Clinical validation of a novel biomarker assay to characterise bradykinin-mediated angioedema in prospective and biobank plasma samples

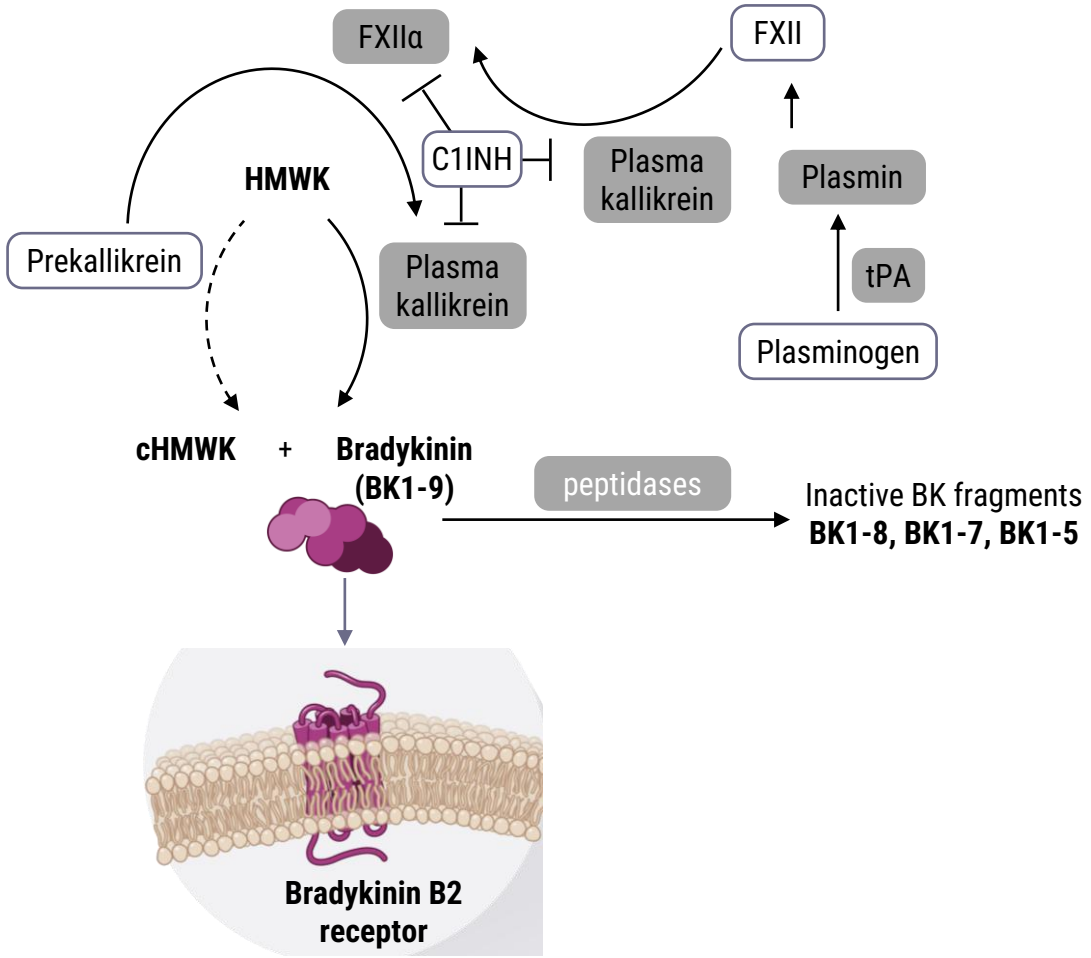
E. Pardali, H.R. Horváth, O. Domenig, D. van Oyen, D. Sexton, G. Zahn, A. Lesage, H. Farkas

14th C1-inhibitor Deficiency & Angioedema Workshop
Budapest, Hungary, 29 May - 1 June 2025

Conflicts of interest disclosure

E.P.: employee of Pharvaris, holds stocks/stock options in Pharvaris; **H.R.H.:** received travel grants from Takeda and CSL Behring; **O.D.:** CEO of Attoquant Diagnostics GmbH; **D.v.O.:** employee of Attoquant Diagnostics GmbH; **D.S.:** Principal at Sexton Bio Consulting, LLC; **G.Z.:** employee of Globalization Partners GmbH, consultant to Pharvaris, holds RSU; **A.L.:** employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma; **H.F.:** received research grants from CSL Behring, Pharming, Takeda and served as an advisor for these companies and BioCryst, Intellia, KalVista, ONO Pharmaceutical, Pharvaris; has participated in clinical trials/registries for BioCryst, CSL Behring, KalVista, Pharming, Pharvaris, Takeda.

