Clinical validation of a kinin biomarker assay to characterize bradykinin-mediated angioedema

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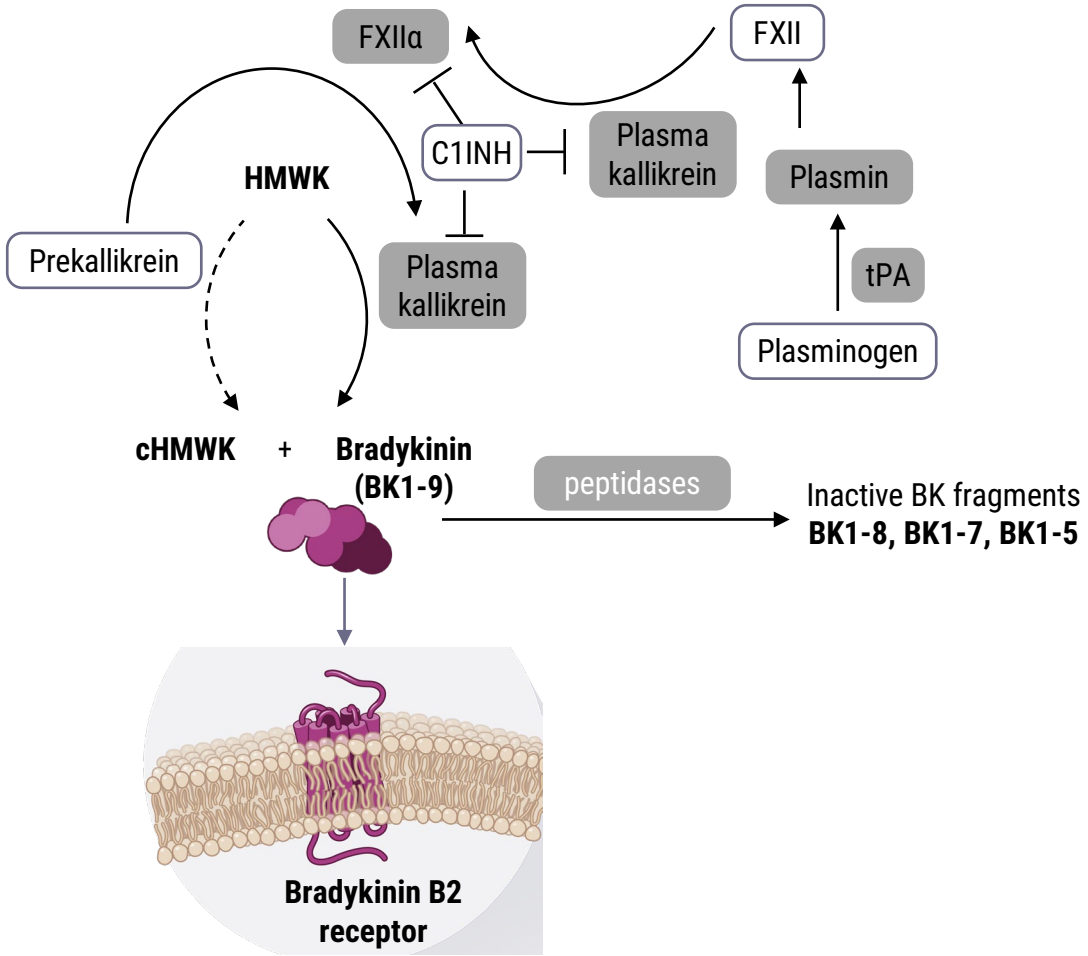
Conflicts of interest disclosure

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.

Availability of a kinin biomarker assay could facilitate the diagnosis of BK-mediated AE with normal C1INH



