Abstract ID: 158979

LC-MS particle-based plasma proteomics in bradykinin-mediated angioedema

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Key takeaways

- The proteomic coverage achieved by nanoparticle-coupled liquid chromatography-mass spectrometry (LC-MS) expanded the identification of putative plasma proteins associated with bradykinin (BK)-mediated angioedema (AE-BK).
- Common proteins identified in AE-BK by LC-MS and by SomaLogic[1] exhibited cross-platform concordance.
- Cross-platform validation supports biomarker candidate prioritization in studies constrained by small cohorts.

Introduction

Bradykinin (BK)-mediated angioedema (AE-BK) is a disorder characterized by recurrent episodes of edema and swelling driven by bradykinin signaling through the bradykinin B2 receptor. While the roles of bradykinin and bradykinin B2 receptor are central to AE-BK pathophysiology, systemic proteomic alterations and their relationship to disease pathophysiology remain to be further defined. Liquid chromatography-mass spectrometry (LC-MS)-based proteomics is limited by the dynamic range of plasma proteins, hindering detection of low-abundance targets. Unlike targeted affinity platforms (e.g., Olink, SomaLogic), MS enables unbiased peptide detection and may provide broader mechanistic insights. Here, we leverage next-generation LC-MS proteomics to profile the plasma proteome in AE-BK and demonstrate its application to biomarker discovery.

Materials & Methods

- Blood samples were collected from people with AE-BK (n=9) during the absence of clinical manifestations of AE attacks and healthy volunteers (HVs, n=7). All participants provided their written informed consent before enrolment. Note donor-level information in **Table 1**.
- Plasma was isolated from blood collected into tubes containing ethylenediaminetetraacetic acid (EDTA).

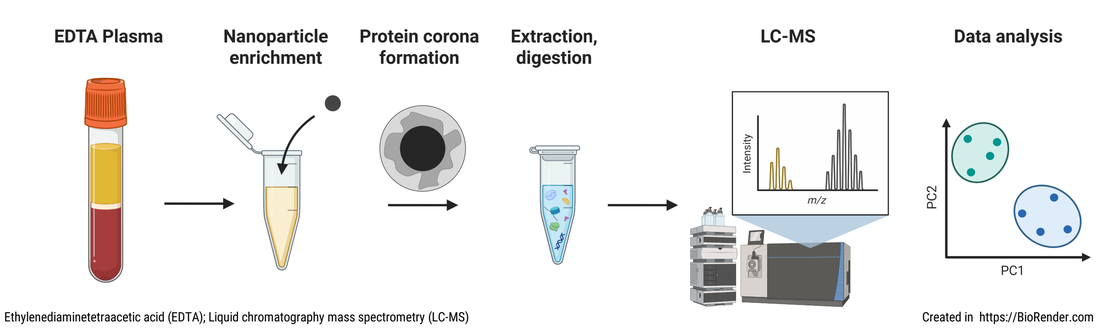
Table 1. Individual-level information of samples used in proteomic study

Code	Subgroup	Age	Sex	Attack rate/yr
HV 1	Healthy volunteer	32	F	N/A
HV 2	Healthy volunteer	28	F	N/A
HV 3	Healthy volunteer	38	F	N/A
HV 4	Healthy volunteer	32	F	N/A
HV 5	Healthy volunteer	41	F	N/A
HV 6	Healthy volunteer	41	F	N/A
HV 7	Healthy volunteer	38	M	N/A
AE-BK 1	HAE-C1INH (HAE1)	26	M	9
AE-BK 2	HAE-C1INH (HAE1)	52	F	18
AE-BK 3	HAE-C1INH (HAE1)	20	F	30
AE-BK 4	HAE-C1INH (HAE1)	59	F	24
AE-BK 5	HAE-C1INH (HAE1)	33	F	2.5
AE-BK 6	HAE-C1INH (HAE1)	63	F	1
AE-BK 7	HAE-C1INH (HAE1)	34	F	52
AE-BK 8	HAE-nC1INH (HAE3)	49	F	6
AE-BK 9	AE-BK	42	F	5

Hereditary angioedema with C1 inhibitor (C1INH) deficiency (HAE-C1INH); Hereditary angioedema with normal C1INH (HAE-nC1INH); Bradykinin (BK)-mediated angioedema (AE-BK; note: genetic testing not performed)

- Proteomic profiling was performed using the P2 UltraDeep proteomic platform (Biognosys AG), which combines particle-based protein enrichment with next-generation data-independent acquisition liquid chromatography–tandem mass spectrometry (LC-MS) (**Figure 1**).
- For testing of differential protein abundance, the following thresholds were applied: q-value < 0.05; absolute average fold-change > 1.5. Proteins meeting these thresholds were submitted to Ingenuity Pathway Analysis (IPA; QIAGEN).

