

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

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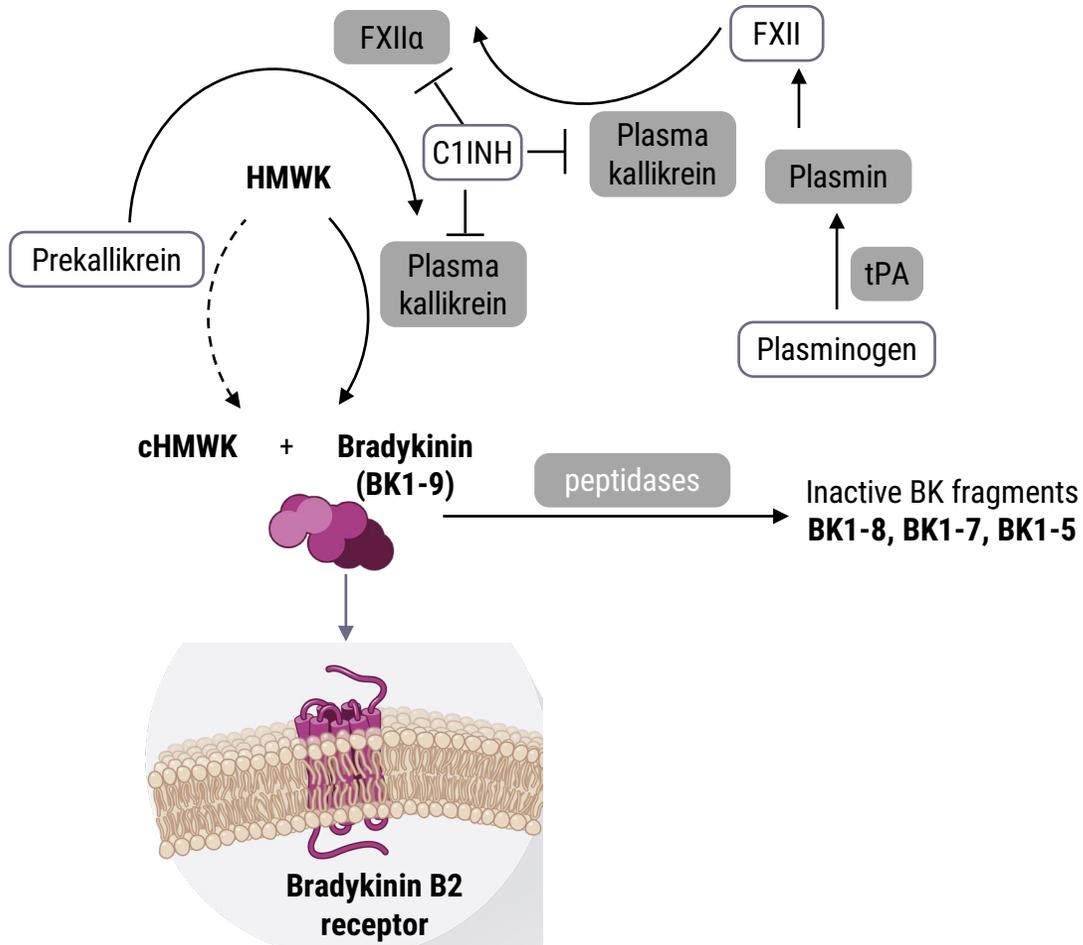
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# Conflicts of interest disclosure