There is no biomarker assay to diagnose BK-mediated AE with normal C1INH



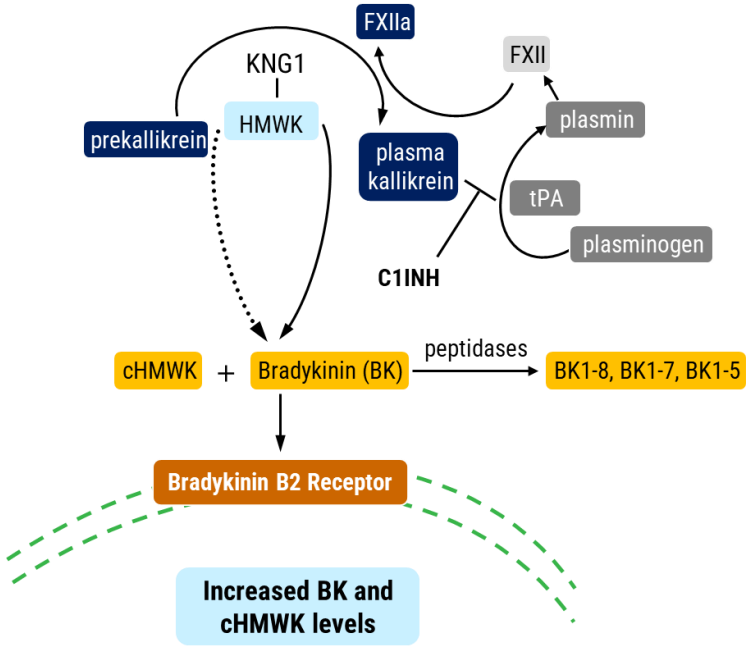
- BK plays a key role in the pathophysiology of different types of angioedema
  - HAE-C1INH
  - AAE-C1INH
  - HAE-nC1INH (e.g. HAE-FXII, HAE-KNG1, HAE-PLG)
  - AE-UNK?
- Diagnostic assays for early diagnosis of BK-AE with normal C1INH are lacking
- KKS biomarker detection has been challenging due to pathway sensitivity to *ex vivo* activation and instability of kinins[1]

AE: angioedema; AAE-C1INH: Acquired angioedema with C1INH deficiency; AE-UNK: Angioedema of unknown etiology; BK: Bradykinin; C1INH: C1 inhibitor; cHMWK: cleaved High molecular weight kininogen; FXII(a): Factor 12(a); HAE-C1INH: Hereditary angioedema with C1INH deficiency; HAE-nC1INH: HAE with normal C1INH; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-KNG: HAE with genetic variant in kininogen *KNG* gene; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HMWK: High molecular weight kininogen; KKS: Kallikrein kinin system; tPA: tissue plasminogen activator;

1. Kaplan AP, Maas C, The Search for Biomarkers in Hereditary Angioedema, Front Med (Lausanne), 2017; 2. Reshef A, et al. J Allergy Clin Immunol. 2024;154(2):398-411.e1.

