *SERPING1* gene location (GRCh38) is 11:57,597,685–57,614,848 at 11q12.1 (2, 3). C1INH serves as the primary regulator of multiple proteolytic cascade systems, including the complement, contact, coagulation, and fibrinolytic pathways. Its deficiency leads to uncontrolled activation of the contact system, resulting in excessive bradykinin generation, increased vascular permeability, and subsequent angioedema formation (1, 4).

Based on biochemical phenotype, HAE with C1INH deficiency is classified into two principal subtypes. Type 1 HAE, accounting for approximately 85% of cases, is characterized by reduced C1INH antigen levels and functional activity. Type 2 HAE, comprising 15% of cases, presents with normal or elevated C1INH antigen levels but diminished functional activity (2, 3). A third category, HAE with normal C1INH (HAE-nC1INH), has been identified in patients with typical clinical manifestations but normal C1INH parameters and no detectable *SERPING1* variants, often associated with variants in other genes. In recent years, researchers have successively identified several new pathogenic genes associated with HAE, including the genes encoding coagulation factor XII (*F12*) (5–7), angiopoietin-1 (*ANGPT1*) (8), plasminogen (*PLG*) (9, 10), kininogen-1 heavy chain (*KNG1*) (11), myoferlin (*MYOF*) (12), heparan sulfate glucosamine 3-O-sulfotransferase 6 (*HS3ST6*) (13), carboxypeptidase N (*CPN*) (14), and DAB2 interacting protein (*DAB2IP*) (15). Additionally, some patients still have unidentified pathogenic genes (HAE-unknown, HAE-UNK).

Over 700 pathogenic *SERPING1* gene variants have been identified to date (16). The predominant variant types are missense variants, followed by frameshift variants, small insertions and deletions, large genomic rearrangements, splice site defects, nonsense variants, and regulatory variants (3). In the Chinese population, variants underlying HAE exhibit considerable diversity. Missense variants and in-frame deletions represent the most prevalent types, comprising 36.8% of all variants, followed by frameshift variants (28.9%), nonsense variants (14.5%), splice site variants (13.2%), and large insertions and deletions (6.6%) (17). The mutational spectrum exhibits considerable heterogeneity, with approximately 30%–34% of cases arising from *de novo* variants, presenting diagnostic challenges in patients without positive family history (17, 18).

In China, HAE research commenced in 1980, with subsequent studies revealing that virtually all confirmed cases are attributable to *SERPING1* variants. Chinese cohort studies indicate a predominance of type 1 HAE (98.73%) with type 2 representing only 1.27% of cases (19). Notably, the incidence of laryngeal edema among Chinese HAE patients reaches as high as 55.06%, with an average time from edema onset to asphyxia of 4.6 h, a maximum mortality rate of 40%, and a misdiagnosis rate of 75%. Untreated individuals exhibit marked disease progression between 20 and 29 years of age or succumb to sudden laryngeal edema at a median age of 46 years (17, 19, 20), underscoring the paramount importance of early diagnosis and appropriate management.

Recent advances in genetic testing methodologies have facilitated the identification of cryptic pathogenic variants, including deep intronic variants, non-canonical splicing variants, and dominant-negative missense variants that may be missed by conventional sequencing approaches (21, 22). These findings have expanded our understanding of HAE molecular pathogenesis and highlighted the importance of comprehensive genetic analysis in suspected cases.

This study presents a proof-of-concept model of HAE diagnosis and management in a resource-limited healthcare environment. Specifically, we established a comprehensive HAE diagnosis and management program at a regional medical center in central China, we typify the systemic diagnostic obstacles: patients experiencing persistent misdiagnoses, unnecessary surgical interventions, and substantial economic burdens associated with seeking specialized medical care. These real-world challenges underscore the urgent need for establishing a professional diagnostic and treatment system. To address these challenges, the project innovatively integrated a multidimensional diagnostic and therapeutic strategy: standardized clinical assessment, real-time biochemical screening (C4, C1INH concentration/function), and targeted *SERPING1* sequencing, embedding family cascade screening and genetic counseling. Our innovation rationale lies in integrating standardized testing, variant classification, cascade screening, personalized treatment, and electronic follow-up into an "affordable, implementable, and evaluable" continuous service within a resource-limited regional environment, demonstrating what is possible in addressing the core challenge of this community case study.

Notably, on May 11, 2018, the National Health Commission and five other departments jointly formulated the "First List of Rare Diseases," which officially incorporated HAE, providing a critical policy foundation for the development of disease diagnosis and treatment systems and creating institutional support for our research work.

Materials and methods

Study setting

Henan Provincial People's Hospital Department of Allergic Diseases (Provincial Allergy and Immunological Disease Diagnosis, Treatment and Quality Control Center), serving a population >99 million. Before program initiation, our hospital lacked systematic HAE diagnostic testing, requiring suspected patients to seek care elsewhere, imposing significant economic and accessibility burdens. To address this situation, the hospital implemented multiple strategies: First, the hospital has independently developed serum C4 and C1INH concentration testing (approximately $7.4). Second, by participating in the China Primary Healthcare Foundation's "Prescription Care—Collaborative Screening—C1 Esterase Inhibitor Detection Project," free serum C4 and C1INH concentration/functional testing is provided for eligible patients.

This study represents a proof-of-concept pilot project aimed at demonstrating what is possible in establishing HAE diagnostic and management services within resource-constrained healthcare environments, with the explicit understanding that the model is not immediately generalizable. The project specifically focused on workflow feasibility and family-based case ascertainment through referred kindreds and cascade screening, deliberately diverging from population-based epidemiological investigations. Despite the extensive administrative catchment area of the medical center, active screening was intentionally restricted in scope. Consequently, the limited number of confirmed cases primarily reflects the region's known systemic diagnostic underestimation and referral constraints, rather than representing the true epidemiological prevalence of HAE.

The study protocol was approved by the Henan Provincial People's Hospital Institutional Ethics Committee (Approval No.: 2022-2211273107). Written informed consent was obtained from adults; parental consent and age-appropriate assent were obtained for minors.

Study subjects: From September 2022 to August 2025, three unrelated families totaling 45 individuals were enrolled, with complete three-generation pedigrees constructed.

Diagnostic criteria

HAE diagnosis was established according to the Chinese Expert Consensus on Diagnosis and Treatment of Hereditary Angioedema (2020) and the International WAO/EAACI Guideline for Hereditary Angioedema Management—2021 Revision and Update (4, 23).