Figure 1. Particle-based enrichment-coupled mass spectrometry



Ethylenediaminetetraacetic acid (EDTA); Liquid chromatography mass spectrometry (LC-MS)

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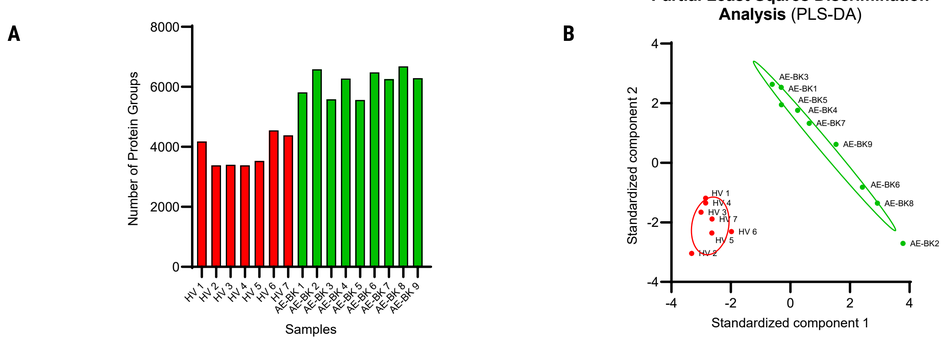
Results

- In total, 6998 protein groups represented by 89336 peptide ion variants were detected across all samples.
- On average 5147 proteins were quantified per sample (**Figure 2A**), spanning a 12-log dynamic range in abundance.
- Partial Least Squares Discriminant Analysis (PLS-DA) was performed by fitting a model onto the study groups. PLS-DA regression analysis sharpens differences between conditions by projecting corresponding data variance onto hyperplanes (components). Separation between controls and AE-BK proteomes is observed (**Figure 2B**).

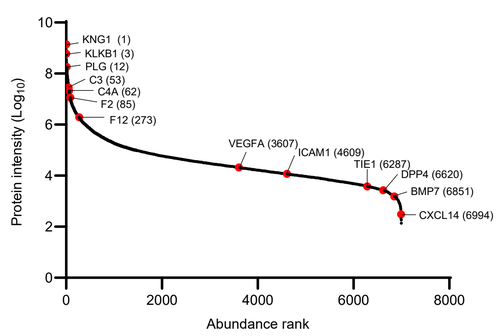
Figure 2. Proteome-wide profiling by LC-MS



Liquid chromatography mass spectrometry (LC-MS); Healthy volunteers (HV; **red**); Bradykinin (BK)-mediated angioedema (AE-BK; **green**)

- The broad range of peptides detected included abundant plasma proteins and low-abundance tissue-derived proteins such as growth factors, cytokines and receptors (**Figure 3**).

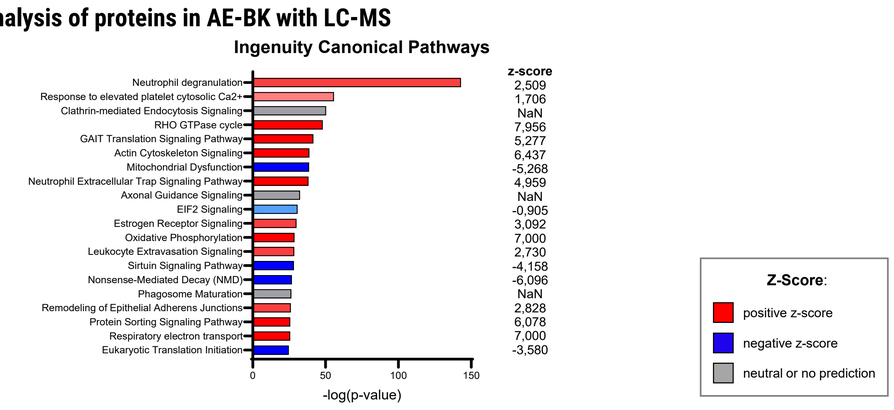
Figure 3. Waterfall plot of quantified plasma proteins demonstrating range of identifications by LC-MS method