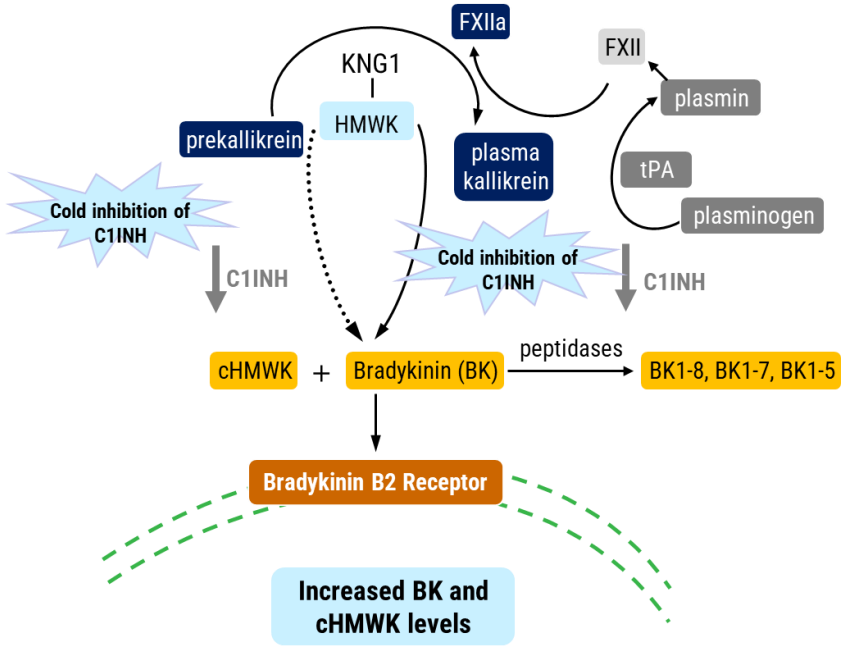
“Dual Approach” to Biomarker Assessment

Absolute KKS Biomarker Levels



Assay kinins and HMWK in EDTA plasma containing protease inhibitors

KKS Pathway Sensitivity to Cold Trigger

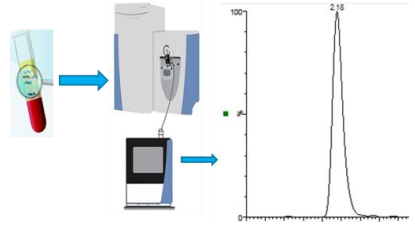


Assay kinins and HMWK in EDTA plasma at baseline and following exposure to cold temperature

Kinin peptides

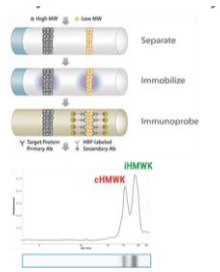
BK1-9, BK1-8, BK1-7, BK1-5, Kallidin

Ultra-high performance liquid chromatography-MS/MS (UPLC-MS/MS)



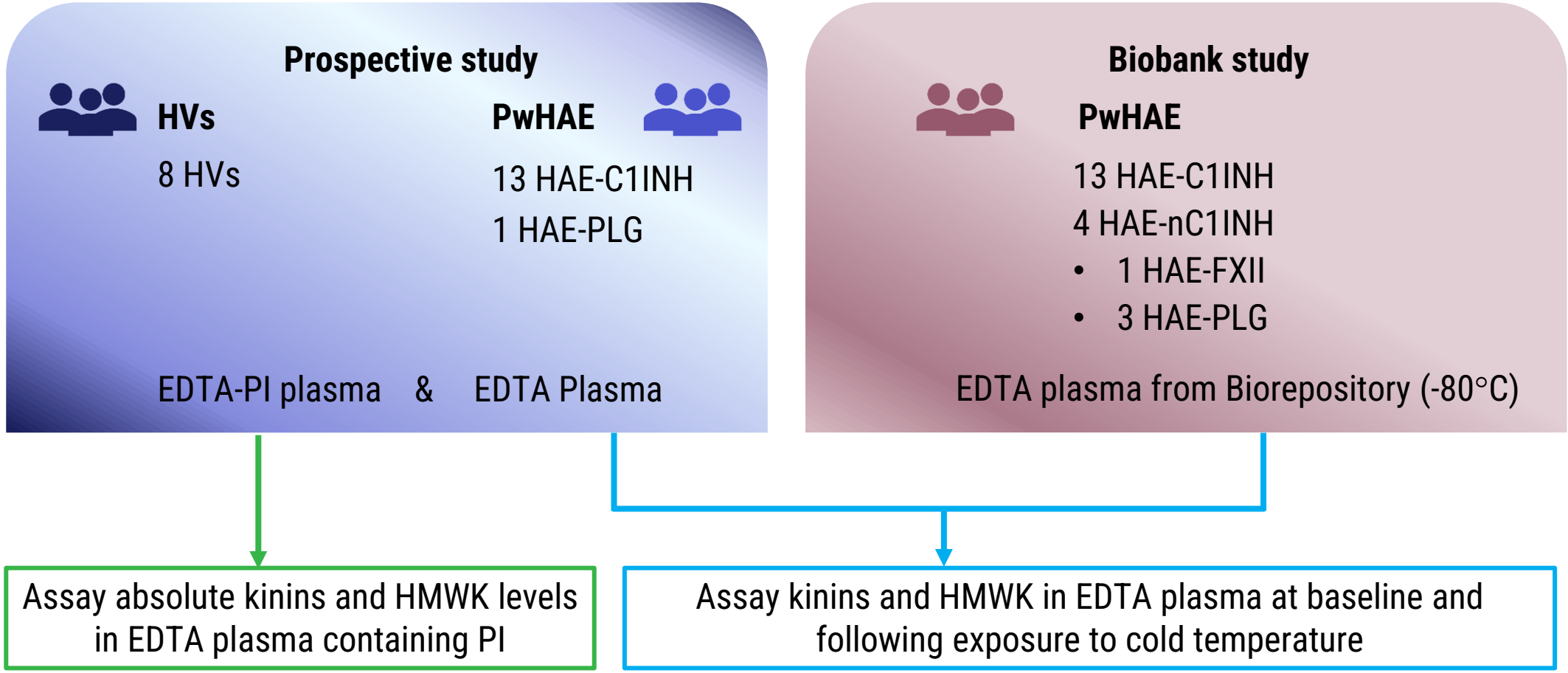
iHMWK and cHMWK

Simple Western Size (SWS) capillary immunoblotting assay



BK: Bradykinin; C1INH: C1 inhibitor; HMWK: high molecular weight kininogen; cHMWK: cleaved high molecular kininogen; iHMWK: intact high molecular kininogen; FXII: Factor 12; SWS: Simple Western Size ; UPLC-MS/MS: Ultra-high performance liquid chromatography-mass spectrometry / mass spectrometry

