Proteomic profiling of interstitial fluid from CIndU and CSU patients highlights the presence of shared MRGPRX2 ligands and inflammatory biomarkers

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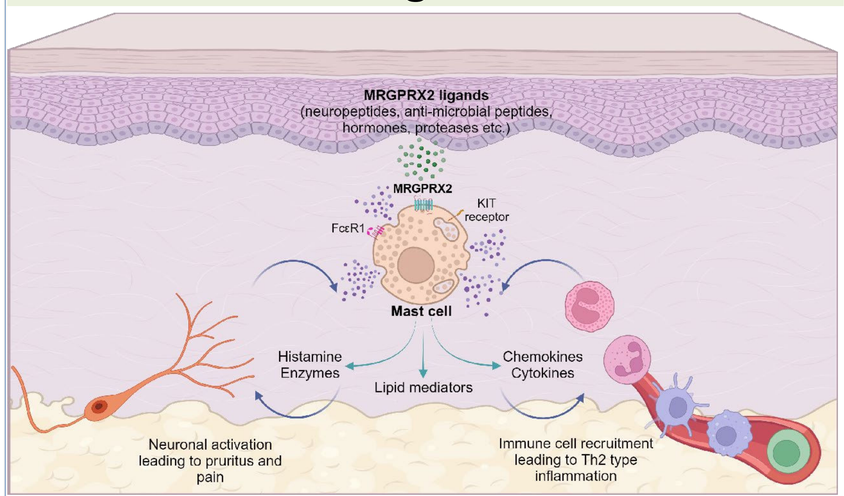
Abstract

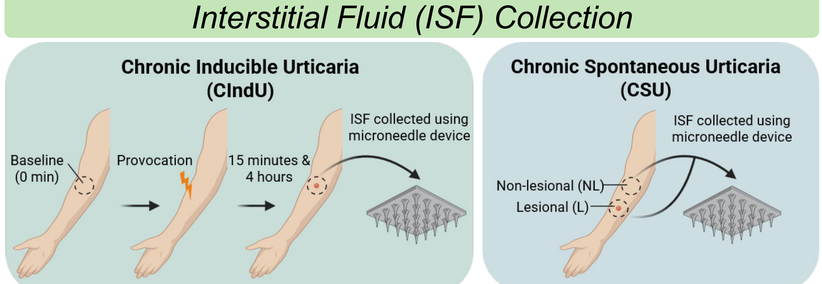
Mast cells are central effectors of many chronic inflammatory skin diseases. MRGPRX2, a receptor expressed on mast cells and sensory neurons, is activated in response to cationic ligands and has been implicated in the pathogenesis of chronic inducible urticaria (CIndU) and chronic spontaneous urticaria (CSU). However, the disease biology remains incompletely understood due to limited access to lesional skin tissue and skin-relevant biofluids. Additionally, peripheral blood analyses often fail to reflect localized cutaneous inflammation. To directly interrogate the lesional microenvironment, we collected dermal interstitial fluid (ISF) using a novel microneedle device. In patients with cold CIndU, ISF was obtained from non-lesional skin and from lesional skin at 15 minutes and 4 hours following cold provocation. In CSU patients, ISF was collected from both lesional and non-lesional sites. Proteomic profiling was performed using mass spectrometry and Olink analyses. Lesional ISF from both CIndU and CSU patients showed increased levels of mast cell activation markers, including tryptase and CD107a, compared to non-lesional skin. Importantly, endogenous MRGPRX2 ligands such as LL-37, PAMP, and Cathepsin S were enriched in lesional samples. Olink analysis revealed elevated cytokines and chemokines associated with immune cell recruitment and inflammatory amplification in lesional skin. Notably, these proteomic signatures were consistently observed across both CIndU and CSU. Together, our findings support a model in which MRGPRX2-driven mast cell activation represents a shared pathogenic mechanism contributing to both diseases.