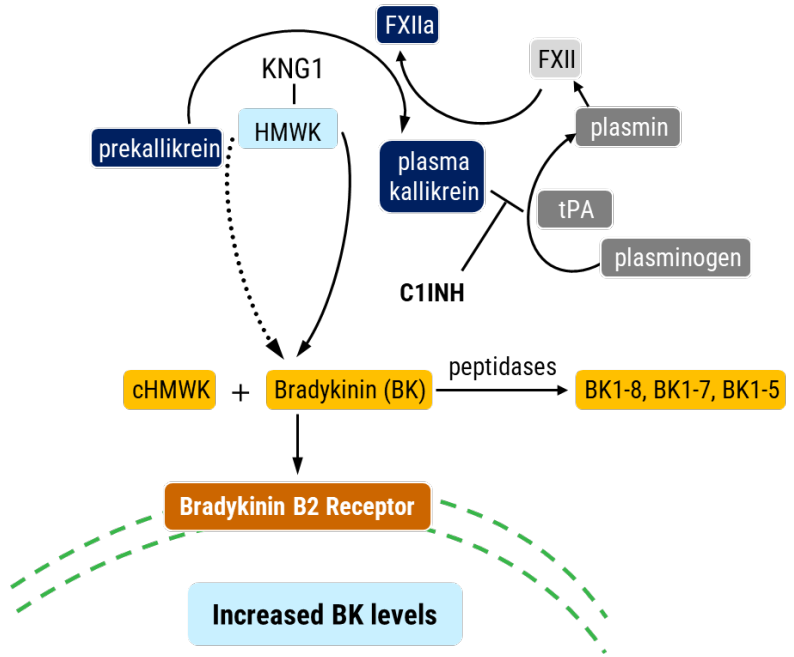
- BK plays a key role in the pathophysiology of different types of angioedema
  - HAE-C1INH
  - AAE-C1INH
  - HAE-nC1INH (e.g. HAE-FXII, HAE-KNG, HAE-PLG)
  - (H)AE-UNK?
- Diagnostic assays for AE-BK with normal C1INH are lacking[1] ➔ higher disease burden[1]
- Detection of kinin biomarkers has been challenging due to pathway sensitivity to *ex vivo* activation and instability of kinins[2]

AAE-C1INH: Acquired angioedema due to C1INH deficiency; AE: angioedema; AE-BK: Bradykinin-mediated AE; BK: Bradykinin; C1INH: C1 inhibitor; cHMWK: cleaved High molecular weight kininogen; FXII(a): factor 12 (activated); HAE-C1INH: Hereditary angioedema due to C1INH deficiency; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-KNG: HAE with genetic variant in kininogen *KNG* gene; HAE-nC1INH: HAE with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; (H)AE-UNK: (hereditary) angioedema of unknown etiology; HMWK: High molecular weight kininogen; tPA: tissue plasminogen activator.

1. Jones D et al. J Asthma Allergy 2023; 2. Kaplan AP, Maas C, Front Med (Lausanne), 2017;

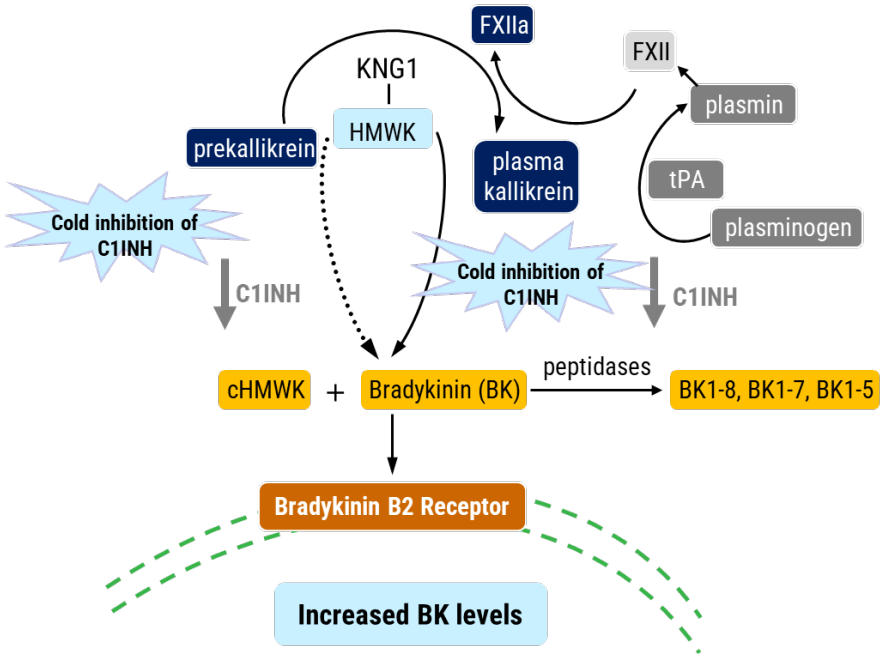
“Dual Approach” to kinin biomarker assessment

Absolute kinin levels



Assay absolute kinin peptides in EDTA plasma containing protease inhibitors (PI) at baseline

BK-forming Pathway Sensitivity to Cold



Assay kinin peptides in EDTA plasma at baseline and following exposure to cold temperature (cold activation)

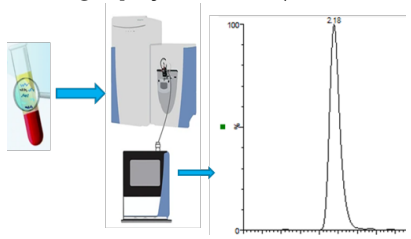
BK: Bradykinin; C1INH: C1 inhibitor; cHMWK: cleaved high molecular kininogen; EDTA: ethylenediaminetetraacetic acid; FXII(a): factor 12 (activated); HMWK: high molecular weight kininogen; KNG1: kininogen gene; PI: protease inhibitors; tPA: tissue plasminogen activator

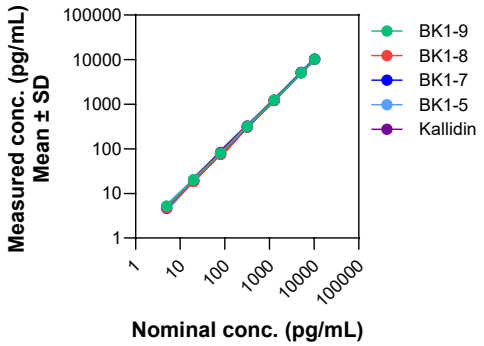
UPLC-MS/MS kinin biomarkers developed and analytically qualified