Clinical validation of KKS biomarker assays
Study design



BK: Bradykinin; EDTA: ethylenediaminetetraacetic acid; HAE-C1INH: Hereditary angioedema with C1 inhibitor deficiency; HAE-nC1INH: HAE with normal C1 inhibitor; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HMWK: high molecular weight kininogen; KKS: Kallikrein kinin system; PwHAE: people with HAE; PI: protease inhibitor cocktail

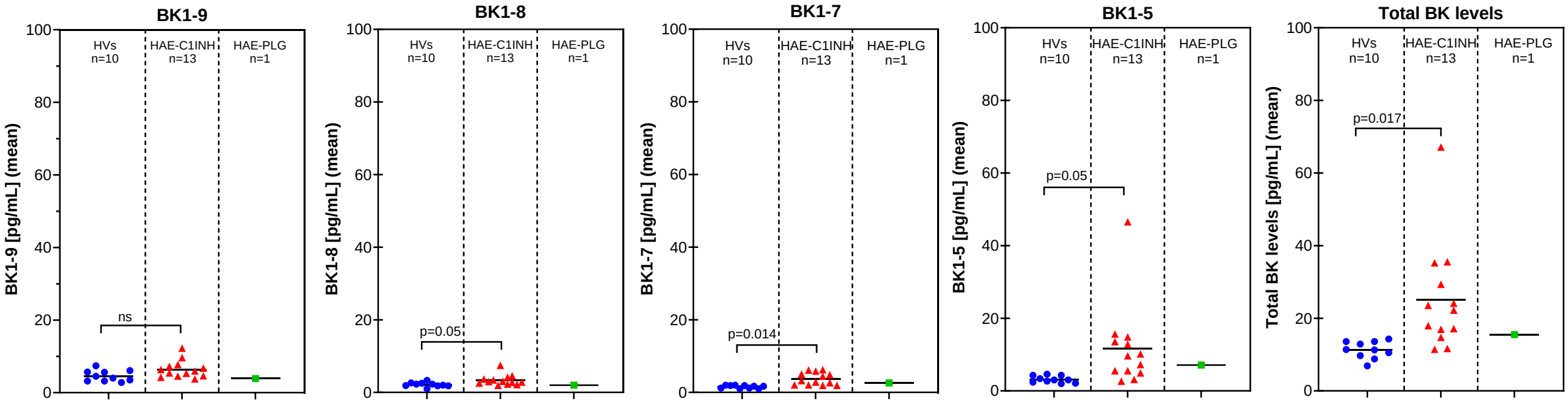
Demographics of participants in prospective study at the Hungarian Angioedema Center of Reference and Excellence

- EDTA plasma samples with and without protease inhibitors were obtained from PwHAE at remission and HVs
- At time of blood collection, pw HAE
  - were not on LTP therapies
  - did not receive ODT, at least 4 days prior to sample collection

Demographics	HVs n=8	HAE-C1INH n=13	HAE-nC1INH HAE-PLG n=1
Age in years, mean (SD)	37.2 (10.4)	40.7 (10.7)	39
Sex: Male/female, n (%)	4 (40.0) / 6 (60.0)	6 (46.2) / 7 (53.8)	1(100.0) / 0 (0.0)
Race: White/other, n	10 / 0	12 / 1	1 / 0
HAE type, n (%)			
HAE-1	n/a	12 (92.3)	n/a
HAE-2	n/a	1 (7.7)	n/a
HAE-PLG	n/a	n/a	1 (100)

EDTA: ethylenediaminetetraacetic acid; HAE: hereditary angioedema; HAE-C1INH: hereditary angioedema with C1 inhibitor deficiency; HAE-nC1INH: hereditary angioedema with normal C1 inhibitor; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; LTP: long term prophylaxis; ODT: on demand therapies; PwHAE: people with HAE; SD: standard deviation

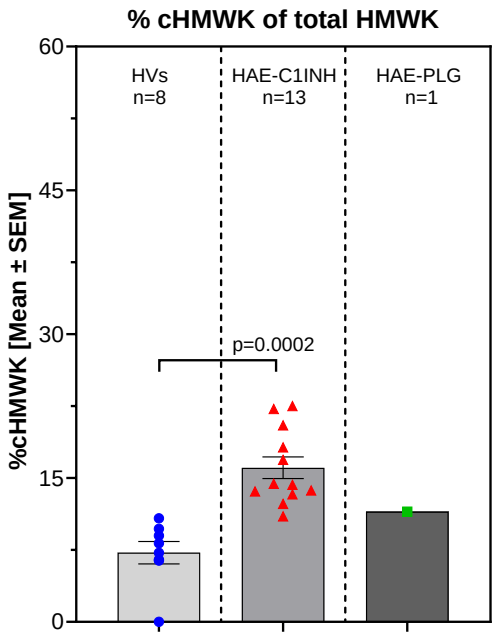
Low absolute kinin levels in plasma from people with HAE-C1INH and HAE-nC1INH at remission

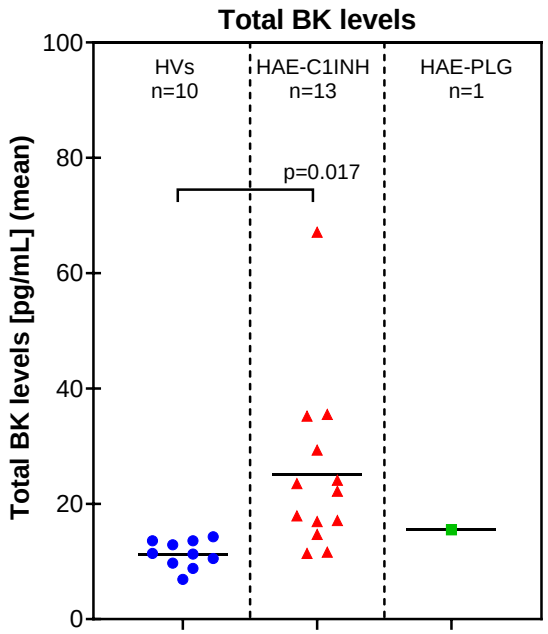


- Absolute kinin levels were analysed in EDTA-PI plasma using the qualified UPLC-MS/MS method
- Mean levels of BK1-9 slightly increased in plasma samples from PwHAE-C1INH vs HVs
- Mean levels of BK1-5, BK1-7, BK1-8 and total kinin levels increased in plasma from PwHAE-C1INH vs HVs
- Kinin levels in an individual with HAE-PLG that never experienced AE attacks, were not increased vs HVs

BK: bradykinin; EDTA-PI: ethylenediaminetetraacetic acid with protease inhibitors; HAE-C1INH: hereditary angioedema with C1INH deficiency; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; PwHAE: people with HAE; UPLC-MS/MS: Ultra-high performance liquid chromatography-mass spectrometry / mass spectrometry

Increased cHMWK levels in plasma from PwHAE-C1INH compared to HVs

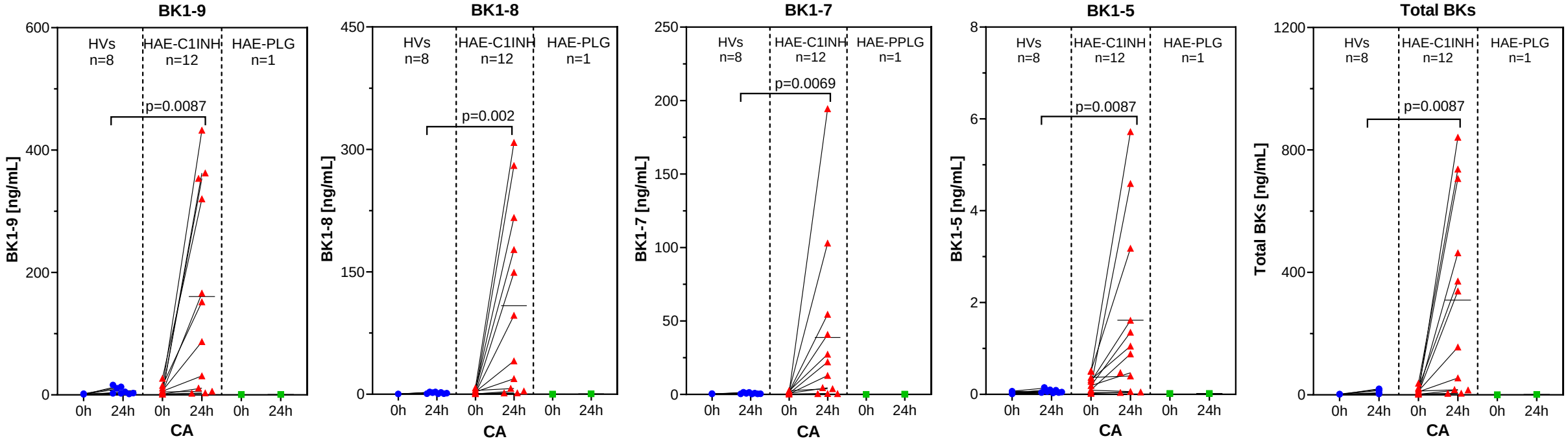




- Absolute iHMWK and cHMWK levels were analysed in EDTA-PI plasma using the capillary immunoassay
- cHMWK levels significantly increased in plasma samples from PwHAE-C1INH at remission vs HVs
- Results from iHMWK and cHMWK analysis are in line with the results from kinin analysis in plasma samples from prospective study