Workflow and diagnostic pathway

Standardized clinical assessment: Structured history (age of onset, attack frequency/location/triggers, previous misdiagnoses and treatments, family history). Physical examination performed during both symptomatic and asymptomatic periods; attack-phase imaging obtained when feasible.

Serum C4, C1INH concentration and function determination: C4 and C1INH concentrations quantified by nephelometry; C1INH functional activity measured by ELISA. Peripheral venous blood (2 mL) collected from probands and all family members in three families for these tests.

DNA extraction: Peripheral venous blood (2 mL) collected from probands and all family members in three families. Genomic DNA extracted according to kit instructions (DNeasy Blood & Tissue Kit, Qiagen, Germany, Cat. No. 69504).

PCR Amplification: Primers were designed using the online software Primer3 (https://bioinfo.ut.ee/primer3-0.4.0/) to amplify the promoter, exon, and intron regions of the *SERPING1* gene. Primer sequences and amplicon lengths shown in Table 1 (all reactions 35 cycles).

Sanger Sequencing: PCR products were purified, and bidirectional sequencing was performed on the purified amplification products using an ABI-3500DX sequencer (Applied Biosystems, USA).

Bioinformatics Analysis: REVEL (https://sites.google.com/site/revelgenomics/) was used to predict variant pathogenicity. Variants were interpreted according to the 2015 ACMG Standards and Guidelines for the Interpretation of Sequence Variants (24). Evidence strength categories were weighted as very strong (PVS), strong (PS), moderate (PM), and supporting (PP).

Detailed diagnostic and therapeutic flowchart (Figure 1).

10.3389/falgy.2025.1696666

TABLE 1 Primer sequences and PCR conditions.

Region	Primer Sequence (5′→3′)	PCR product length (bp)	Ta Opt (°C)
5UTR	GGCCAGCCAATAGCTAAGAC	246	58
	AGTCCCAGGTGGAAGCAAG		
Exon1	GAGGAGCCAGGGAGAAGGT	391	58
	ACCATCTACGAGTGCGTGTG		
Intron1	ACTCTCTACTCAGTCTGCACT	658	60
	GACCACATACCCCAGCCAG		
Exon2	CCACACCTTCTCTTCCTGCT	599	59
	CTTCTTCCCAGAGGCATGG		
Intron2	CTGACCCTGCTGACCCTC	1,814	58
	GGTTGTGTGGTGGGTTCATC		
Exon3	GATCTGTGATCCCCTCCAAA	360	58
	GCTCCAACATTCCCTCTGTC		
Intron3	CTTCTGCCCAGGACCTGTTA	1,986	60
	TGGGCTGTGGAAGATCTGAG		
Exon4	CCACCATGCCGTATTCACTA	491	59
	TGCAGGTAGGAAAAGGATGAA		
Intron4-1	ACTTCACCTGTGTCCACCAG	1,938	58
	AGGCGCGTGCCACCACACCTG		
Intron4-2	GTGACAGACCGAGACTCTGTCTC	1,976	58
	TGTTGCTTAGGACTCTGGGG		
Exon5	AGCCCACTTAACCCCAAGTT	356	58
	CCCCAAAATGATGGGACTAC		
Intron5	GACAGTCTGCCCTCCGATAC	290	60
	CATTCTGGTTTTCTTGGGATCAA		
Exon6	CAGGAGAGAGATGCGGTAGG	396	58
	AGGGTTGCAGGACAAACTGA		
Intron6-1	TGTGGCCCATTTCATTGACC	1,392	58
	AGATGGGTGGATAAGTCCAT		
Intron6-2	GCCCGCCACCACACCTGGCT	2,117	58
	TTCAACATCATGTTAAAACC		
Intron6-3	CATCTAATACCTGACAGGTC	1,898	58
	TCACTTTGATGCGGGGTAGT		
Exon7	AGAGAGCACTGGGACTCAGG	376	60
	GGAGCCCTTTTGGTGGATAG		
Intron7	ACTACCCCGCATCAAAGTGA	2,563	59
	CACCCCAGTCTCTGTCAGTT		
Exon8 + 3UTR	AGCAGCACAAGTTCCCTGTC	399	58
	AGCCTGGGTGACAGATTGAG		

Materials and methods—genetic testing and segregation

Cascade screening strategy: Construct a pedigree spanning at least three generations; for co-segregation analysis, include only biological relatives. Spouses and other non–blood-related individuals may be tested for counseling purposes but are excluded from co-segregation counts. Prioritize first-degree relatives; with informed consent, perform C4 and C1INH concentration/function assays, and conduct targeted genetic testing for the family-specific variant established in the proband.

Counseling points: 50% autosomal dominant inheritance risk, variable expressivity and age-related expression, reproductive options, trigger avoidance and prodrome recognition, personalized emergency plans, and compliant communication with written notification of potential insurance and employment implications.

Stratified personalized treatment and local adaptation

Mild/low burden: On-demand treatment (icatibant 30 mg subcutaneous), with pre-filled syringes and family emergency plans provided.

Severe/high risk: Those with laryngeal or gastrointestinal involvement prioritized for long-term prophylaxis (lanadelumab 300 mg subcutaneous every 2 weeks; extended to every 4 weeks under economic constraints with close AECT monitoring and breakthrough treatment assurance).

Monitoring tools: Regular assessment with AECT (disease control) and AE-QoL (quality of life); electronic recording of attacks and medications.

Quality and data systems

SOP standardization: End-to-end SOP and version management from blood collection/transport, testing and reporting, ACMG interpretation to follow-up and medication pathways.

Provincial EQA (planning): Regular proficiency testing and closed-loop improvement to enhance inter-institutional result comparability.

Electronic follow-up and dashboard: Structured capture of attack rates, laryngeal events, emergency/hospitalization, medications and adverse events; key quality indicators [Turnaround Time (TAT), EQA scores, AECT completion rate/scores, emergency rates, prophylaxis adherence, etc.] visualized, supporting Plan–Do–Check–Act (PDCA) continuous improvement.

Accessibility and payment strategies

Payment and assistance: Through the China Primary Health Care Foundation, we implemented an innovative co-payment model integrating medical insurance and foundation assistance, strategically designed to mitigate out-of-pocket expenses for long-term prophylaxis and emergency medications in HAE treatment. This financial intervention significantly reduced patients' economic burden, successfully limiting patient self-payment proportions to 10%–30% for critical treatments, with medication costs specifically reduced to approximately $207 per dose for Lanadelumab and $194 per dose for Icatibant. Economic evaluation: Conducting cost-effectiveness and equity evaluation of stratified prevention strategies (e.g., cost per laryngeal event avoided, payment burden by income stratification) to provide evidence for policy decisions.