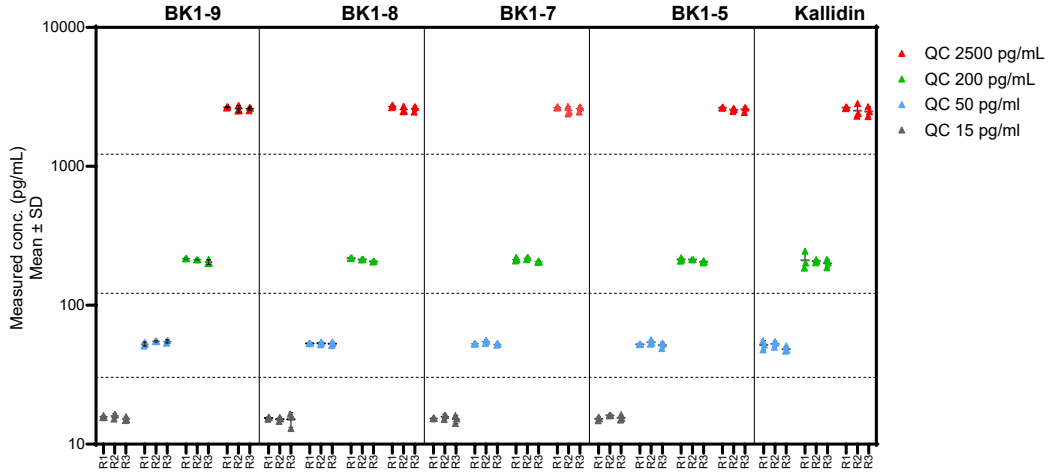
Kinin peptides

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

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





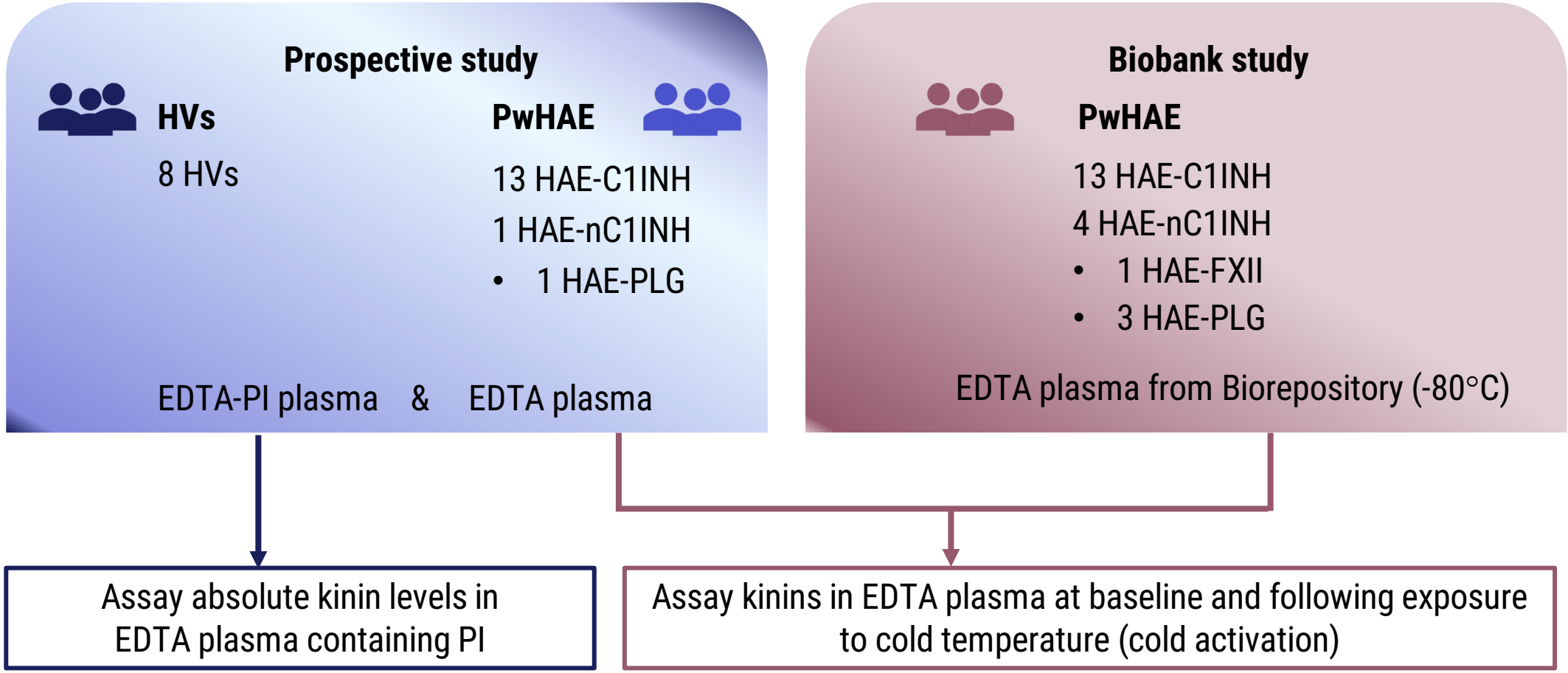


Analytical method	Ultra-high performance liquid chromatography-MS/MS (UPLC-MS/MS)
Analytes	BK1-9, BK1-8, BK1-7, BK1-5, kallidin
LLoQ	BK1-9, BK1-8, BK1-7, BK1-5: 5 pg/mL Kallidin: 20 pg/mL
ULoQ	10240 pg/mL
Carry over	<20%
Intra-run & Inter-run Accuracy and Precision	Δ conc. <15% nominal value; CV<15%

Validated assay detects main kinin metabolites with high sensitivity and wide dynamic range

BK: Bradykinin; CV: Coefficient of Variation; LLoQ: Low limit of quantification; QC: Quality control sample; SD: Standard deviation; ULoQ: Upper limit of quantification: UPLC-MS/MS: Ultra-high performance liquid chromatography-mass spectrometry / mass spectrometry

Clinical validation of kinin biomarker assays
Study design



BK: Bradykinin; C1INH: C1 inhibitor; EDTA: ethylenediaminetetraacetic acid; HAE-C1INH: Hereditary angioedema due to C1INH deficiency; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-nC1INH: HAE with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; KKS: Kallikrein kinin system; PI: protease inhibitor cocktail; PwHAE: people with HAE;

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

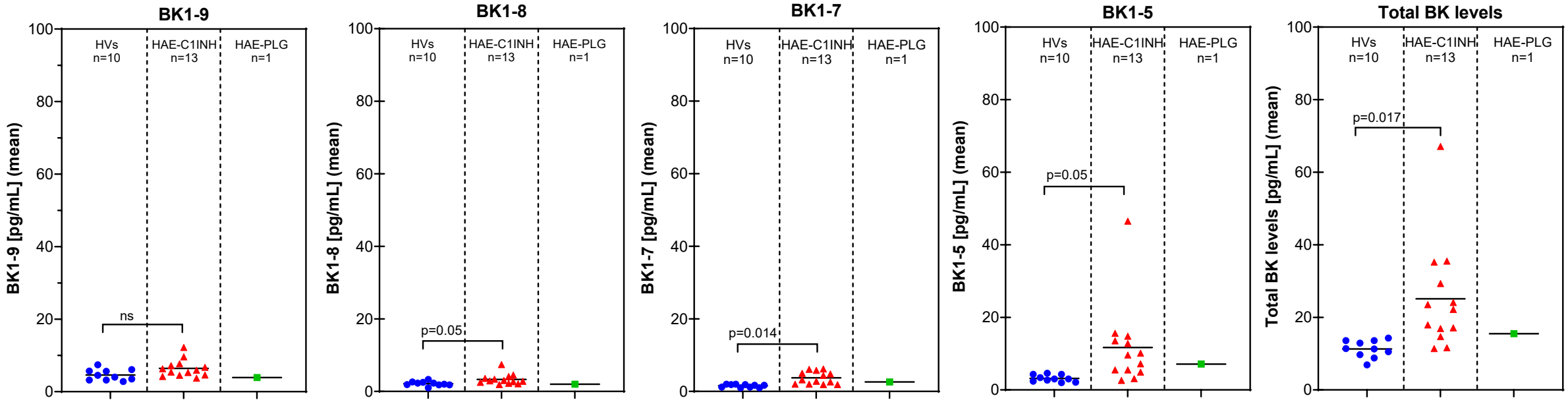
- EDTA plasma samples with and without PI were obtained from PwHAE at remission and from HVs
- At time of blood collection, PwHAE:
  - Were not on LTP therapies
  - Had not experienced attacks and had not received ODT, at least 4 days prior to sample collection