BK: bradykinin; EDTA-PI: ethylenediaminetetraacetic acid with protease inhibitors; HAE-C1INH: hereditary angioedema with C1 inhibitor deficiency; HAE-nC1INH: hereditary angioedema with normal C1 inhibitor levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; cHMWK: cleaved high molecular weight kininogen; iHMWK: intact high molecular weight kininogen; PwHAE: people with HAE

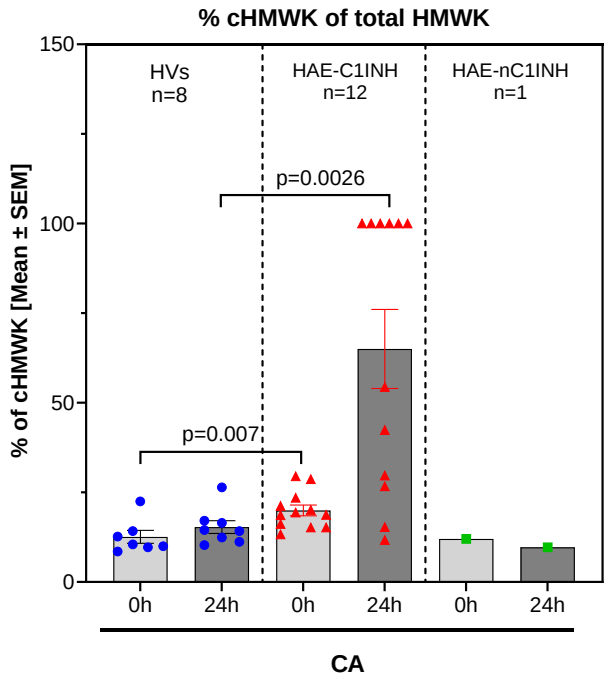
Cold activation revealed increased KKS sensitivity in PwHAE-C1INH at remission

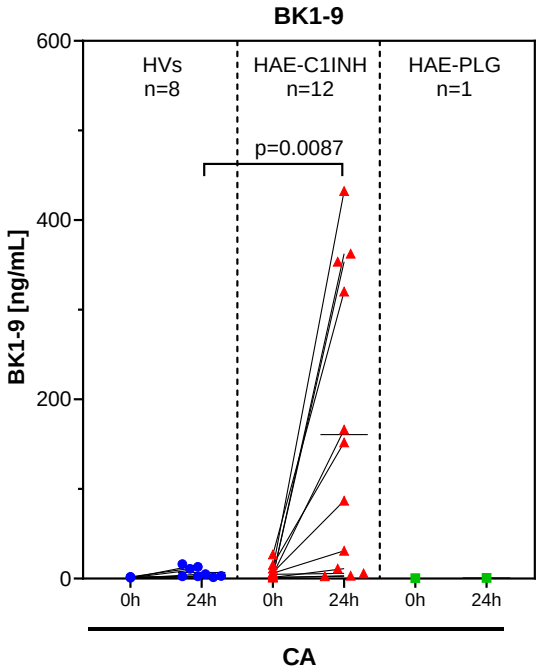


- Kinin levels were analysed before (baseline) and following exposure to cold temperature (4°C) for 24 hours
- Cold activation caused elevated BK levels, indicative of KKS pathway hypersensitivity in PwHAE-C1INH
- PwHAE-C1INH showed a remarkably sensitive KKS cascade compared to HVs
- One individual with HAE-PLG without history of AE attacks did not respond to cold activation

AE: angioedema; BK: bradykinin; CA: cold activation; HAE-C1INH: hereditary angioedema with C1 inhibitor deficiency; HAE-nC1INH: hereditary angioedema with normal C1 inhibitor levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; KKS: Kallikrein kinin system; PwHAE: people with HAE

Cold activation resulted in increased cleavage of iHMWK in plasma from Pw HAE-C1INH and HAE-nC1INH at remission in prospective study





- iHMWK and cHMWK levels were analysed before and following exposure to cold temperature (4°C) for 24 hours
- Cold activation induced cleavage of HMWK resulting in increased cHMWK levels in Pw HAE-C1INH vs HVs
- One asymptomatic Pw HAE-PLG did not respond to cold activation
- Results from iHWMK and cHMWK analysis were in line with the results from kinin analysis