Background



Experimental Workflow

Interstitial Fluid (ISF) Collection



Analysis

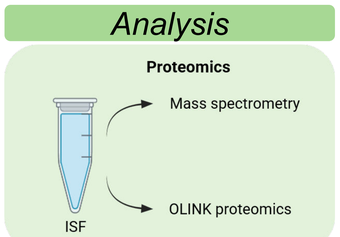
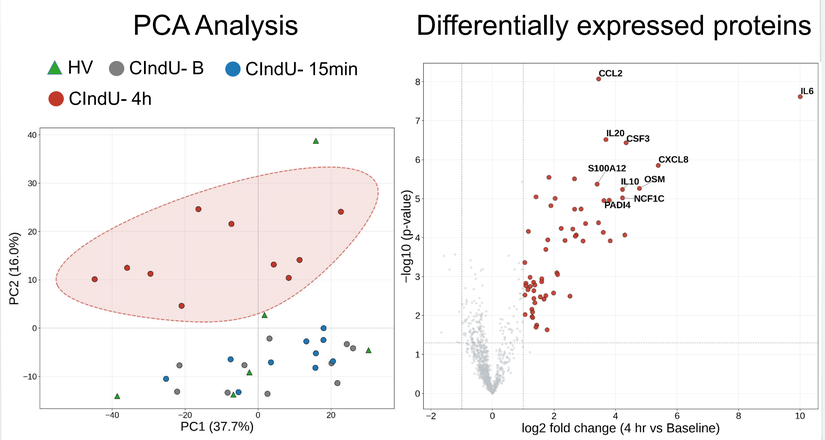


Figure 1. ISF samples were collected from patients with CIndU and CSU using a microneedle-based collection device (PELSA device from Ascilion). In CIndU patients, ISF was collected at baseline and at 15 minutes and 4 hours following provocation. In CSU patients, ISF was collected from non-lesional (NL) and lesional (L) skin. Samples were subsequently analyzed by mass spectrometry and Olink proteomics to characterize protein expression profiles associated with skin inflammation and disease state.

An acute inflammatory program is enriched in lesional CIndU ISF samples

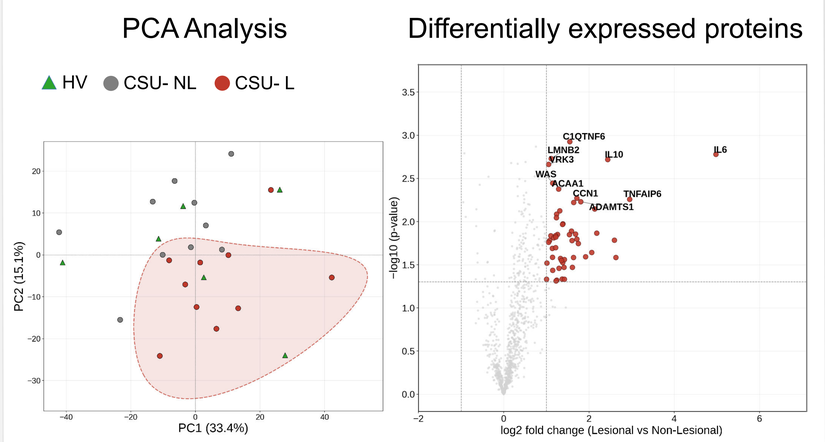


GO Biological Processes

Functional category	Upregulated proteins
Innate immune signaling *ITAM-axis kinases, adapters & cytoskeletal effectors*	FGR · LAT2 · INPP5D · CRACR2A · HCLS1 · SASH3 · IRAG2 · FMNL1 · LRCH4 · RAB44 · PSTPIP1 · APBB1IP · IPCEF1 · SIPA1 · IQGAP2
Cytokines *IL-6 family, type-2/3, TNF family, transcription factors*	IL6 · OSM · LIF · IL10 · IL13 · IL16 · IL17C · IL20 · TNFSF14 · TNFAIP6 · CEBPB
Chemokines & growth factors *Leukocyte chemotaxis & granulopoiesis*	CXCL8 · CXCL1 · CCL2 · CCL3 · CCL4 · CCL7 · CCL8 · CCL20 · CSF3 · S100A12
Neutrophil effector function *Degranulation, ROS, NETs, myeloid receptors*	NCF1 · PADI4 · PADI2 · CEACAM8 · FCAR · VNN2 · ADGRE3 · ADGRG3 · CLEC12A · CLEC4D · CLEC6A · C1QTNF6
Programmed cell death; DNA damage *PANoptosis, p21, lamin release, PARP*	ZBP1 · CDKN1A · BCL2L15 · VRK3 · PARP1 · LMNB1 · LMNB2
Tissue remodeling; wound healing *ECM proteolysis, matricellular cues, EGF-family*	ADAMTS1 · CCN1 · HBEGF · ACAA1
Nuclear transport *Nuclear pore complex*	TPR · NUP50

Figure 2. Proteomic profiling of ISF samples from CIndU patients at baseline and 4 hours following provocation using the Olink Reveal platform. Principal component analysis (PCA; left) shows clustering of healthy (HV), CIndU baseline (CIndU- B), 15 min (CIndU- 15min) and 4-hour (CIndU- 4h) post-provocation samples. Volcano plot (right) shows differentially expressed proteins comparing 4 hour versus baseline ISF samples, with 61 significantly increased proteins (≥2-fold increase and p <0.05, paired t-test, FDR) highlighted in red. Functional pathway enrichment analysis (bottom) of significantly upregulated proteins using Gene Ontology (GO) annotation.

Innate immune pathways and wound healing responses are upregulated in lesional CSU ISF samples



GO Biological Processes

Functional category	Upregulated proteins
Innate immune signaling *ITAM-axis kinases, adapters & cytoskeletal effectors*	FGR · LAT2 · INPP5D · CRACR2A · HCLS1 · SASH3 · IRAG2 · FMNL1 · LRCH4 · RAB44 · WAS
Cytokines *IL-6 family, type-2/3, TNF family*	IL6 · OSM · IL10 · IL13 · IL16 · TNFSF14 · TNFAIP6 · TNFRSF11B · TNFRSF6B
Chemokines & growth factors *Leukocyte chemotaxis & granulopoiesis*	CXCL8 · CCL2 · CCL7 · CCL19 · CSF2 · CSF3 · S100A12
Neutrophil effector function *Degranulation, ROS, NETs, myeloid receptors*	NCF1 · PADI4 · CEACAM8 · FCAR · VNN2 · ADGRE3 · CLEC12A · CLEC4D · CLEC4C · C1QTNF6 · MAFB
Programmed cell death; DNA damage *PANoptosis, pyroptosis, p21, lamin release*	ZBP1 · GSDMD · CDKN1A · BCL2L15 · VRK3 · LMNB1 · LMNB2
Tissue remodeling; wound healing & metabolism *ECM proteolysis, immune metabolism*	ADAMTS1 · CCN1 · DKK4 · ACAA1 · IDO1
Neuropeptide & hormone signaling *Neurogenic inflammation, hormonal mediators*	CALCA · POMC · PSPN

Figure 3. Proteomic profiling of ISF samples from non-lesional and lesional CSU patients using the Olink Reveal platform. Principal component analysis (PCA; left) shows clustering of healthy (HV), non-lesional CSU (CSU- NL), and lesional CSU (CSU- L) samples. Volcano plot (right) shows differentially expressed proteins comparing lesional versus non-lesional ISF samples, with 53 significantly increased proteins (≥2-fold increase and p <0.05, paired t-test, FDR) highlighted in red. Functional pathway enrichment analysis (bottom) of significantly upregulated proteins using Gene Ontology (GO) annotation.