Demographics	HVs n=8	HAE-C1INH n=13	HAE-nC1INH HAE-PLG[1] n=1
Age in years, mean (SD)	37.2 (10.4)	40.7 (10.7)	39
Sex: Male/female, n (%)	4 (50.0) / 4 (50.0)	6 (46.2) / 7 (53.8)	1(100) / 0 (0)
Race: White/other, n	8 / 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)

[1]The individual with HAE-PLG did not have a history of AE attacks

AE: angioedema; C1INH: C1 inhibitor; EDTA: ethylenediaminetetraacetic acid; HAE-1: hereditary angioedema type 1: HAE-2: hereditary angioedema type 2; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; LTP: long-term prophylaxis; ODT: on-demand treatment; PI: protease inhibitors; PwHAE: people with HAE; SD: standard deviation

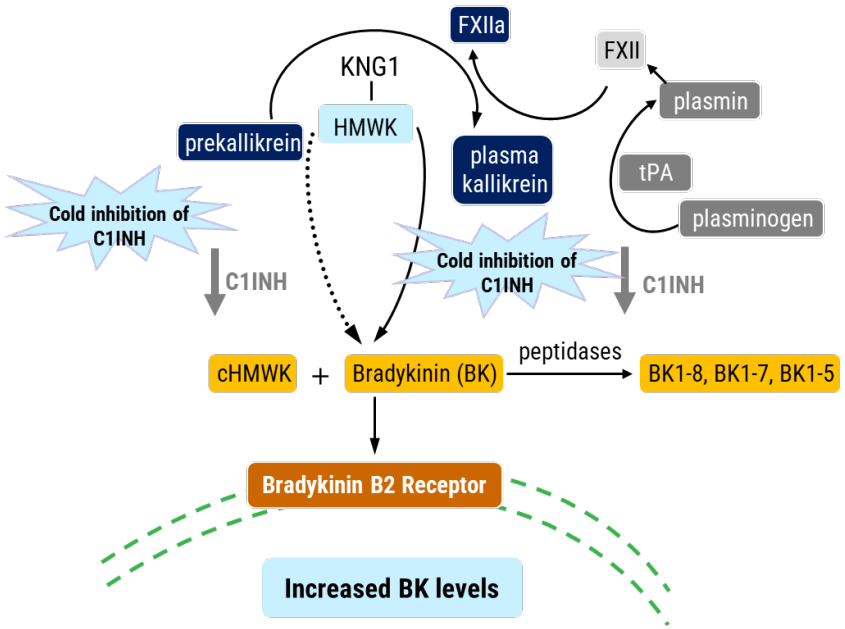
Low absolute kinin levels in plasma from PwHAE-C1INH and PwHAE-nC1INH at remission

