A novel kinin biomarker assay for characterization of different types of bradykinin-mediated angioedema

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Key takeaways

- **The kinin biomarker assay can be used to assess bradykinin-forming cascade sensitivity in plasma samples from people with hereditary angioedema due to C1 inhibitor deficiency (HAE-C1INH) and angioedema due to acquired C1INH deficiency (AAE-C1INH) using cold activation**
- **Importantly, the assay allows evaluation of BK-forming pathway sensitivity in plasma samples from people with HAE with normal C1INH (HAE-nC1INH) and with HAE or AE of unknown etiology (H)AE-UNK**
- **The clinically validated kinin biomarker assay may become a key tool for identifying, studying, and managing BK-mediated diseases including angioedema**

Background

- Angioedema (AE) is a predominant manifestation in multiple medical conditions and is generally mediated by bradykinin (BK) and/or histamine.[1]
- Differentiating BK-mediated from histamine-mediated AE and assessing the role of BK in the pathogenesis of other conditions by measuring kinin peptides remains a challenge due to proteolytic instability of the kinin peptides and limitations of current analytical assays.[2,3]
- Establishment and clinical validation of a method to accurately measure BK and kinin related peptides (BK-metabolites) could aid in identifying, studying, and managing BK-mediated pathologies, including BK-mediated angioedema.

Methods

- Blood samples were collected from people with hereditary AE (HAE) due to C1 inhibitor deficiency (HAE-C1INH), AE due to acquired C1INH deficiency (AAE-C1INH), HAE with normal C1INH function (HAE-nC1INH, with variants in F12 or PLG genes), HAE-nC1INH of unknown etiology (HAE-UNK), AE with of unknown etiology (AE-UNK) and healthy volunteers (HVs), in tubes containing ethylenediaminetetraacetic acid (EDTA) and plasma was prepared (**Table 1**).
- All participants provided their informed consent.

Table 1. Demographics of study population

	HVs n=30	HAE-C1INH n=30	AAE-C1INH n=4	HAE-nC1INH n=4	HAE-UNK n=3	AE-UNK n=3
Age in years, mean (SD)	34.9 (8.8)	36.7 (12.8)	53 (13.0)	38.5 (12.8)	40.0 (10.1)	43.3 (18.8)
Sex: male/female, n	17 / 13	13 / 17	4 / 0	2 / 2	0 / 3	1 / 2
Race: white/other, n	26 / 4	29 / 3	4 / 0	4 / 0	0 / 3	2 / 1
HAE-1/HAE-2, n	n.a.	28 / 2	n.a.	n.a.	n.a.	n.a.
HAE-nC1INH type, n						
HAE-FXII	n.a.	n.a.	n.a.	1	n.a.	n.a.
HAE-PLG	n.a	n.a.	n.a.	3	n.a.	n.a.

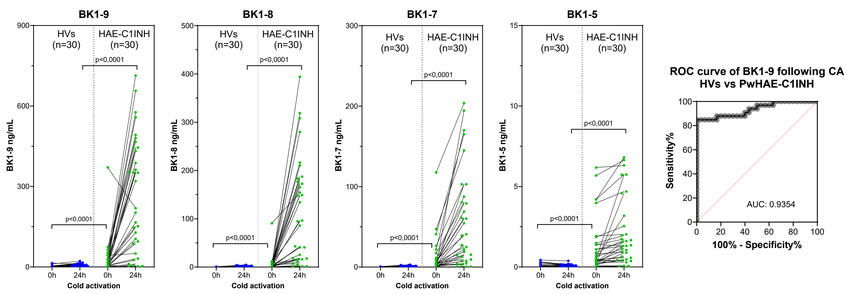
AE: angioedema; AAE-C1INH: AE with acquired C1INH deficiency; AE-UNK: AE of unknown etiology; C1INH: C1 inhibitor; FXII: factor XII; HAE: hereditary angioedema; HAE-1: HAE type 1; HAE-2: HAE type 2; HAE-C1INH: HAE with C1INH deficiency; HAE-FXII: HAE-nC1INH with genetic variant in FXII; HAE-nC1INH: HAE with normal C1INH; HAE-PLG: HAE-nC1INH with genetic variant in PLG; HAE-UNK: HAE-nC1INH of unknown etiology; n: number of individuals; n.a.: not applicable; PLG: plasminogen; SD: standard deviation

- An ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS/MS) method was optimized to measure BK1-9, BK1-8, BK1-7, BK1-5 in plasma.[4]
- Kinin levels were analyzed in plasma before and after cold activation (exposure to 4°C for 24 hours).

Results

- Kinin levels were analyzed in EDTA plasma before (baseline) and following cold activation (**Figure 1**).
- Cold activation led to significant increase in BK and kinin related peptide levels in EDTA plasma from people with HAE-C1INH vs HVs.
- BK and other kinin related peptide levels were significantly higher in plasma from people with HAE-C1INH also at baseline (no cold activation).