CIndU and CSU share a common inflammatory program

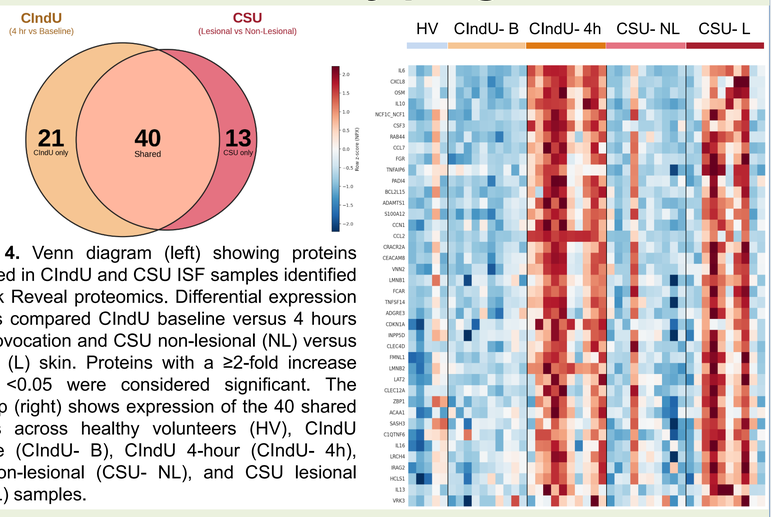


Figure 4. Venn diagram (left) showing proteins increased in CIndU and CSU ISF samples identified by Olink Reveal proteomics. Differential expression analysis compared CIndU baseline versus 4 hours post-provocation and CSU non-lesional (NL) versus lesional (L) skin. Proteins with a ≥2-fold increase and p <0.05 were considered significant. The heatmap (right) shows expression of the 40 shared proteins across healthy volunteers (HV), CIndU baseline (CIndU- B), CIndU 4-hour (CIndU- 4h), CSU non-lesional (CSU- NL), and CSU lesional (CSU- L) samples.

Increased mast cell activation and MRGPRX2 ligands in CSU and CIndU

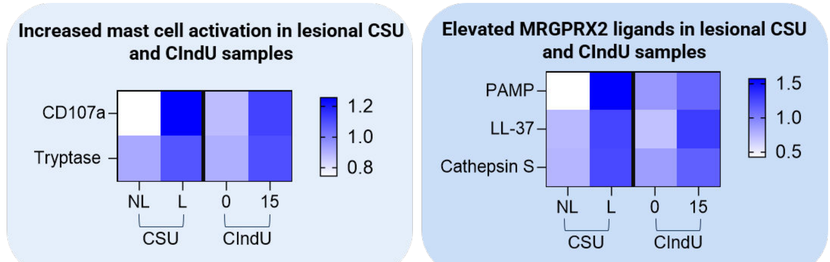
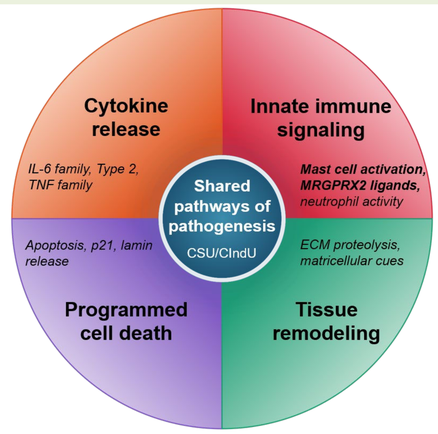


Figure 5. Heatmap of proteins detected by mass spectrometry in ISF from CSU and CIndU patients. ISF was collected from CSU non-lesional (NL) and lesional (L) skin and from CIndU patients at baseline (0) and 15 minutes post provocation. The left panel shows mast cell activation markers, while the right panel shows MRGPRX2 ligands. Heatmaps are row mean normalized.

Conclusion



In both CSU and CIndU, the lesional protein signature reflects a dominant innate immune response, ***characterized by increased mast cell activation markers, MRGPRX2 ligands, and cytokines/chemokines of the IL-6 family.*** *This inflammatory response is counterbalanced by an active tissue-repair program.*

Acknowledgements

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Disclosures

SB, LRB and JLH are employees of, and hold stock in, Evommune.