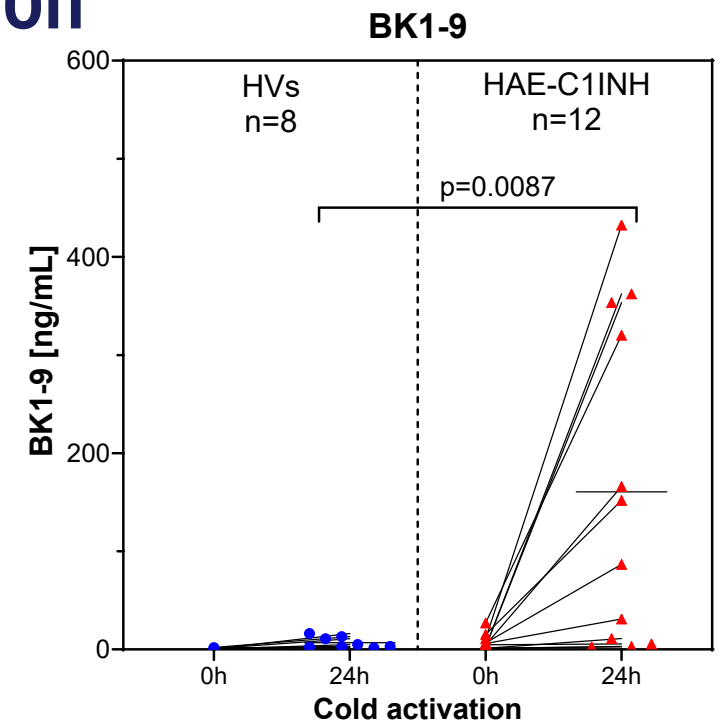


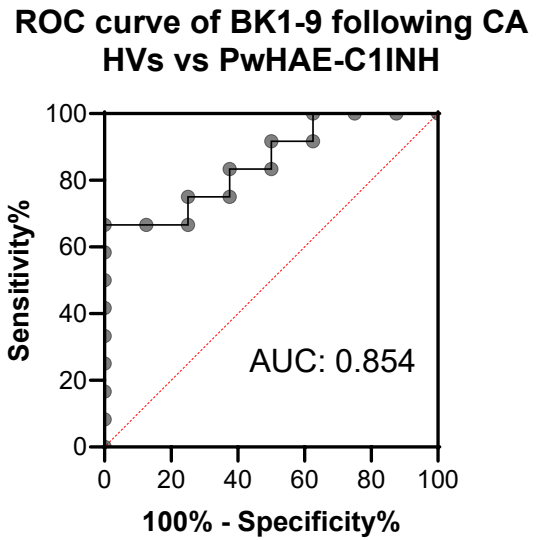
- Absolute kinin levels were analysed in EDTA-PI plasma using the qualified UPLC-MS/MS method
- Kallidin levels were below the limit of detection in all types of samples (not shown)
- Mean levels of kinin peptides were slightly increased in plasma from PwHAE-C1INH
- No increase in kinin levels in an individual with HAE-PLG who had never experienced AE attacks

AE: angioedema; BK: bradykinin; C1INH: C1 inhibitor; EDTA-PI: ethylenediaminetetraacetic acid with protease inhibitors; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; PwHAE-C1INH: people with hereditary angioedema due to C1INH deficiency; PwHAE-nC1INH: people with hereditary angioedema with normal C1INH levels; UPLC-MS/MS: Ultra-high performance liquid chromatography-mass spectrometry / mass spectrometry

Cold activation revealed increased BK-forming cascade sensitivity in PwHAE-C1INH at remission







- Kinin levels were analysed before (baseline) and following exposure to cold temperature
- Cold activation caused elevated BK levels, indicative of BK-forming cascade sensitivity in PwHAE-C1INH
- PwHAE-C1INH showed a remarkably sensitive BK-forming cascade compared to HVs
- One individual with HAE-PLG without history of AE attacks did not respond to cold activation (not shown)

AE: angioedema; AUC: area under the curve; BK: bradykinin; C1INH: C1 inhibitor; cHMWK: cleaved high molecular kininogen; FXII(a): factor 12 (activated); HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HMWK: high molecular weight kininogen; HVs: healthy volunteers; KNG1: kininogen gene; PwHAE-C1INH: people with HAE-C1INH; ROC: Receiver operator characteristic; tPA: tissue plasminogen activator

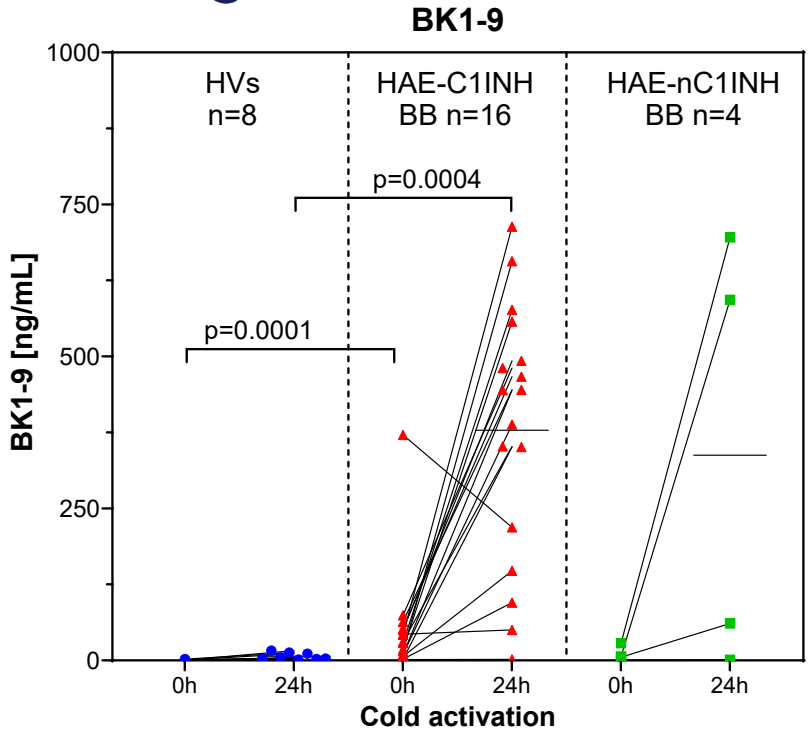
Demographics of patients in biobank study with plasma samples from the Biorepository of the Hungarian Angioedema Center of Reference and Excellence

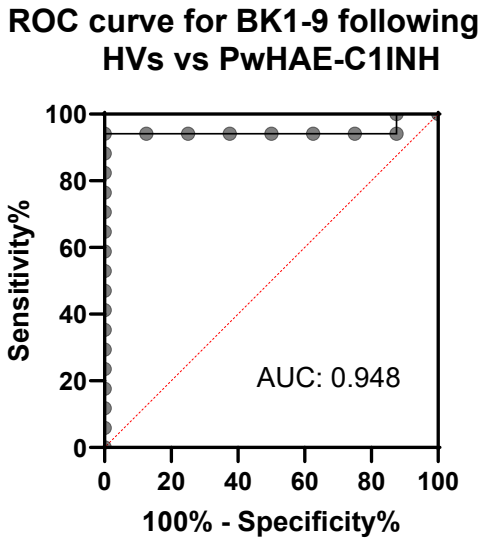
- EDTA plasma samples from PwHAE, stored at -80°C, were obtained from the Biorepository of the Hungarian Angioedema Center of Reference and Excellence
- Samples were obtained at remission
- At the time of blood collection, PwHAE:
  - Were not on LTP therapies
  - Had not experienced AE attacks and had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

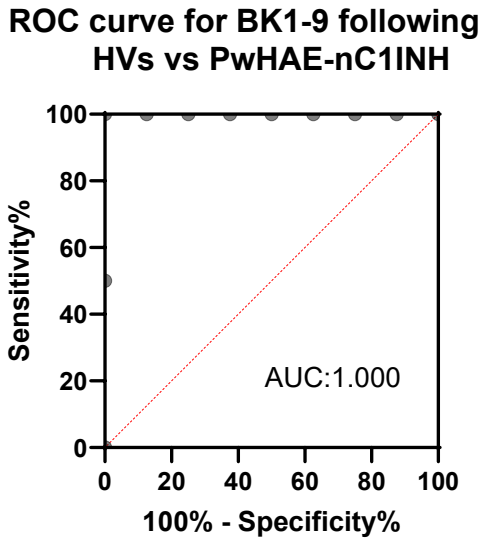
Demographics	HVs n=8	HAE-C1INH n=16	HAE-nC1INH n=4
Age in years, mean (SD)	37.2 (10.4)	32.9 (12.3)	38.5 (12.8)
Sex: Male / female, n (%)	4 (50.0) / 4 (50.0)	7 (43.7) / 9 (56.3)	2 (50.0) / 2 (50.0)
Race: White/other, n	8 / 0	15 / 1	4 / 0
HAE-C1INH type, n (%)			
HAE-1	n/a	14 (87.5)	n/a
HAE-2	n/a	2 (12.5)	n/a
HAE-nC1INH type, n (%)			
HAE-FXII	n/a	n/a	1 (25.0)
HAE-PLG	n/a	n/a	3 (75.0)

AE: angioedema; C1INH: C1 inhibitor; EDTA: ethylenediaminetetraacetic acid; HAE-1: hereditary angioedema type 1; HAE-2: hereditary angioedema type 2; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; LTP: long-term prophylaxis; ODT: on-demand treatment; PwHAE: people with HAE; SD: standard deviation

Biobank plasma samples from PwHAE can be used to study BK-forming cascade sensitivity