Results

Clinical characteristics of families

Family 1 ($n = 14$): Three-generation family with 14 members evaluated (Figure 2A). Proband (III-5), male, 24 years old,

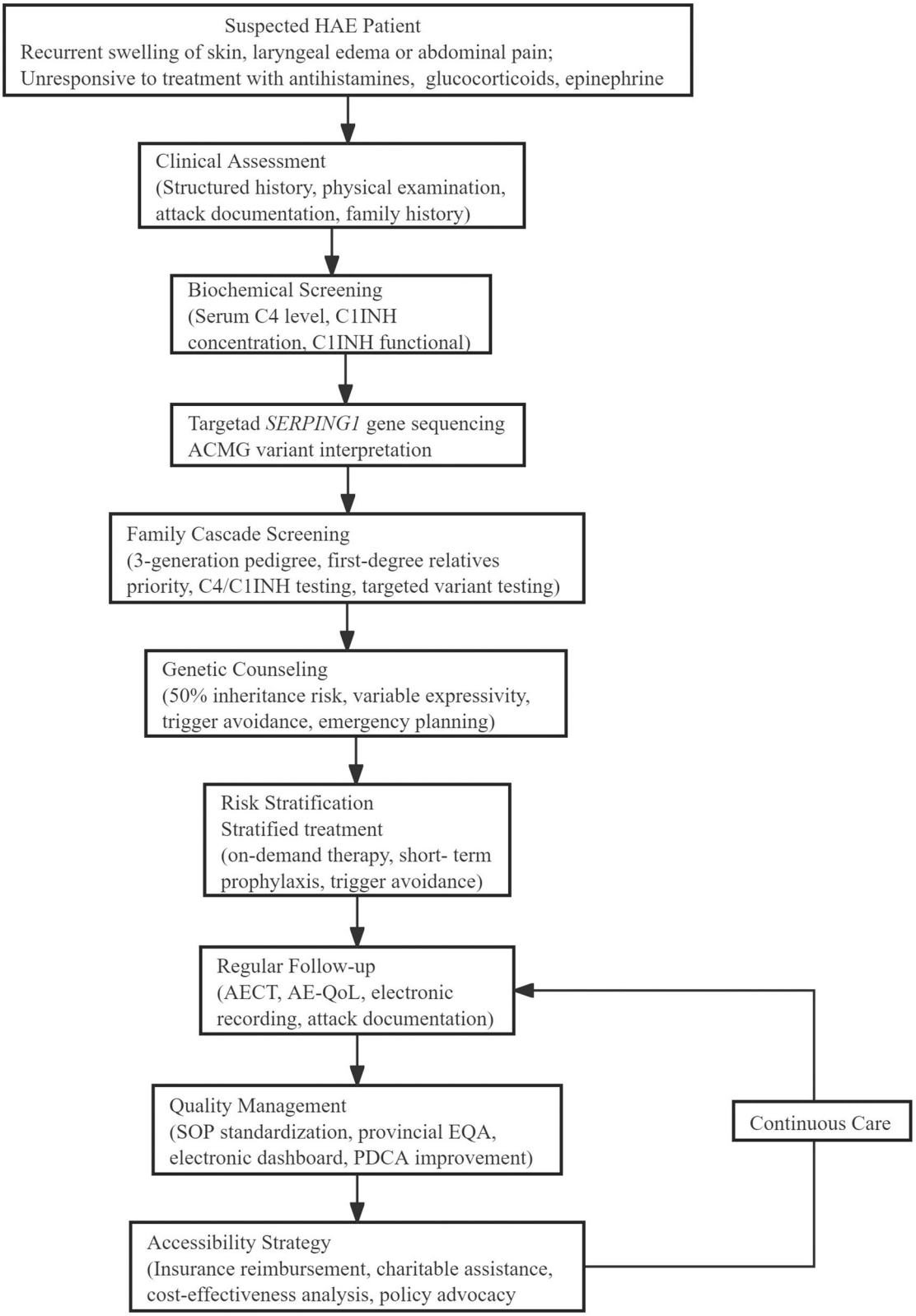

FIGURE 1
Study flow chart.

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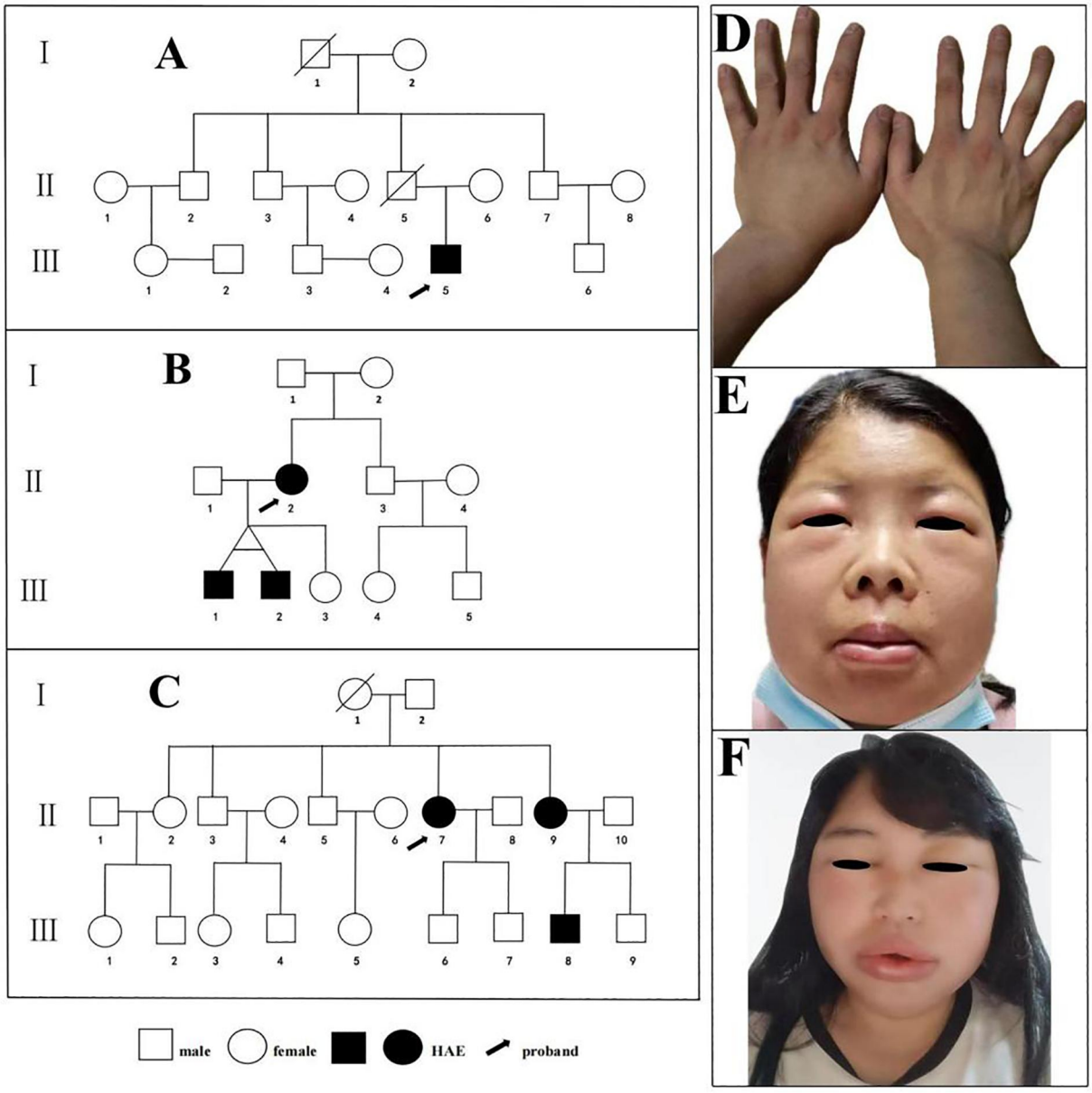