**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. **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

# Availability of novel biomarker assays may aid diagnosis of BK-mediated angioedema with normal C1INH



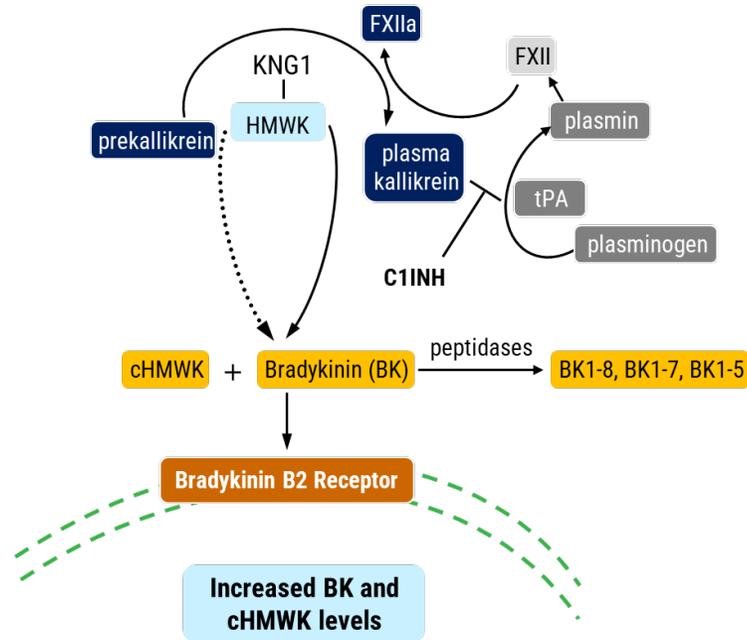
- BK plays a key role in the pathophysiology of different types of angioedema
  - HAE-C1INH
  - AAE-C1INH
  - HAE-nC1INH (e.g. HAE-FXII, HAE-KNG, HAE-PLG)
  - (H)AE-UNK?
  
- Diagnostic assays for early diagnosis of AE-BK with normal C1INH are lacking
  
- Detection of biomarkers related to bradykinin pathway has been challenging due to pathway sensitivity to *ex vivo* activation and instability of kinins<sup>1</sup>

AAE-C1INH: acquired angioedema due to C1INH deficiency; AE: angioedema; AE-BK: bradykinin mediated angioedema; 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. Kaplan AP, Maas C. *Front Med (Lausanne)*. 2017;2. Reshef A, et al. *J Allergy Clin Immunol*. 2024;154(2):398-411.e1.

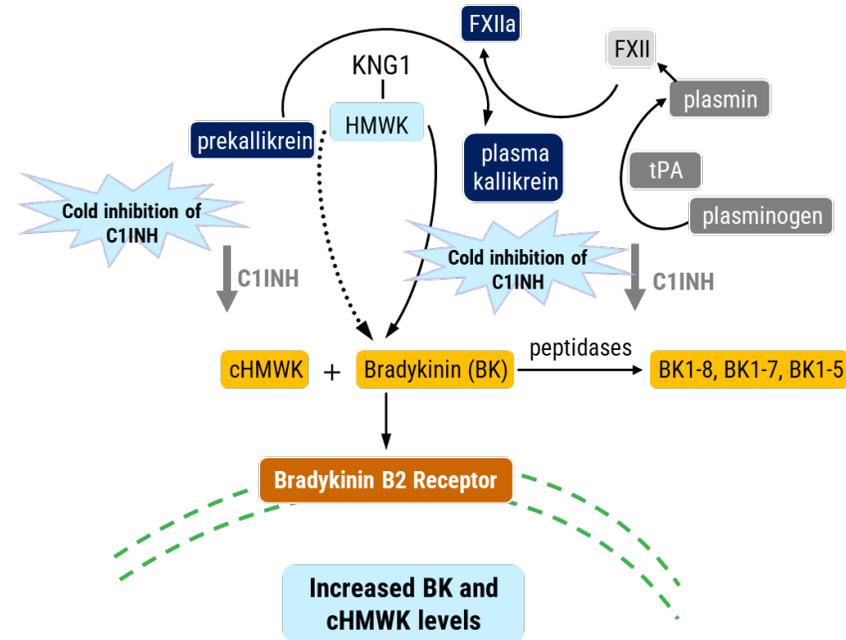
# “Dual Approach” to biomarker assessment

## Absolute biomarker levels



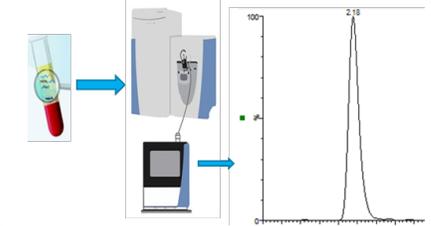
Assay kinins and HMWK in EDTA plasma containing protease inhibitors

## BK-forming pathway sensitivity to cold

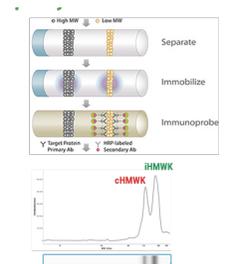


Assay kinins and HMWK in EDTA plasma at baseline and following exposure to cold temperature (cold activation)

**Kinin peptides**  
BK1-9, BK1-8, BK1-7, BK1-5, Kallidin  
**Ultra-high performance liquid chromatography-MS/MS (UPLC-MS/MS)**



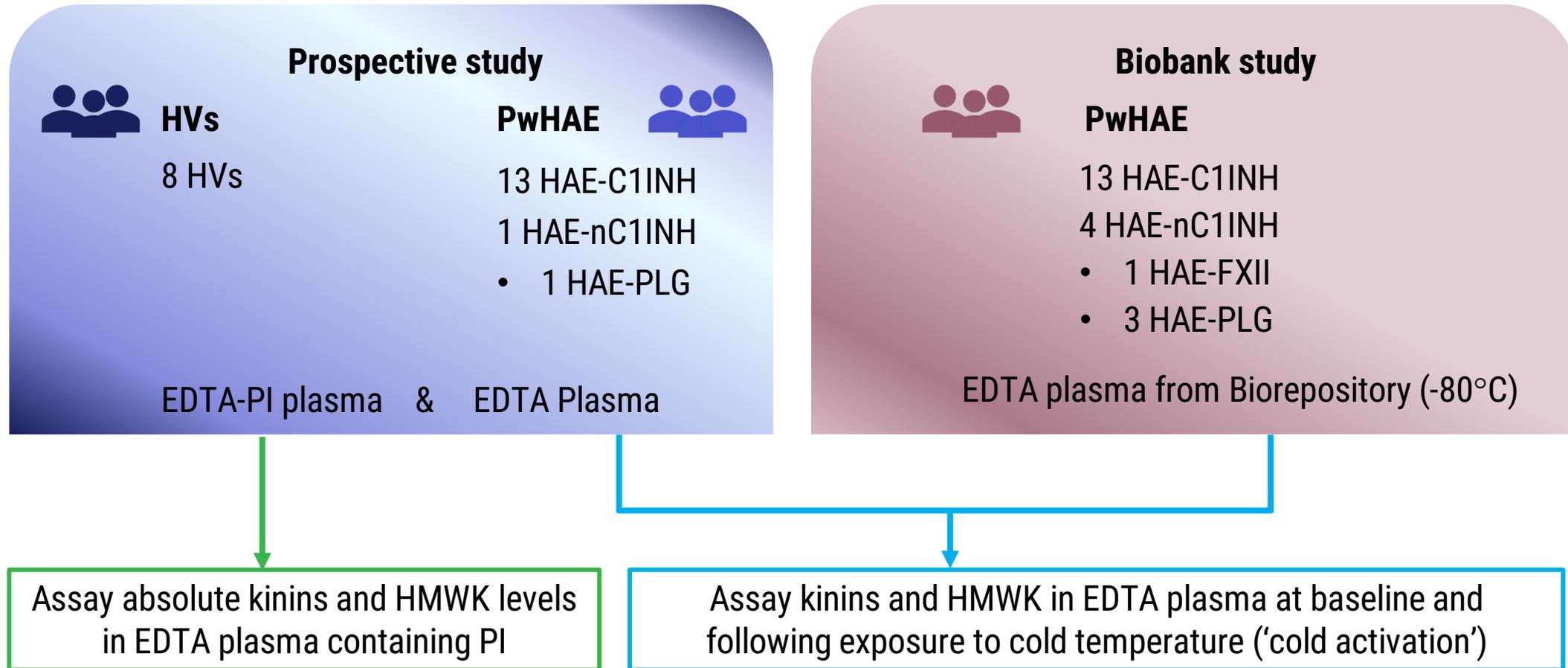
**iHMWK and cHMWK**  
**Simple Western Size (SWS) capillary immunoblotting assay**



BK: Bradykinin; C1INH: C1 inhibitor; cHMWK: cleaved high molecular kininogen; EDTA: EDTA: ethylenediaminetetraacetic acid; FXII: Factor 12; FXII(a): Factor 12 (activated); HMWK: high molecular weight kininogen; iHMWK: intact high molecular kininogen; KNG1: kininogen gene; SWS: simple western size; tPA: tissue plasminogen activator; UPLC-MS/MS: Ultra-high performance liquid chromatography-mass spectrometry / mass spectrometry