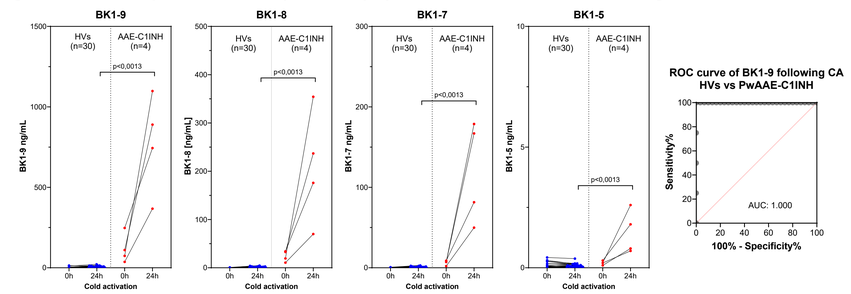
Figure 1. Increased bradykinin levels in EDTA plasma from people with HAE-C1INH at baseline and following cold activation



AUC: area under the curve; BK: Bradykinin; C1INH: C1 inhibitor; HAE-C1INH: Hereditary angioedema with C1INH deficiency; HVs: Healthy volunteers; PwHAE-C1INH: people with HAE-C1INH; ROC: Receiver operator characteristic

- BK and kinin related peptides were significantly increased in plasma from people with AAE-C1INH following cold activation (**Figure 2**).
- Clear differentiation between healthy volunteers and people with AAE-C1INH.

Figure 2. Increased bradykinin levels in EDTA plasma from people with AAE-C1INH at baseline and following cold activation

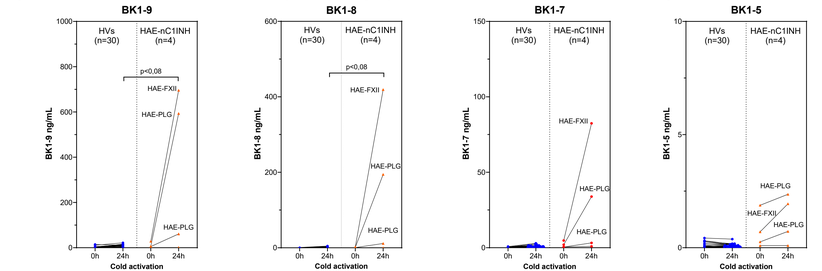


AUC: area under the curve; BK: Bradykinin; C1INH: C1 inhibitor; AAE-C1INH: angioedema with acquired C1INH deficiency; HVs: Healthy volunteers; PwAAE-C1INH: people with AAE-C1INH; ROC: Receiver operator characteristic

Results

- Cold activation caused elevated BK and kinin related peptide levels in samples from people with HAE-FXII and HAE-PLG, indicative of BK-forming cascade sensitivity to triggers (**Figure 3**).
- Two individuals with HAE-PLG without history of AE attacks did not respond to cold activation.

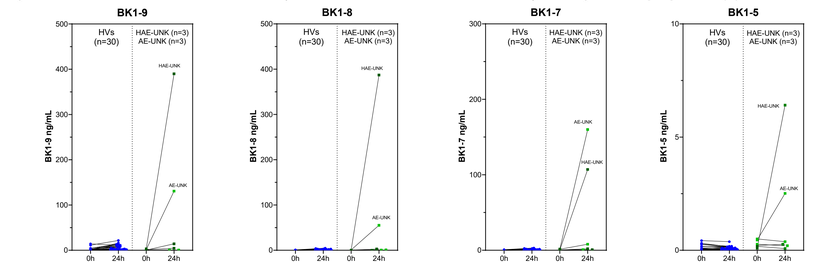
Figure 3. Increased bradykinin levels in EDTA plasma from people with HAE-nC1INH at baseline and following cold activation



BK: Bradykinin; C1INH: C1 inhibitor; FXII: factor XII; HAE: hereditary angioedema; HAE-FXII: HAE-nC1INH with genetic variant in FXII; HAE-nC1INH: HAE with normal C1INH; HAE-PLG: HAE-nC1INH with genetic variant in PLG; HVs: Healthy volunteers; PLG: plasminogen

- Cold activation caused increased BK and kinin related peptides levels in samples from people with HAE-UNK and AE-UNK, indicative of BK-forming cascade sensitivity to triggers (**Figure 4**).
- Angioedema attacks in these individuals are expected to be bradykinin-mediated.

Figure 4. Cold activation kinin biomarker assay can be used for identification of a bradykinin signature in people with (H)AE-UNK



AE-UNK: angioedema with normal C1INH deficiency of unknown etiology; BK: Bradykinin; C1INH: C1 inhibitor; HAE: hereditary angioedema; HAE-UNK: Hereditary angioedema with normal C1INH deficiency of unknown etiology; HVs: Healthy volunteers

References

1. Maurer M, et al. Clin Rev Allergy Immunol. 2021;61:40-9. **2.** Kaplan AP, et al. Adv Immunol. 2014;121:41-89. **3.** Kaplan AP, et al. Front Med (Lausanne). 2017;4:206. **4.** Pardali E, et al. Ann Allergy Immunol. 2024; 6:S30: R088.

E.P.: employee of Pharvaris, holds stocks/stock options in Pharvaris; **H.R.H.:** received travel grants from Takeda and CSL Behring; **J.J.Y.:** employee of Virant Diagnostics, no conflicts of interest; **L.W.:** employee of Virant Diagnostics / Institute for Asthma and Allergy / Angioedema Center of Reference and Excellence, no conflicts of interest; **M.d.L.:** no conflicts of interest; **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; **D.M.C.:** has received speaker or consultancy fees from Astria, BioCryst, CSL Behring, Intellia, Ionis, KalVista, Otsuka, Pharvaris, and Takeda and research funding from CSL Behring, Ionis, KalVista and Pharvaris; **H.H.L.:** employee of Virant Diagnostics / Institute for Asthma and Allergy / Angioedema Center of Reference and Excellence, received speaker or consultancy fees from Biocryst, CSL Behring, Ionis, Kalvista, Pharming, and Takeda and research grants from Pharvaris, Ionis, CSL Behring, Biocryst, Kalvista, Intellia, Arpo, and AdalRx; **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; **A.L.:** employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma.