

Mast cell-pain connection in eosinophilic esophagitis

To the Editor,

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease with esophageal dysfunction; symptoms include dysphagia, heartburn, and pain.¹ Although EoE diagnosis includes ≥ 15 eosinophils per high-powered field (eos/HPF),¹ eosinophilia does not correlate well with clinical symptoms,¹ complicating therapeutic decision-making.

Mast cells (MCs) are involved in allergy, including EoE,² and neuromodulation.³ MCs reside in peripheral tissue and communicate with nearby structures, including nerve endings. MC-nerve associations are found in several tissues (e.g., brain, intestine, and skin).³ Although MCs are involved in EoE pathogenesis and MC-associated genes are upregulated in EoE,² the association is understudied.

Study design and demographic characteristics are summarized (Figure 1A, Table S1). We examined a Cincinnati Children's Hospital Medical Center cohort ($n = 43$) of patients with active EoE (≥ 15 eos/HPF; $n = 25$) and normal controls ($n = 18$) who presented for standard-of-care endoscopy with esophageal biopsies. Using Spearman's rank correlation coefficient, we examined the correlations of MC and eosinophil counts and MC counts with expression of 94 EoE-relevant esophageal genes. Esophageal MC counts were associated with eosinophil counts ($r = .54$, $p = .0002$). From these 94 genes, *CPA3* ($r = .72$, $p < .0001$) and *HPGDS* ($r = .76$, $p < .0001$) were most significantly associated with MC counts (per correlation coefficients) (Figure 1B, left and middle). The *CPA3+HPGDS* sum correlated even more highly with MC counts ($r = .77$, $p < .0001$) (Figure 1B, right).

We further examined a Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR) cohort of patients with EoE (active [≥ 15 eos/HPF] $n = 115$, inactive [< 15 eos/HPF] $n = 79$) who had enrolled in OMEGA (NCT02523118), completed patient-reported outcome metrics and EoE Histology Scoring System (HSS) components of a core EoE outcome set⁴; and had expression of 94 EoE-relevant esophageal genes (For detailed information, see the Appendix S1). We divided active EoE into four quartiles

by using *CPA3+HPGDS* sum as a MC count surrogate marker; the highest and lowest quartiles represented MC-high and MC-low, respectively. Although peak eosinophil counts did not differ between MC-high and MC-low, MC-high had a significantly lower EDP score ($p < .0001$), indicating more severe disease (molecular level), and higher histologic score ($p = .022$) (Figure 1C), indicating more severe histologic abnormalities. The presence of pain, but not dysphagia ($p = .530$), was significantly higher in MC-high than in MC-low ($p = .035$) (Figure 1D).

Expression of *TRPV1* (pain-associated gene) and *CPA3+HPGDS* sum (MC surrogate marker) were significantly increased in patients with active/inactive EoE with pain, whereas peak tissue eosinophil count and *CLC* expression (eosinophil surrogate marker) did not significantly differ by pain (Figure 2A–C). *TRPV1* showed significant association with *CPA3+HPGDS* sum in patients with active/inactive EoE (Figure 2D). *TRPV1* and MCs were in close proximity by co-immunofluorescence of esophageal biopsies (Figure 2E), suggesting an interaction.

Our findings indicate that *TRPV1* and MCs may modulate pain in EoE. Pain positively associated with molecular expression of *TRPV1*, *CPA3*, and *HPGDS*, but not eosinophilia, suggesting MC specificity. MCs release mediators that act on the nociceptor subset of sensory neurons, leading to pain sensitization. Nociceptors mediate pain, are present in peripheral tissues (e.g., gastrointestinal tract, skin), and express stimuli-triggered receptors (e.g., *TRPV1*). In an EoE animal model, antigen challenge had a *TRPV1*-mediated, sensitizing effect on neurons.⁵ *TRPV1*-mediated MC activation and neuronal sensitization may underlie visceral hypersensitivity,³ and esophageal mucosal nerve fibers in patients with reflux have increased *TRPV1* immunoreactivity.⁶ We observed proximity of MCs and nerves in EoE esophageal biopsies. Thus, *TRPV1* and MCs likely contribute to pain in EoE. Although using MC genes as a surrogate marker instead of MC counts is a limitation, our findings provide insight into disease pathophysiology and support MCs contributing to specific EoE symptoms, especially pain.

Abbreviations: CCHMC, Cincinnati Children's Hospital Medical Center; CEGIR, Consortium of Eosinophilic Gastrointestinal Disease Researchers; EDP, eosinophilic esophagitis diagnostic panel; EoE, eosinophilic esophagitis; HPF, high-power field; MC, Mast cells; PRO, Patient-reported outcome; EEsAI, eosinophilic esophagitis activity index; PEES, Pediatric Eosinophilic Esophagitis Symptom Scores; HSS, EoE Histology Scoring System; EREFS, EoE endoscopic reference score.

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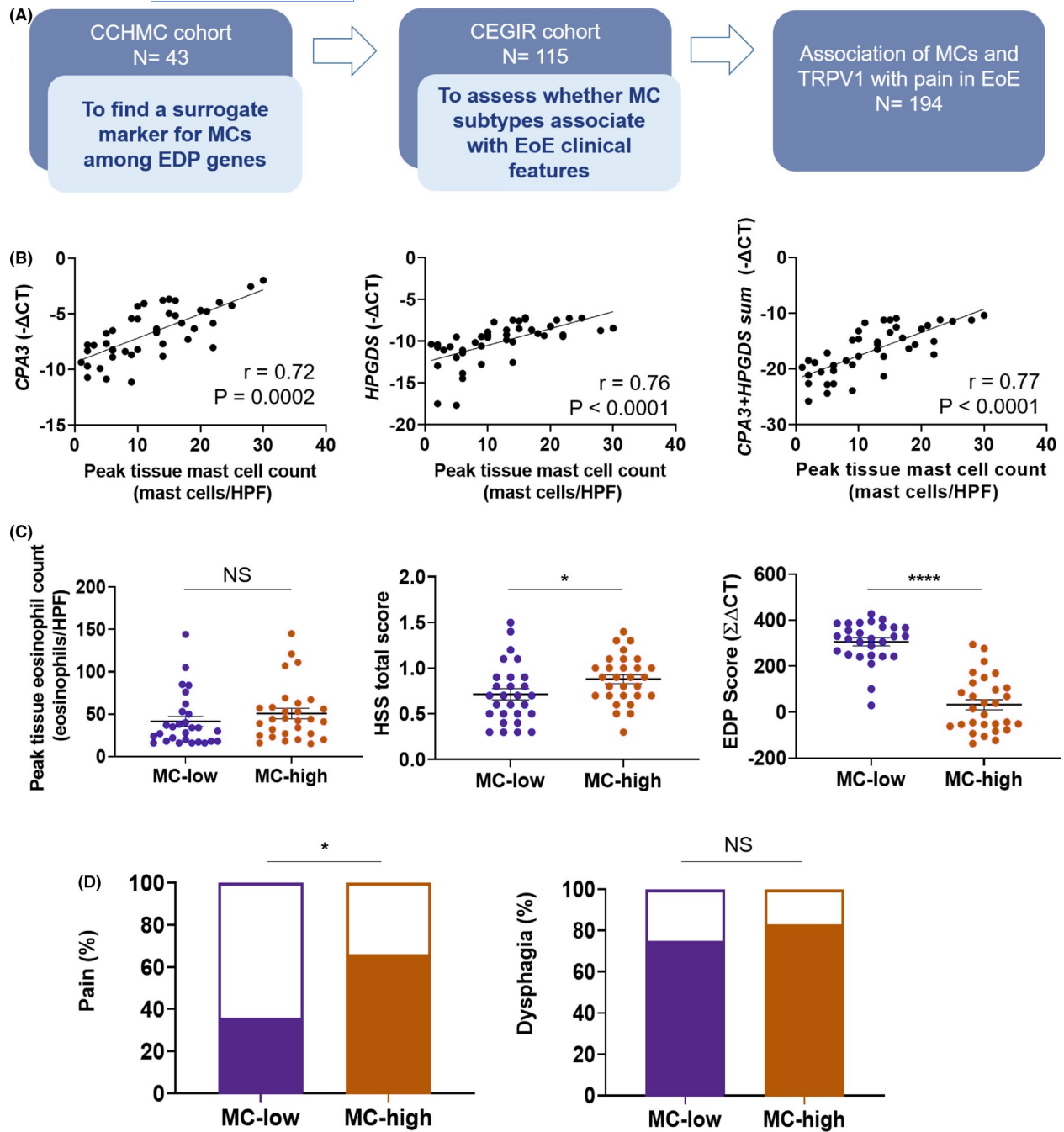


FIGURE 1 MC and clinical feature association in EoE. A, Study methodology. The association of MCs and TRPV1 with pain in EoE included CEGIR cohort patients with active EoE (≥ 15 eosinophils/HPF, $n = 115$) and inactive EoE (< 15 eosinophils/HPF, $n = 79$). B, MC count and MC-associated gene correlation (CPA3, left; HPGDS, middle; CPA3+HPGDS sum, right) (Spearman correlation). Markers represent distinct individuals. C, MC-low and MC-high comparison showing by mean with SEM (standard error of mean) for disease parameters, with markers representing distinct individuals. * $p < .05$ and **** $p < .0001$ (Mann-Whitney U test). D, Percentage of MC-low and MC-high patients with pain (left) and dysphagia (right). * $p < .05$ (Chi-square test). CCHMC, Cincinnati Children's Hospital Medical Center; CEGIR, Consortium of Eosinophilic Gastrointestinal Disease Researchers; MC, mast cell; EoE, eosinophilic esophagitis; EDP, EoE Diagnostic Panel; HSS, EoE Histology Scoring System; HPF, high-power field; NS, not significant