# Clinical validation of bradykinin and HMWK biomarker assays

## Study design



BK: Bradykinin; C1INH: C1 inhibitor; EDTA: ethylenediaminetetraacetic acid; HAE: hereditary angioedema; HAE-FXII: HAE with genetic variant in *F12* gene; HAE-C1INH: HAE due to C1INH deficiency; HAE-nC1INH: HAE with normal C1INH levels; HAE-PLG: HAE with genetic variant in plasminogen *PLG* gene; HMWK: high molecular weight kininogen; HV: Healthy volunteers; PI: protease inhibitor cocktail; PwHAE: people with HAE

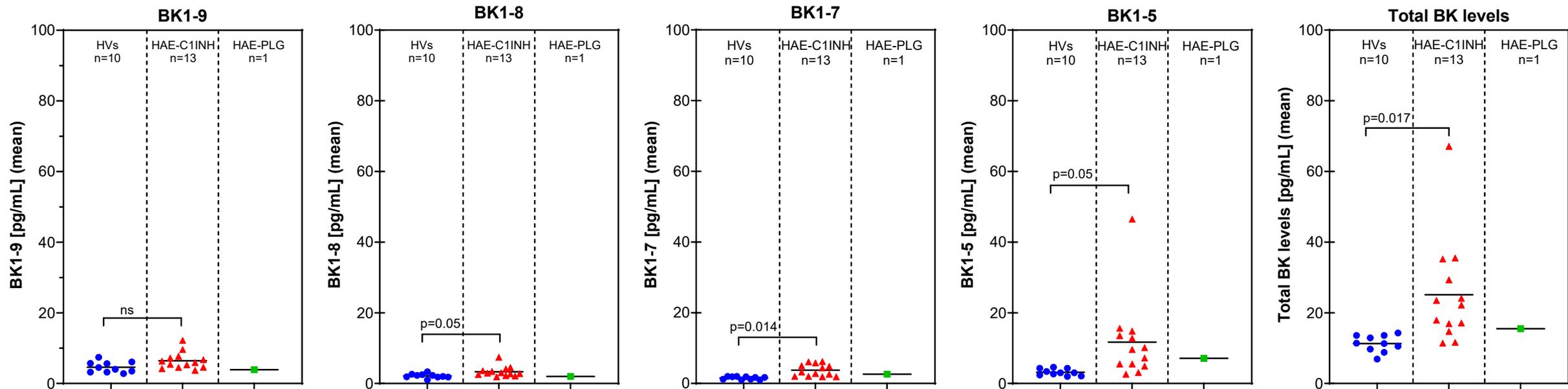
# Demographics of participants in prospective study at the Hungarian Angioedema Center of Reference and Excellence

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

	HVs	HAE-C1INH	HAE-nC1INH HAE-PLG <sup>1</sup>
Demographics	n=8	n=13	n=1
<b>Age in years</b> , mean (SD)	37.2 (10.4)	40.7 (10.7)	39
<b>Sex: Male/female</b> , n (%)	4 (50.0) / 4 (50.0)	6 (46.2) / 7 (53.8)	1(100.0) / 0 (0.0)
<b>Race: White/other</b> , n	8 / 0	12 / 1	1 / 0
<b>HAE type</b> , 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)