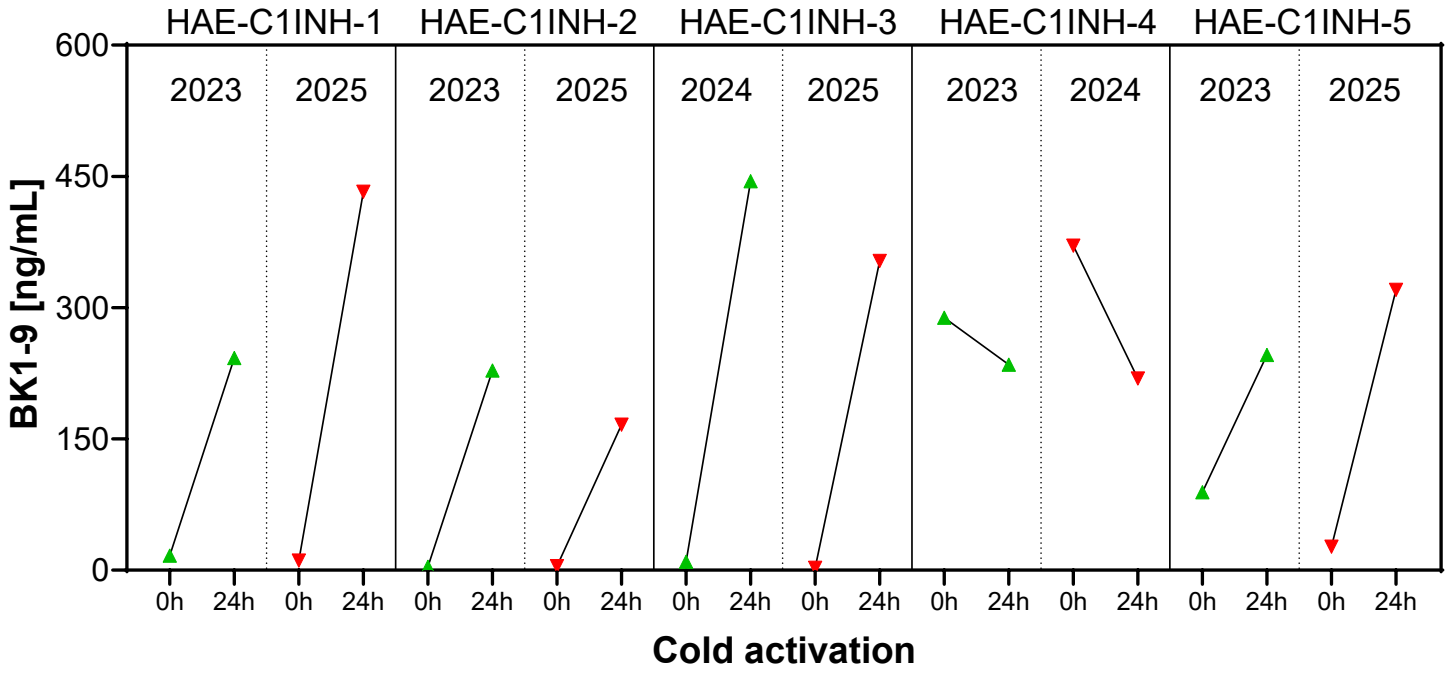




- Kinin levels were analysed before and after exposure to cold temperature in biobank plasma samples
- Cold activation caused elevated BK levels in samples from PwHAE-C1INH and PwHAE-nC1INH with HAE-FXII and HAE-PLG, indicative of BK-forming cascade sensitivity to triggers
- Two individuals with HAE-PLG without history of AE attacks did not respond to cold activation

AE: angioedema; AUC: area under the curve; BB: biobank; BK: bradykinin; CA: cold activation; C1INH: C1 inhibitor; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-FXII: HAE with genetic variant in F12 gene; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; PwHAE-C1INH: people with HAE-C1INH; PwHAE-nC1INH: people with HAE-nC1INH; ROC: Receiver operator characteristic

Cold activation induced comparable increase in BK levels in plasma from PwHAE-C1INH collected at different time points



- Longitudinal analysis of BK-forming cascade hypersensitivity in biobank plasma samples from PwHAE-C1INH
- Kinin levels were analysed before and after exposure to cold temperature in biobank plasma samples from PwHAE-C1INH from different time points

BK: bradykinin; C1INH: C1 inhibitor; HAE-C1INH: hereditary angioedema due to C1INH deficiency; PwHAE-C1INH: people with hereditary angioedema due to C1INH deficiency

Conclusions

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

AE: angioedema; AE-BK: bradykinin–mediated angioedema; BK: bradykinin; C1INH: C1 inhibitor; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-nC1INH: hereditary angioedema with normal C1INH levels