Results (continued)

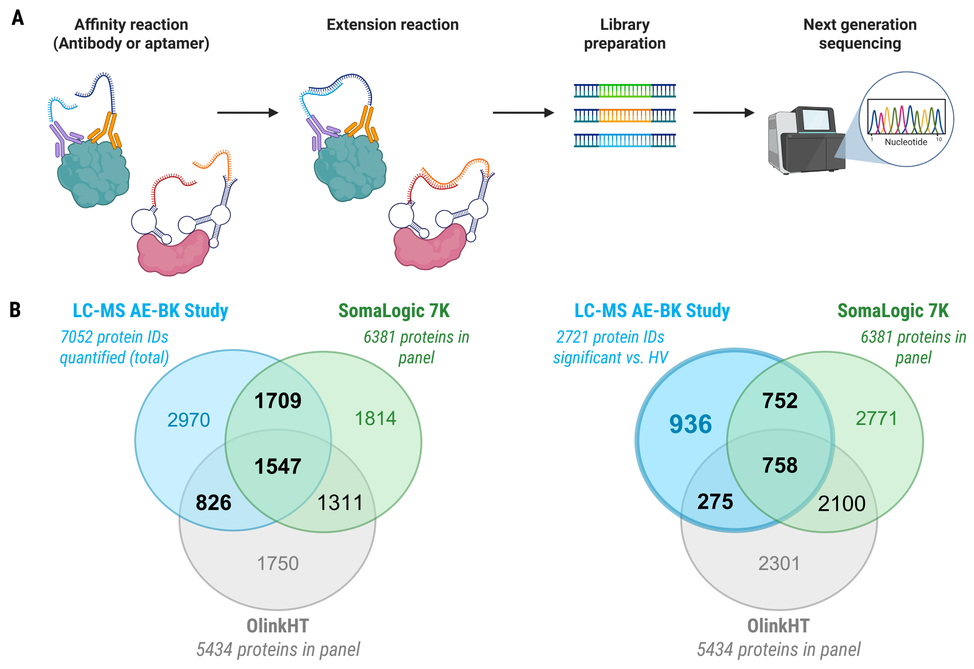
- Analysis of canonical pathways revealed a number of biological pathways associated with AE-BK (**Figure 4**).

Figure 4. Pathway analysis of proteins in AE-BK with LC-MS



- Schematic representation of available affinity-based high-throughput proteomic platforms (**Figure 5A**). Olink and SomaLogic technologies are antibody- and aptamer-based, respectively.
- Among the 7,052 unique protein IDs quantified by LC-MS (6,998 protein groups), 3,256 (46.2%) overlapped with the SomaLogic 7K panel and 2,373 (33.7%) with the Olink HT platform (**Figure 5B, left**).
- Of the 2,721 unique proteins found to be significantly regulated in AE-BK by LC-MS, 1,510 (55.5%) were represented on the SomaLogic 7K platform and 1,033 (38.4%) in OlinkHT. Notably, 936 differentially abundant proteins identified by LC-MS were not covered by either affinity-based panel (**Figure 5B, right**).

Figure 5. Complementarity of proteomic platforms in biomarker studies



Liquid chromatography mass spectrometry (LC-MS); Bradykinin (BK)-mediated angioedema (AE-BK); Healthy volunteers (HV)

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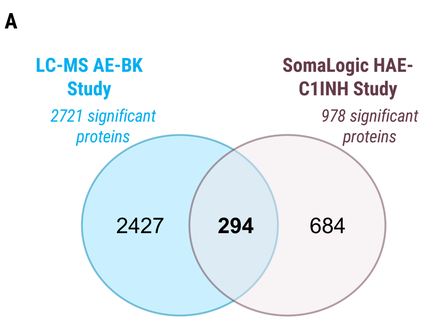
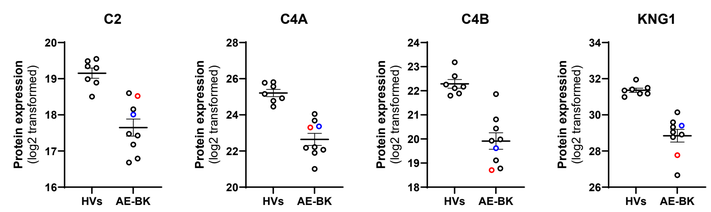
- Comparison of AE-BK LC-MS data with SomaLogic proteomic data from HAE-C1INH reported by Sexton *et al.* 2025[1] revealed 294 overlapping proteins (30% of 978), of which 195 (66%) showed concordant regulation across studies (**Figure 6A**).
- Selected concordantly regulated proteins in AE-BK (LC-MS) and HAE-C1INH (SomaLogic[1]) studies and fold change (Log_2FC). Plots of selected proteins in AE-BK by LC-MS (**Figure 6B**).

Figure 6. Cross platform comparison of selected proteins in AE-BK



B

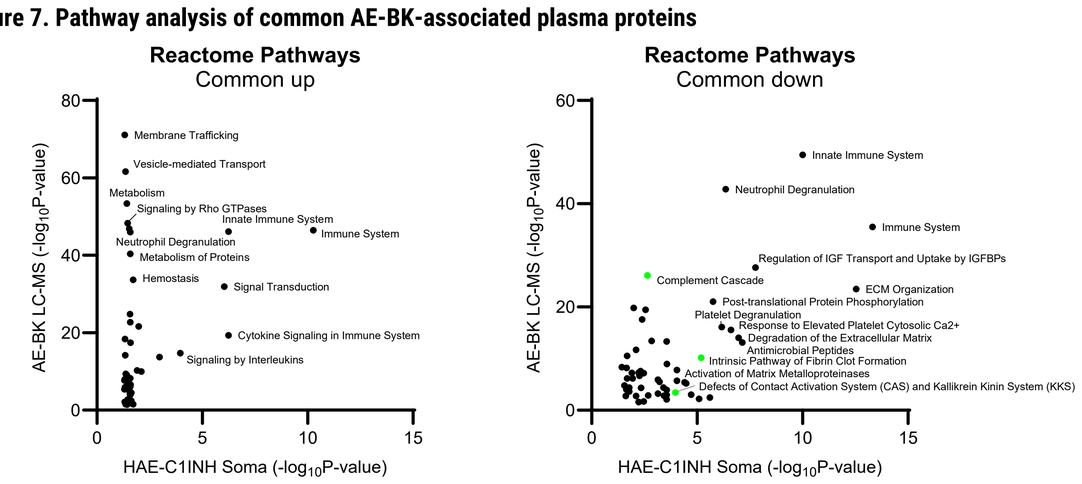
Gene ID	Log2FC AE-BK vs HV (LC-MS)	Log2FC HAE-C1INH vs HV (Sexton *et al.* 2025[1])
C2	-1.503	-0.750
C4A	-2.569	-2.045
C4B	-2.372	-2.045
KNG1	-2.518	-0.315*



Healthy volunteers (HV); Bradykinin (BK)-mediated angioedema (AE-BK); *KNG1 aptamer binding to total KING1; Datapoint references from Table 1: HAE-C1INH (**black** outline), HAE-nC1INH (**red** outline), AE-BK (**blue** outline)

- Concordant pathway analysis across LC-MS and SomaLogic[1] proteomic datasets highlighted dysregulation of the complement, contact, and kallikrein-kinin systems (green), as well as broader immune regulatory processes (**Figure 7**).

Figure 7. Pathway analysis of common AE-BK-associated plasma proteins



Bradykinin (BK)-mediated angioedema (AE-BK); Liquid chromatography mass spectrometry (LC-MS); Hereditary angioedema with normal C1INH (HAE-nC1INH); SomaLogic proteomics (Soma)

Limitations of study

- A key limitation of this study is its small sample size, necessitating validation in larger cohorts.
- LC-MS improved plasma proteome coverage but still lacks sensitivity for ultra-low-abundance proteins including many cytokines. Complementary platforms (e.g., NULISAseq Inflammation panel) may uncover additional candidates.

References

1. Sexton, *et al.* Front Immunol. 2025; 15:1471168.

J.D.: employee of Pharvaris, holds stocks/stock options in Pharvaris; **E.P.:** employee of Pharvaris, holds stocks/stock options in Pharvaris; **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.