FIGURE 2
Pedigrees of the three families with HAE. **(A)** Hereditary Angioedema Family 1. **(B)** Hereditary Angioedema Family 2. **(C)** Hereditary Angioedema Family 3. Images of acute attacks in index patients from three Hereditary Angioedema families. **(D)** The proband of Family 1 presenting with left hand edema; **(E)** The proband of Family 2 presenting with edema of the lips, eyelids, and face. **(F)** The proband of Family 3 presenting with edema of the lips, eyelids, and face.

presented with recurrent non-pitting edema predominantly affecting upper limbs for 4 years, occurring approximately 3–5 times annually, self-resolving without treatment over 1–3 days. Triggers included physical activity, mechanical pressure, and minor trauma. Previously misdiagnosed with chronic urticaria, unresponsive to antihistamines. Presented with left hand non-pitting edema one day before enrollment (Figure 2D). Family history: grandfather (I-1) died of laryngeal edema asphyxiation at age 63; uncle (II-7) had similar but less frequent attacks; father (II-5) died of hepatocellular carcinoma without clear edema history.

Family 2 ($n = 11$): Three-generation family with 11 members evaluated (Figure 2B). Proband (II-3), female, 36 years old, experienced recurrent facial, limb, and gastrointestinal edema for 20 years. Attacks occurred 8–10 times annually, with facial and limb edema lasting 3–5 days; abdominal attacks presented as severe cramping, nausea, and vomiting lasting 2–3 days. Previously underwent exploratory laparotomy for suspected acute abdomen and multiple hospitalizations for misdiagnosed acute pancreatitis or appendicitis. Presented with lip, eyelid, and facial edema 2 days before enrollment (Figure 2E). Her twin sons (III-1, III-2, 9 years old) were asymptomatic at evaluation but showed biochemical abnormalities consistent with HAE.

Family 3 ($n = 20$): Three-generation family with 20 members evaluated (Figure 2C). Proband (II-7), female, 43

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years old, experienced recurrent facial and limb edema with occasional laryngeal edema for 4 years. Attacks occurred 7–10 times annually, with facial and limb edema lasting 3–5 days; underwent tracheal intubation for laryngeal edema 2 years ago, with multiple hospitalizations for misdiagnosed acute laryngitis and edema. Presented with lip, eyelid, and facial edema 10 h before enrollment (Figure 2F). Her sister (III-9, 37 years old), has a 5-year history of recurrent extremity edema occasionally accompanied by abdominal pain, with approximately 5–6 attacks per year, no apparent triggers, and episodes resolving spontaneously in about 2 days without treatment. Her nephew (IV-8, 11 years old), was asymptomatic at evaluation but had biochemical abnormalities consistent with HAE.

Previous medical history, clinical symptoms, treatment regimens, and edema control status of the four patients with HAE are shown in Table 2.

Biochemical parameters

Family 1: Proband's serum C4 and C1INH functional activity were significantly below 50% of normal lower limits, consistent with HAE type 1 diagnosis. Test results for 13 normal controls within the family were all within reference ranges.

Family 2: Proband and her two sons (III-1, III-2, both asymptomatic) showed elevated serum C1INH antigen levels with reduced functional activity, consistent with HAE type 2 diagnosis. Test results for 8 normal controls within the family were all within reference ranges.

Family 3: Proband, her sister, and nephew showed serum C4 and C1INH functional activity significantly below 50% of normal lower limits, consistent with HAE type 1 diagnosis. Test results for 17 normal controls within the family were all within reference ranges.

C4 and C1INH level of serum and C1INH function, for the seven HAE patients across the three families are presented in Table 3.

Genetic analysis

SERPING1 variant identification

SERPING1 gene testing results for probands and confirmed HAE patients in three families (Figure 3).

Family 1 (Figure 3A): Proband carried heterozygous missense variant (NM_000062.3) c.1034G>A (p.Gly345Glu) in *SERPING1* exon 7, causing glycine to glutamic acid substitution at position

TABLE 2 Clinical characteristics of symptomatic patients with HAE.

Clinical Data		Subject			
		Proband 1	Proband 2	Proband 3	Proband 3's younger sister
Sex		Male	Female	Female	Female
Age(years)		26	38	43	37
HAE type		HAE-1	HAE-2	HAE-1	HAE-1
Family history		None	Positive	Positive	Positive
Age at first onset (years)		22	16	39	32
Time from onset to diagnosis (years)		2	20	4	5
Edema attack sites		Limbs	Face, limbs, abdomen, pharynx	Face, larynx, limbs	Abdomen, limbs
Triggering factors		Exertion, compression, minor trauma	Cold exposure, emotional changes	Without apparent trigger	Without apparent trigger
Duration (days)		1–2	2–5	3–5	2–3
Attacks/year	Base-line	5	14	13	9
	Post-treatment	2	1	0	0
AECT scores	Base-line	11	4	2	5
	Post-treatment	12	13	13	14
Edema severity during attacks		Mild	Severe (requiring bed rest)	Severe (requiring bed rest)	Severe
Urticaria during attacks (Yes/No)		No	No	No	No
Current treatment (Danazol/Icatibant/Lanadelumab)		Icatibant for on-demand/acute attack treatment (30 mg per attack/dose)	(1) 2022.09.29-2023.07.30: Danazol 200 mg twice daily (oral), taken inconsistently. (2) 2023.11.20- present: lanadelumab 300 mg subcutaneously every 4 weeks (q4w).	(1) 2024.09.03-2024.12.10: lanadelumab 300 mg subcutaneously every 2 weeks (q2w). (2) 2024.12.27-present: lanadelumab 300 mg subcutaneously every 4 weeks (q4w).	(1) 2024.09.21-2024.12.19: lanadelumab 300 mg subcutaneously every 2 weeks (q2w). (2) 2025.01.07-present: lanadelumab 300 mg subcutaneously every 4 weeks (q4w).

TABLE 3 Results of the C4 and C1INH level of serum and C1INH function in patients with HAE.

Subject		Laboratory test (normal rages)		
		C4 level (g/L)	C1INH level (g/L)	C1INH function (%)
Family 1	Proband 1	0.03	0.11	2.5
Family 2	Proband 2	0.02	0.7	6.3
	Proband 2's elder son	0.08	0.59	60.8
	Proband 2's younger son	0.10	0.62	51.6
Family 3	Proband 3	0.02	0.08	13.1
	Proband 3's younger sister	0.04	0.06	19.7
	Proband 3's nephew	0.06	0.19	53.4

The normal range for C4 levels is 0.10–0.40 g/L, C1INH levels is 0.21–0.39 g/L, and normal C1INH function is defined as equal to or greater than 68%.

345. All 13 normal controls within the family were wild-type, without this variant.

Family 2 (Figure 3B): Proband carried heterozygous missense variant (NM_000062.3) c.1396C>T (p.Arg466Cys) in *SERPING1* exon 8, causing arginine to cysteine substitution at position 466. Her two sons (III-1, III-2) also carried this heterozygous variant. All 8 normal controls within the family were wild-type, without this variant.

Family 3 (Figure 3C): Proband carried heterozygous missense variant (NM_000062.3) c.1483G > A (p.Val495Ile) in *SERPING1* exon 8, causing valine to isoleucine acid substitution at position 495. Her sister and nephew (III-9, IV-8) also carried this heterozygous variant. All 17 normal controls within the family were wild-type at this locus, without this variant.

Bioinformatics analysis and variant classification

Family 1: *SERPING1* c.1034G > A. Annotated as "Pathogenic" in ClinVar (Variation ID: 2137099). No carrier frequency observed in East Asian populations in gnomAD_exome database (PM2_Supporting). Multiple pathogenic variants reported near this locus (PM1_Supporting) (25, 26). This variant detected in an unrelated HAE patient (PS4_Supporting) (27). REVEL prediction score 0.794, meeting supportive pathogenic evidence (PP3_Moderate). The proband exhibits typical C1INH deficiency and reduced functional activity, accompanied by a characteristic clinical phenotyp (PP4_Supporting). According to ACMG variant classification standards, this variant classified as "Likely Pathogenic" (PM2_Supporting + PP3_Moderate + PS4_Supporting + PM1_Supporting + PP4_Supporting).

Family 2: *SERPING1* c.1396C > T. Variant occurs in functional domain (PM1_Moderate). Annotated as "Pathogenic" in ClinVar (Variation ID: 3947) and "Disease-causing variant" in HGMD. No carrier frequency in East Asian populations in gnomAD_exome (PM2_Supporting). Variant detected in multiple unrelated HAE patients (PS4_Strong) (17, 28–31). REVEL score 0.721, meeting supportive pathogenic evidence (PP3_Supporting). The proband had elevated C1INH antigen levels with markedly reduced functional activity and presented with severe, HAE-specific clinical manifestations, including facial angioedema and colicky abdominal pain (PP4_Supporting). According to ACMG standards, this variant classified as "Pathogenic" (PM1_Moderate + PM2_Supporting + PP3_Supporting + PS4_Strong + PP1_Supporting + PP4_Supporting).

Family 3: *SERPING1* c.1483G > A not recorded in ClinVar, HGMD, or LOVD, with no carrier frequency in East Asian populations in gnomAD_exome database (PM2_Supporting). The variant perfectly co-segregated with HAE in a three-generation family (PP1_Supporting). The proband presented with severe, HAE-specific manifestations including laryngeal edema (PP4_Supporting). However, computational prediction yielded a low REVEL score of 0.12, suggesting benign impact (BP4_Supporting). According to ACMG standards, this variant classified as "Variant of Uncertain Significance" (PP1_Supporting + PM2_Supporting + PP4_Supporting + BP4_Supporting).

Local adaptation of treatment protocols

Personalized management strategies

Treatment selection comprehensively considered disease severity, attack frequency, laryngeal involvement history, and economic factors:

Mild phenotype (Family 1): Acute attacks treated on-demand with icatibant 30 mg subcutaneous. Patient trigger avoidance education with pre-filled syringes and emergency action plans.

Severe phenotype (Family 2): Previous laryngeal and gastrointestinal involvement, initiated lanadelumab long-term prophylaxis (300 mg subcutaneous every 4 weeks due to economic reasons; standard interval every 2 weeks). Breakthrough therapy: icatibant availability; ongoing monitoring with the AECT and the AE-QoL questionnaire.

Severe phenotype (Family 3): Previous laryngeal and facial involvement, initiated lanadelumab long-term prophylaxis (300 mg subcutaneous every 2 weeks, adjusted to every 4 weeks after 6 months). Breakthrough therapy: icatibant availability; AECT. Her sister, with prior extremity and gastrointestinal involvement, likewise initiated long-term prophylaxis with the same lanadelumab regimen, with availability of icatibant for breakthrough attacks and ongoing monitoring using the AECT and the AE-QoL questionnaire.

Follow-up and outcome monitoring

Family 1: During 25 months follow-up, proband experienced 4 mild limb edema episodes, all successfully controlled with icatibant, with symptom resolution within 2–4 h post-administration.

Family 2: During 21 months follow-up, proband experienced only 1 buttock edema episode, possibly related to prolonged

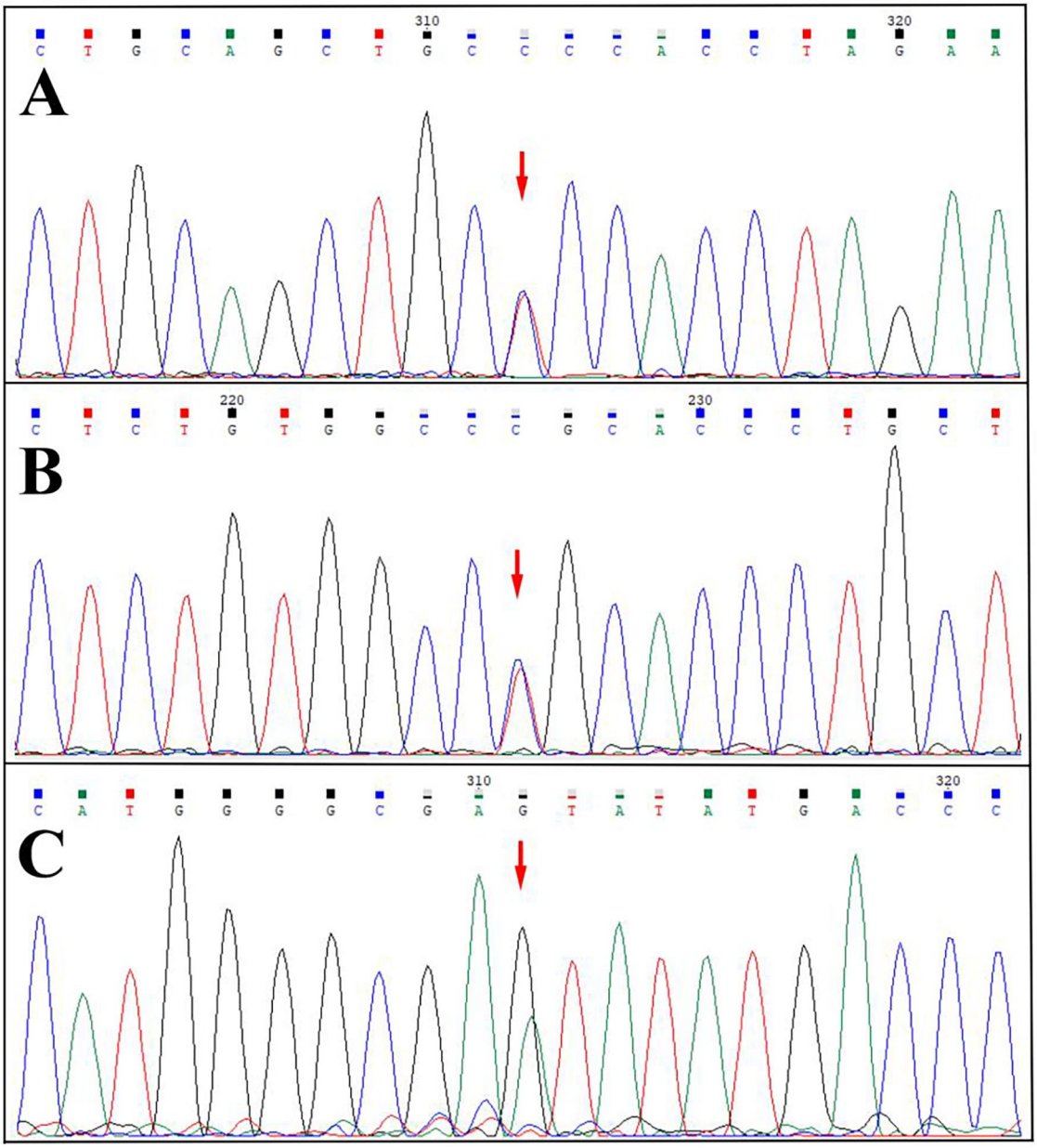

FIGURE 3
Sequencing map. **(A)** Heterozygous missense variant of the *SERPING1* gene (NM_000062.3) c.1034G>A (p.Gly345Glu) in the proband 1 (reverse sequencing). **(B)** Heterozygous missense variant of the *SERPING1* gene (NM_000062.3) c.1396C>T (p.Arg466Cys) in the proband 2. **(C)** Heterozygous missense variant (NM_000062.3) c.1483G > A (p.Val495Ile) in the proband 3.

sitting, resolving within approximately 2 h. At each visit over the past 6 months, AECT scores were ≥13, and the 4-week AE-QoL score was <11, indicating good control. Icatibant available for breakthrough attacks. Two asymptomatic sons received no prophylactic treatment but comprehensive trigger avoidance and early symptom recognition education with continued observation.

Family 3: During 12 months of follow-up, the proband had no angioedema attacks; at each visit over the past 6 months, AECT scores were ≥13, and the 4-week AE-QoLscore was <6, indicating good control, and icatibant was available for breakthrough attacks. Her sister likewise had no angioedema attacks during 12 months of follow-up (AECT scores at each visit were ≥14, and the 4-week AE-QoL score was <3). Her nephew was asymptomatic and not on prophylactic therapy; alongside disease education and follow-up management, he was provided with on-demand emergency medication (icatibant) for immediate access.

Discussion

This community case study established an integrated service pathway combining "standardized biochemistry—targeted

genetics—cascade screening—personalized treatment—electronic follow-up—quality management" at a regional center hub, demonstrating the potential feasibility of establishing specialized HAE services in resource-limited settings within a pilot framework. Evaluation of three unrelated families revealed HAE's phenotypic heterogeneity and age-related variability, demonstrating that family-directed testing can enhance early identification and enable risk-based management.

Our findings highlight major diagnostic gaps in resource-limited settings—including frequent misdiagnoses, unnecessary surgeries, and high economic burden—underscoring the need for standardized diagnostic and management frameworks. Since March 2023, our center has participated in the national HAE Diagnostic Capacity Standardization Project, aiming to improve clinical outcomes and quality of life. The project remains exploratory, requiring additional resource and policy support for broader implementation. In developing countries, genetic testing cost and accessibility pose specific challenges. Our approach establishes a pragmatic diagnostic pathway that prioritizes clinical phenotypes and biochemical markers (C4, C1INH concentration/function) as the first-line diagnostic basis, with genetic testing reserved for risk stratification and long-term management. This stepwise model balances cost, accessibility, and clinical accuracy, enabling feasible and equitable HAE diagnosis and treatment in constrained health systems. The clinical presentations in our cohort exemplified HAE phenotypic heterogeneity. The three probands' earliest onset was at age 16, with the longest interval from onset to diagnosis reaching 20 years, consistent with domestic reports: average onset age 21.25 years, approximately 75% experiencing first attacks at ages 10–30, average diagnostic delay approximately 12.64 years (19). Delays reflect HAE's rarity and variable presentations, easily confused with common diseases. Due to low HAE awareness among physicians and patients, patients often seek multiple consultations without diagnosis. Initial presentations may involve airways or abdomen, risking asphyxiation or unnecessary surgical intervention. International studies show HAE patients average 4.4 physician consultations before diagnosis, 65% experience misdiagnosis, and 24% undergo unnecessary exploratory abdominal surgery (32). In Family 2, the proband underwent exploratory laparotomy for suspected acute abdomen and multiple hospitalizations for misdiagnosed acute pancreatitis/appendicitis. In Family 3, the proband underwent tracheal intubation for laryngeal edema. Many undiagnosed patients face higher mortality risk due to lack of timely diagnosis and treatment (33). China currently lacks data on emergency department HAE acute attack presentations.