BK: bradykinin; CA: cold activation; cHMWK: cleaved high molecular weight kininogen; HAE-C1INH: hereditary angioedema with C1INH deficiency; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy controls; iHMWK: intact high molecular weight kininogen; Pw HAE: people with HAE; SEM: standard error of the mean

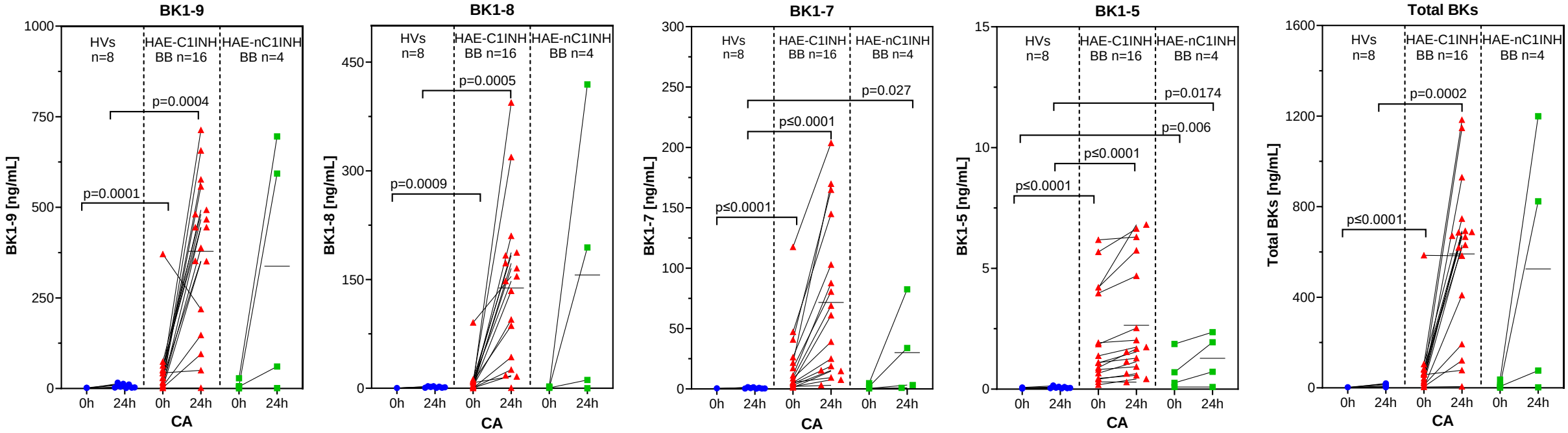
Demographics of Biobank samples from the Biorepository of the Hungarian Angioedema Center of Reference and Excellence

- EDTA plasma samples from PwHAE, stored at -80°C, were obtained from the Biorepository of the Hungarian Angioedema Center of Reference and Excellence
- Samples were obtained at remission
- At the time of blood collection, PwHAE
  - were not on LTP therapies
  - did not receive ODT, at least 4 days prior to sample collection

Demographics	HVs n=8	HAE-C1INH n=16	HAE-nC1INH n=4
Age in years, mean (SD)	37.2 (10.4)	32.9 (12.3)	38.5 (12.8)
Sex: Male / female, n (%)	4 (40.0) / 6 (60.0)	7 (43.7) / 9 (56.3)	2 (50.0) / 2 (50.0)
Race: White/other, n	10 / 0	15 / 1	4 / 0
HAE-C1INH type, n (%)			
HAE-1	n/a	14 (87.5)	n/a
HAE-2	n/a	2 (12.5)	n/a
HAE-nC1INH type, n (%)			
HAE-FXII	n/a	n/a	1 (25)
HAE-PLG	n/a	n/a	3 (75)

AE: angioedema; EDTA: ethylenediaminetetraacetic acid; HAE: hereditary angioedema; HAE-C1INH: hereditary angioedema with C1 inhibitor deficiency; HAE-nC1INH: hereditary angioedema with normal C1 inhibitor; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; LTP: long term prophylaxis; ODT: on demand therapies; PwHAE: people with HAE; SD: standard deviation