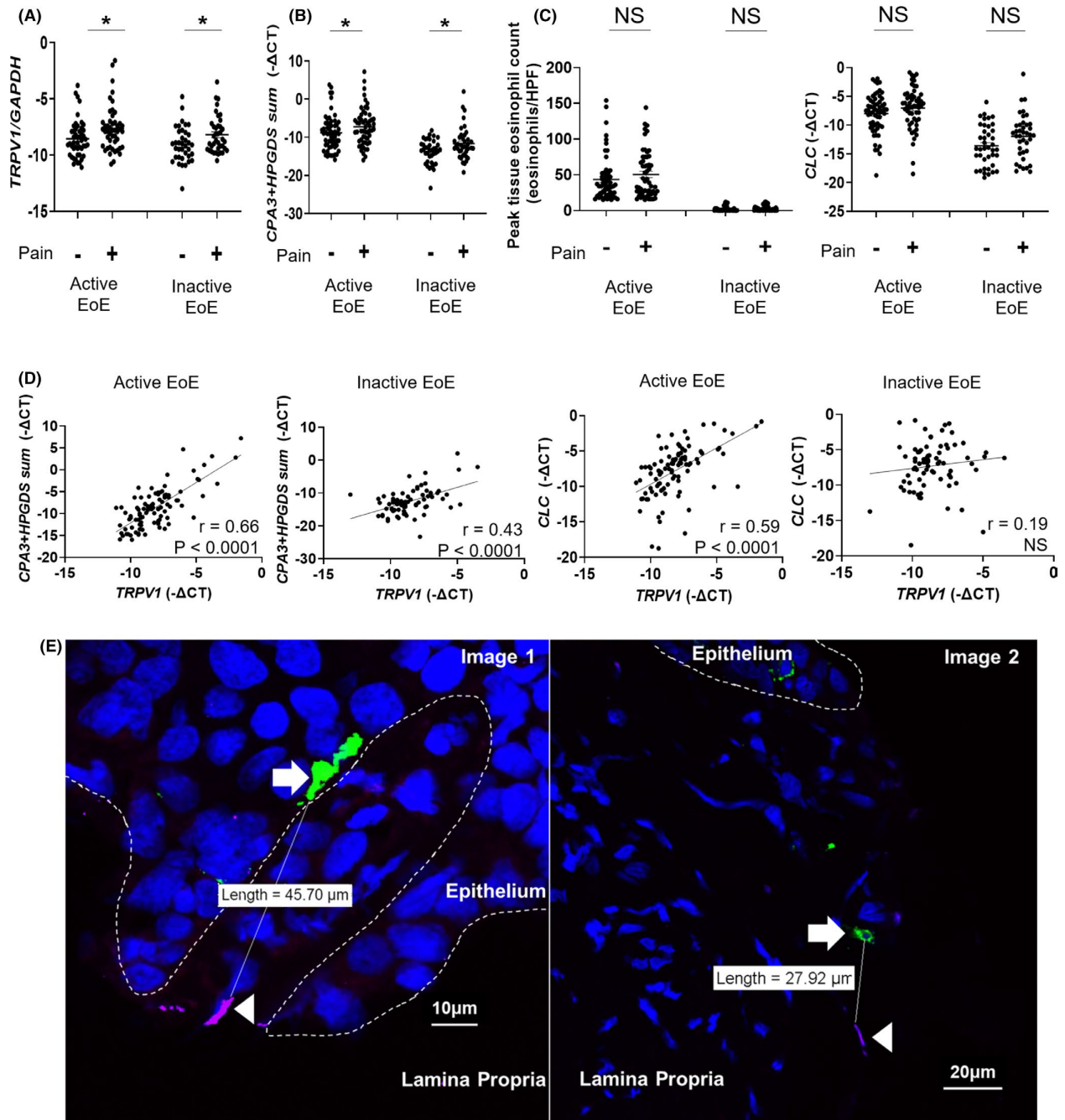


FIGURE 2 MCs and TRPV1 associate with pain in EoE. A-C, TRPV1 (A), CPA3+HPGDS sum (MCs surrogate marker) (B), and eosinophil parameters (C) in EoE by pain. * $p < .05$ and NS (Mann-Whitney U test). D, TRPV1 correlates with MC-associated (CPA3+HPGDS sum) and eosinophil-associated genes (CLC) (Spearman correlation). A-D, Markers represent distinct individuals. E, Immunofluorescence staining of esophageal tissue for tryptase (green), TRPV1 (magenta), and DAPI (blue) ($n = 2$). The MC (arrows)-nerve (arrowheads) distance is 45.70 μm (image 1) and 27.92 μm (image 2). MC, mast cell; EoE, eosinophilic esophagitis; HPF, high-power field; NS, not significant

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CONFLICT OF INTEREST




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SUPPORTING INFORMATION

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