<sup>1</sup>The individual with HAE-PLG did not have an history of AE attacks

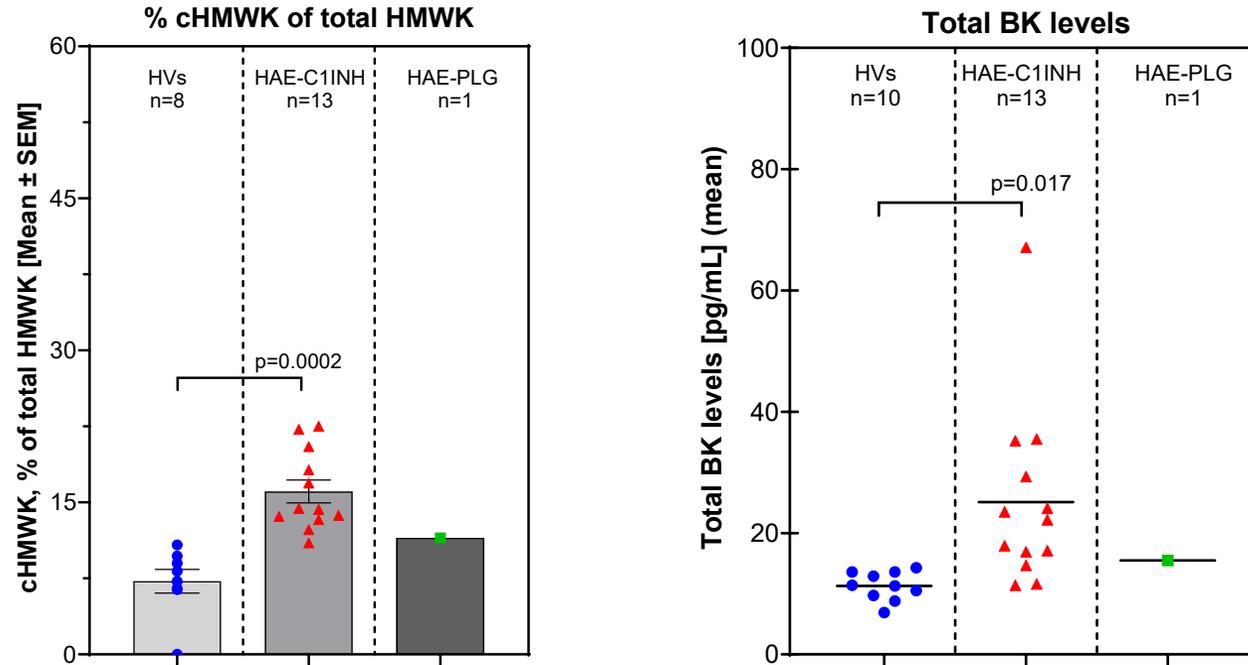
# Low absolute kinin levels in plasma from people with HAE-C1INH and HAE-nC1INH at remission



- Absolute kinin levels were analysed in EDTA-PI plasma from the prospective study using the qualified UPLC-MS/MS method
- Mean levels of kinin peptides were slightly increased in plasma from PwHAE-C1INH at remission in absence of AE attacks
- No increase in kinin levels in an individual with HAE-PLG who 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: hereditary angioedema with genetic variant in plasminogen *PLG* gene; HVs: healthy volunteers; PwHAE-C1INH: people with HAE-C1INH; UPLC-MS/MS: ultra-high performance liquid chromatography-mass spectrometry / mass spectrometry

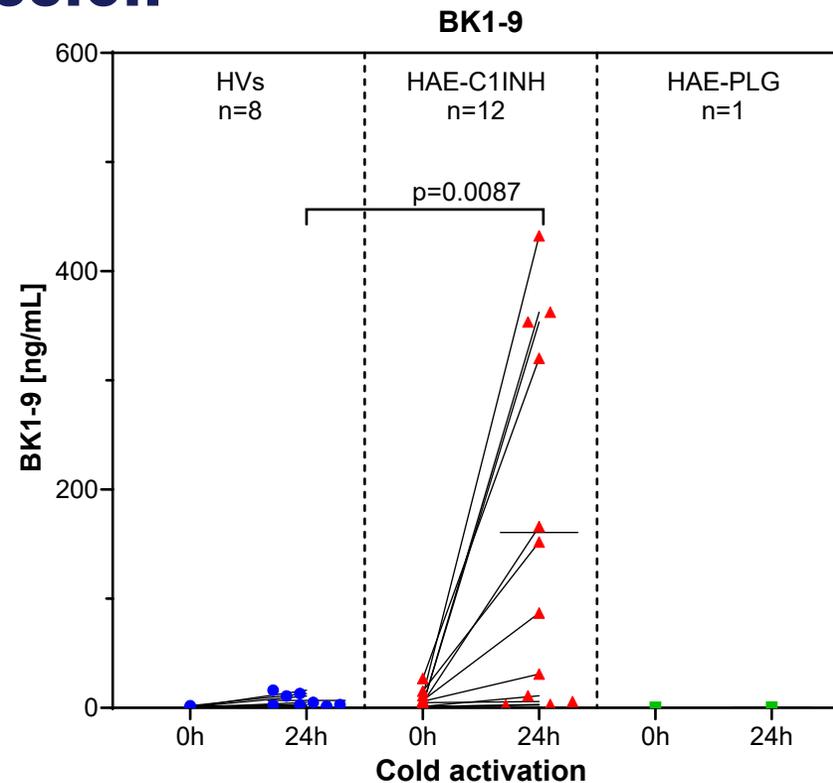
# Increased cHMWK levels in plasma from PwHAE-C1INH compared to HVs



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

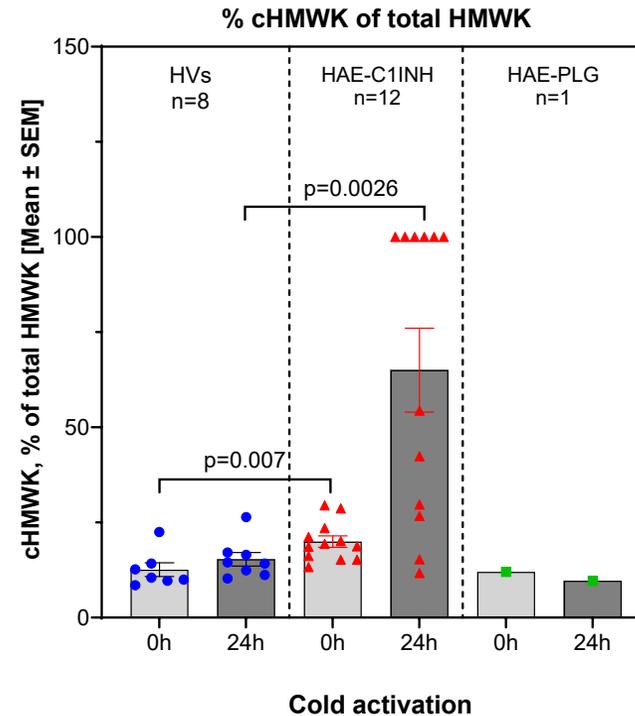
BK: bradykinin; cHMWK: cleaved high molecular weight kininogen; EDTA-PI: ethylenediaminetetraacetic acid with protease inhibitors; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-PLG: hereditary angioedema with genetic variant in plasminogen *PLG* gene; HMWK: intact high molecular weight kininogen; HVs: healthy volunteers; iHMWK: intact high molecular weight kininogen; PwHAE-C1INH: people with HAE-C1INH; SEM: standard error of the mean

# 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 (4°C) for 24 hours
- 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

# Cold activation resulted in increased cHMWK levels in plasma from PwHAE-C1INH 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 PwHAE-C1INH vs HVs

BK: bradykinin; C1INH: C1 inhibitor; cHMWK: cleaved high molecular weight kininogen; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: hereditary angioedema with genetic variant in plasminogen *PLG* gene; HMWK: high molecular weight kininogen; HVs: healthy volunteers; iHMWK: intact high molecular weight kininogen; PwHAE-C1INH: people with HAE-C1INH; SEM: standard error of the mean

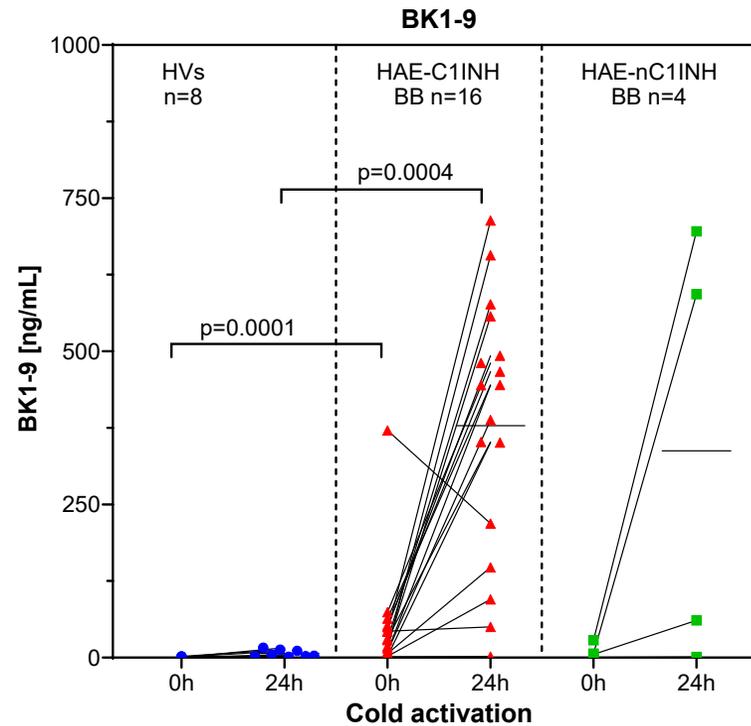
# Demographics of patients in biobank study with plasma samples from the Biorepository of the Hungarian Angioedema Center of Reference and Excellence