Using three-generation pedigrees to identify high-risk relatives, implementing biochemical-genetic targeted testing for at-risk members, and embedding counseling into outpatient workflows significantly improved adherence and completion rates. We found two genetically confirmed children in Family 2 (III-1, III-2, twins, 9 years old) and one genetically confirmed patients in Family 3 (IV-8, 11 years old) had biochemical abnormalities but were clinically asymptomatic, demonstrating HAE's variable expressivity and age-related expression. HAE's clinical spectrum is extremely broad, from completely asymptomatic to life-threatening attacks; attack frequency and severity can differ significantly within the same family (19). Onset age varies greatly, with over 75% experiencing first attacks before age 30, typically worsening during ages 20–29 (19, 34). Previous studies suggest up to 30%–34% are *de novo* pathogenic variants, possibly without positive family history (17, 18, 35), thus negative family history does not exclude HAE. This supports early, parallel gene–biochemistry testing for at-risk relatives regardless of current symptom status. Once a familial pathogenic variant is defined, targeted Sanger sequencing provides a cost-effective cascade testing method, reducing turnaround time and unnecessary next-generation sequencing expenses. Integrating biochemical results and genetic confirmation allows same-visit triage, rapid decision-making, and implementation at regional laboratories with minimal infrastructure. From a public health standpoint, this family-directed dual-track strategy enhances early detection and cost-effectiveness in resource-limited settings. In our pilot, three asymptomatic carriers were identified prospectively, enabling risk stratification and preventive education. Beyond diagnostic yield, this strategy strengthens long-term management through anticipatory guidance and emergency readiness, reducing risk of severe attacks and emergency burden. Importantly, it complements rather than replaces comprehensive genetic testing, providing a phased and scalable framework adaptable to clinical and economic realities.

Treatment response differences among the three probands emphasized the importance of personalized treatment. In this study, Family 1's proband achieved success with on-demand icatibant for mild attacks, while Family 2 and 3 probands required long-term lanadelumab prophylaxis due to severe phenotypes. This aligns with current international guidelines advocating personalized treatment based on disease burden, attack frequency, and quality of life impact (4). Extending lanadelumab dosing intervals to every 4 weeks (standard every 2 weeks) due to economic considerations while still achieving adequate disease control highlights treatment accessibility issues in resource-limited settings. This underscores the critical issue of treatment access in resource-limited settings: with appropriate risk communication and close monitoring, dose-interval extension may serve as a context-specific trade-off under resource constraints. Although Non-standard dosing was effective in these cases; however, expanding access to modern HAE treatments remains urgent.

Management of HAE should be phenotype driven, implementing guideline-concordant on-demand therapy and indicated prophylaxis; a VUS should not serve as the sole basis for clinical decision making, and current molecular findings should be integrated into the evidence base with periodic reinterpretation. For patients with strong clinical and biochemical evidence of HAE but negative conventional sequencing, we recognize the importance of additional genetic analyses such as MLPA to detect large deletions/duplications; our future standard protocol will incorporate these comprehensive methods to identify all possible pathogenic mechanisms, ensuring complete molecular characterization while maintaining phenotype-based management decisions. Identifying pathogenic variants in asymptomatic carriers presents genetic counseling challenges. Among Families 2

and 3, three asymptomatic individuals carrying pathogenic variants should be enrolled in longitudinal, family-centered follow-up. In addition to systematic education on trigger avoidance, prodrome recognition, and emergency management, psychosocial support should be emphasized, including communication around uncertainty and anxiety with stress-reduction strategies, individualized guidance on academic and sports participation, facilitation of effective intra-family communication and caregiver stress management, and a readily actionable, always-available written emergency plan to mitigate fear and helplessness. For minors and their families, we will refine counseling points (trigger avoidance, prodrome recognition, emergency planning, and psychological coping) and specify the follow-up cadence: reassessment every 6–12 months during stable periods; every 3–6 months during puberty or other major physiologic/environmental transitions, prior to elective surgery or dental procedures, and at initiation or discontinuation of estrogen-containing therapies or angiotensin-converting enzyme inhibitors; with immediate evaluation for any prodromal symptoms or suspected attacks. Follow-up will include baseline profiling (definition of the familial subtype and individual risk; documentation of family history of attacks, potential trigger exposures, past medical and medication history), psychosocial screening (anxiety/depression, school and peer interactions, family stress), and health literacy assessment; laboratory monitoring will be tailored to subtype, with clinical surveillance prioritized in normal–C1INH forms and testing frequency adjusted pragmatically. At each visit, we will review symptom/trigger diaries, quality-of-life measures, and participation in learning and sports, and update the individualized emergency plan. Patient education and support systems will be strengthened through standardized materials and family meetings, training in stress-management and coping skills, and referral to psychological counseling or peer support as needed; we will also liaise with schools or childcare institutions, providing physician letters and emergency cards to reduce absenteeism and stigma and to optimize participation in school activities. Ongoing trigger avoidance and medication review will be maintained, with personalized travel and exercise advice and perioperative planning; access to and training in the use of on-demand medications will be ensured, medical alert identification provided, red-flag symptoms and care-seeking thresholds defined, and emergency contact and follow-up pathways established (including telehealth or rapid-access appointments). Finally, we will plan a smooth transition from pediatric to adult care and provide counseling on contraception and pregnancy management, favoring non-estrogen options and individualized care during pregnancy.

Notably, our findings expand the Chinese *SERPING1* pathogenic variant spectrum to 98 variants (17, 36–40), with c.1034G > A and c.1483G > A identified in Chinese patients for the first time, suggesting the domestic variant spectrum is broader than previously recognized. Our program also represents one of the first coordinated HAE service model in China. This further emphasizes that targeted locus testing within families can significantly reduce costs and turnaround time, suitable for large-scale cascade screening in resource-limited scenarios.

Key program elements demonstrated in this pilot and considerations for pathway optimization

This proof-of-concept program successfully implemented several integrated elements under specific enablers—intensive case management, foundation-subsidized drug access, and strong institutional support. These measures demonstrate feasibility within a controlled pilot environment rather than universal applicability. Scaling up will require context-tailored adaptation, resource assessment, and capacity building aligned with local healthcare and economic realities.