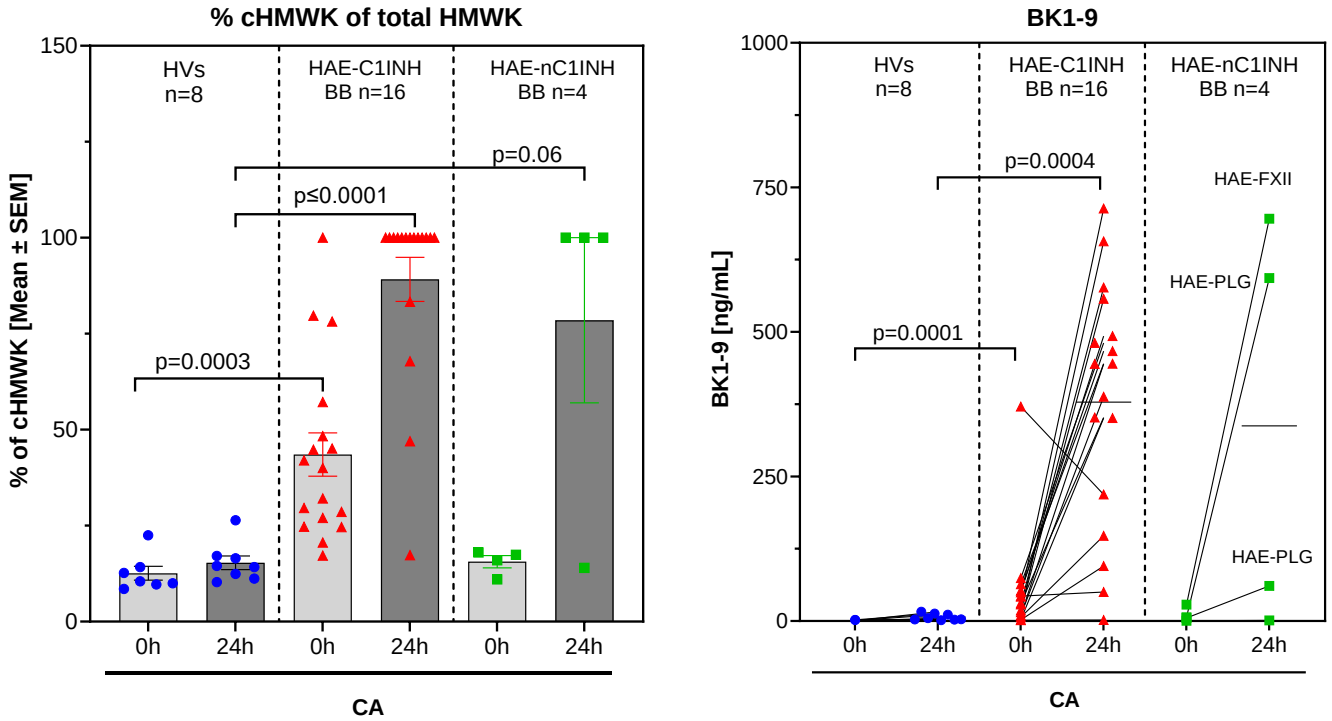
Analysis of biobank plasma samples from PwHAE-C1INH and HAE-nC1INH can be used to study KKS hypersensitivity revealed by cold activation



- Kinin levels were analysed before and after exposure to cold temperature (4°C) for 24 hours
- Cold activation caused elevated BK levels in samples from PwHAE-C1INH and HAE-nC1INH, indicative of KKS pathway sensitivity to triggers
- Clear differentiation of HVs and individuals with HAE-C1INH and HAE-nC1INH
- Significantly higher BK1-9 and total BK levels in EDTA plasma from PwHAE-C1INH also at baseline (no CA)

BB: biobank; BK: bradykinin; CA: cold activation; HAE-C1INH: hereditary angioedema with C1 inhibitor deficiency; HAE-nC1INH: hereditary angioedema with normal C1 inhibitor levels; HVs: healthy volunteers; KKS: Kallikrein kinin system; PwHAE: people with HAE

Cold activation revealed KKS sensitivity resulting in increased cleavage of HMWK in biobank samples from PwHAE-C1INH and HAE-nC1INH



- iHMWK and cHMWK levels were analysed before and after exposure to cold temperature (4°C) for 24 hours
- cHMWK levels, relative to total levels were increased in PwHAE
- Results from iHWMK and cHMWK analysis were in line with the results from kinin analysis

BK: bradykinin; CA: cold activation; cHMWK: cleaved high molecular weight kininogen; HAE-C1INH: hereditary angioedema with C1 inhibitor deficiency; HAE-C1INH: hereditary angioedema with normal C1 inhibitor levels; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy controls; iHMWK: intact high molecular weight kininogen; KKS: Kallikrein kinin system; Pw HAE: people with HAE

Conclusions

- The qualified kinin assay can be used to reliably measure bradykinin levels and characterise people with BK mediated angioedema
- Results are supported by the iHMWK and cHMWK biomarker assay
- The kinin biomarker assay can be used to assess KKS sensitivity in HAE-C1INH and HAE-nC1INH using cold activation
- Importantly, the assay allows evaluation of KKS pathway hypersensitivity in plasma samples from biorepositories for patients with different AE types
- The clinically validated BK assay may become a key tool for identifying, studying, and managing BK-mediated pathologies including angioedema