- 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 collected at remission
- At the time of blood collection, PwHAE
  - were not on LTP therapies
  - did not experience AE attacks and did not receive ODT, at least 4 days prior to sample collection

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

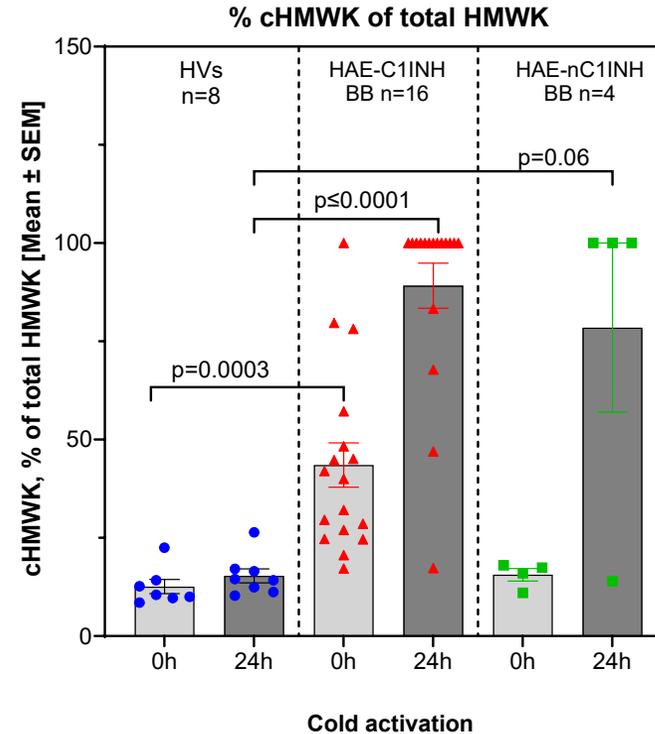
AE: angioedema; C1INH: C1 inhibitor; EDTA: ethylenediaminetetraacetic acid; HAE-1: hereditary angioedema type 1; HAE-2: hereditary angioedema type 2; HAE-C1INH: hereditary angioedema due to C1INH deficiency; HAE-FXII: hereditary angioedema with genetic variant in *F12* gene; HAE-nC1INH: hereditary angioedema with normal C1INH levels; HAE-PLG: hereditary angioedema 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

# Analysis of biobank plasma samples from PwHAE can be used to study BK-forming cascade sensitivity revealed by cold activation



- Kinin levels were analysed in biobank plasma samples before and after exposure to cold temperature (4°C) for 24 hours
- Cold activation caused elevated BK levels in samples from PwHAE-C1INH and PwHAE-nC1INH, indicative of BK-forming cascade sensitivity to triggers
- Clear differentiation of HVs and PwHAE-C1INH and PwHAE-nC1INH with HAE-FXII and HAE-PLG
- Two individuals with HAE-PLG without history of AE attacks did not respond to cold activation

# Cold activation revealed BK-forming cascade sensitivity resulting in increased cHMWK levels in biobank samples from PwHAE



- iHMWK and cHMWK levels were analysed before and after exposure to cold temperature
- cHMWK levels, relative to total levels were increased in PwHAE-C1INH and PwHAE-nC1INH with HAE-FXII and HAE-PLG

BB: biobank; BK: bradykinin; cHMWK: cleaved high molecular weight kininogen; HAE-C1INH: hereditary angioedema with C1 inhibitor 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; HMWK: high molecular weight kininogen; HVs: healthy volunteers; iHMWK: intact high molecular weight kininogen; PwHAE-C1INH: people with HAE-C1INH; PwHAE-nC1INH: people with HAE-nC1INH; SEM: standard error of the mean

# 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 BK-forming cascade sensitivity in HAE-C1INH and HAE-nC1INH to cold activation
- Importantly, the assay allows evaluation of BK-forming cascade hypersensitivity in plasma samples from biorepositories for patients with different AE types
- We plan to use this assay to evaluate the role of BK in other pathologies including recurrent angioedema