Prioritizing C4 and C1INH function testing in suspected cases and family screening as low-cost, highly accessible "triage tools," followed by family-directed sequencing, improves efficiency and affordability. Based on attack frequency, involved sites (especially laryngeal/gastrointestinal), AECT, AE-QoL and previous crisis history, adopting a stepwise strategy of "on-demand icatibant + breakthrough treatment assurance + lanadelumab prophylaxis"; under economic constraints, cautiously extending lanadelumab dosing intervals with close monitoring. Setting electronic medical record (EMR) embedded alerts for patients with "recurrent non-pitting edema," "abdominal cramps with vomiting but inconsistent imaging/enzymes," or "emergency laryngeal event history" triggers HAE screening workflows, reducing unnecessary exploratory surgery and intubation risks. Implement multidisciplinary, tiered care with coordinated primary-level referral pathways and teleconsultation to minimize repeat visits and avoidable interventions. A digital follow-up program should integrate attack events, emergency utilization, and medication responses into a visual dashboard to enable bidirectional alerts and early intervention. Scale-up should concurrently advance workforce training and infrastructure, drug-access strategies, and practical implementation at the awareness/cultural level, with concrete elements including SOP templates, training modules for primary hospitals, telemedicine support, EMR flags, and participation in EQA to ensure testing consistency. Address access barriers to icatibant and lanadelumab; through medical insurance coverage and foundation assistance, we significantly reduced patient economic burdens: the out-of-pocket cost for icatibant is approximately $194 (originally $607), and the out-of-pocket cost of lanadelumab is approximately $207 (originally $1,989).

Limitations and risk control

Sample size and representativeness: Only 3 families (with 7 confirmed patients) were enrolled; referral-based sampling may overestimate severe phenotype proportions, limiting generalizability.

Follow-up duration: 12–25 months insufficient to assess long-term outcomes and complications; continued tracking needed to verify sustainability and safety.

Dosing strategy bias: Some individuals adopted non-standard interval prophylactic regimens; while effective under monitoring, cannot replace evidence-based standards; requires validation in larger samples with longer follow-up.

Finally, as a pre–post observational implementation study, this work is subject to inherent limitations related to small sample size, potential severity-related referral bias, and constraints on causal inference.

Conclusion and future directions

This community case study demonstrates successful implementation of a comprehensive HAE diagnosis and management program at a Chinese regional center. Through systematic family screening, genetic characterization, and localized treatment protocols, we improved clinical outcomes and, for the first time in Chinese patients, identified two novel *SERPING1* variants.The program illustrates what is achievable in HAE service development within a resource-limited setting when supported by dedicated institutional commitment, subsidized medication access, and intensive case management. Our experience emphasizes the importance of local adaptation of international guidelines and provides insights into the opportunities and challenges of implementing specialized rare disease services in similar contexts. Notably, the gap between the administrative catchment population and the number of confirmed cases reflects underdiagnosis and early-phase program constraints rather than true prevalence. Continued expansion and optimization of such programs is needed to address the significant disease burden caused by HAE underdiagnosis and inadequate treatment in China.

Future research directions

Multicenter prospective cohorts: Collaborating with inter-provincial centers to unify quantified outcomes (attack rates, laryngeal events, AECT/AE-QoL, emergency/hospitalization, and resource utilization) to generate real-world evidence.

Standardization and quality systems: Establishing provincial EQA, unified SOPs, digital follow-up systems, and quality indicator dashboards to make testing and treatment "more consistent, comparable, and sustainable."

Accessibility and equity: Linking with insurance/charitable programs to systematically evaluate cost-effectiveness and equity of stratified prevention strategies, expanding modern HAE treatment accessibility through policy and payment innovations.

Progress and plans: Multicenter expansion and a prospective registry are underway; the multicenter cohort framework, harmonized outcome set, and registry timeline have been defined to further enhance external validity and dissemination/implementation value.

Data availability statement

The datasets generated for this study have been deposited in the ClinVar database under accession numbers Variation ID: 2137099 and Variation ID: 3947. These data are publicly available through the ClinVar repository at https://www.ncbi.nlm.nih.gov/clinvar/.

Ethics statement

The studies involving humans were approved by Institutional Ethics Committee of Henan Provincial People's Hospital (Ethics Approval Number: 2022-2211273107). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

WD: Data curation, Resources, Formal analysis, Project administration, Investigation, Writing – review & editing, Methodology, Supervision, Validation, Writing – original draft. ZM: Methodology, Visualization, Data curation, Conceptualization, Writing – review & editing. KY: Investigation, Conceptualization, Validation, Methodology, Supervision, Writing – review & editing. QZ: Methodology, Formal analysis, Writing – review & editing, Conceptualization, Investigation. XL: Formal analysis, Methodology, Writing – review & editing, Validation. WZ: Writing – review & editing, Validation, Methodology. WG: Investigation, Methodology, Writing – review & editing. SW: Methodology, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition.

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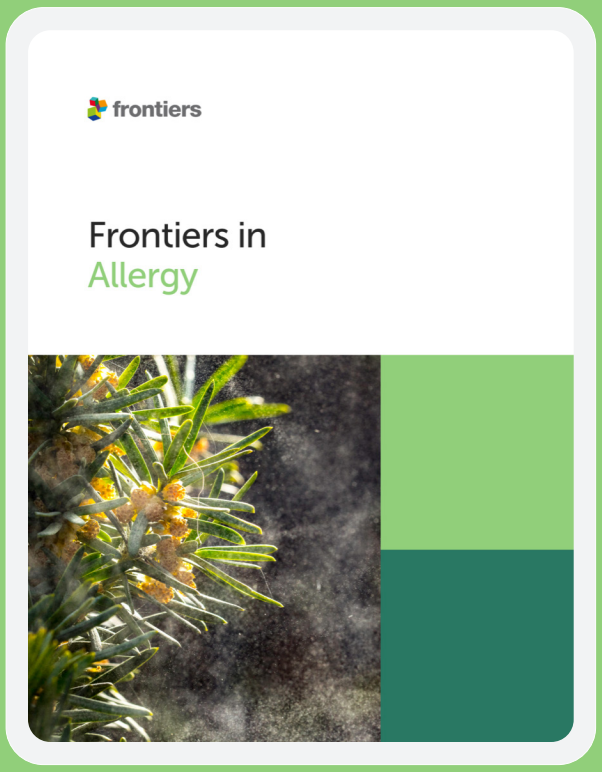

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