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GATA-3 and T-bet as diagnostic markers of non-esophageal eosinophilic gastrointestinal disease

Joseph L. Neely, Annika Reimers, Steve Taylor, Shipra Garg, Mia Y. Masuda, Shauna Schroeder, Benjamin L. Wright, Alfred D. Doyle 

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To the Editor,

Eosinophilic gastrointestinal diseases (EGIDs) represent a spectrum of disorders classically defined by chronic eosinophil-predominant inflammation. Currently, there are no consensus diagnostic criteria for non-esophageal EGIDs. In contrast to the esophagus, eosinophils are resident cells in the gastrointestinal mucosa; therefore, establishing histologic thresholds indicative of pathology is more challenging. Further complicating the diagnosis, a significant proportion of patients with eosinophilic gastritis (EG) and/or eosinophilic duodenitis (EoD) develop disease remission.¹ Disappointing results of clinical trials exclusively targeting eosinophils suggest that other cell types (eg, mast cells and T cells) likely play an important role in driving disease pathogenesis.² This highlights the need for new disease markers.

While eosinophilic esophagitis (EoE) is the most common EGID, this spectrum of disorders includes Type 2 immunopathology that may occur anywhere along the GI tract. The master transcriptional regulators of Type 1 and Type 2 immune responses are T-bet and GATA-3, respectively. We recently examined T-bet and GATA-3 expression in biopsies from pediatric subjects with EoE, proton pump inhibitor-responsive EoE, and controls and found that both T-bet and GATA-3 expression were increased in EoE subjects.³ This study expands on our initial findings by examining these nuclear transcription factors in subjects with eosinophilic gastritis and/or duodenitis. We hypothesized that GATA-3 expression levels would be elevated in EG/EoD and that the ratio of GATA-3+ vs. T-bet+nuclei (G:T ratio) could serve as a potential diagnostic marker of EG/EoD.

We performed a retrospective study of gastrointestinal biopsies from pediatric subjects with EG/EoD ($n = 26$) and controls ($n = 40$) (See flow diagram in Figure S1). This study was approved by the Phoenix Children's Hospital IRB (PCH IRB #16-040). Inclusion/exclusion criteria and clinical characteristics, including endoscopic and histopathologic findings, were reported previously⁴ and are summarized in the Supplemental Methods and Table S1. Histologic diagnosis of EGID required ≥ 30 eosinophils per high-power field (eos/hpf) in ≥ 5 hpf for EG (gastric body or antrum) and ≥ 3 hpf for EoD (duodenal bulb or body).^{5, 6} Serial sections were stained for T-bet and GATA-3 by immunohistochemistry (Figure 1). Slides were digitized and analyzed as previously described.³ Percentages of T-bet+ and GATA-3+ nuclei were correlated to tissue eosinophil counts and compared between EG/EoD cases and controls.

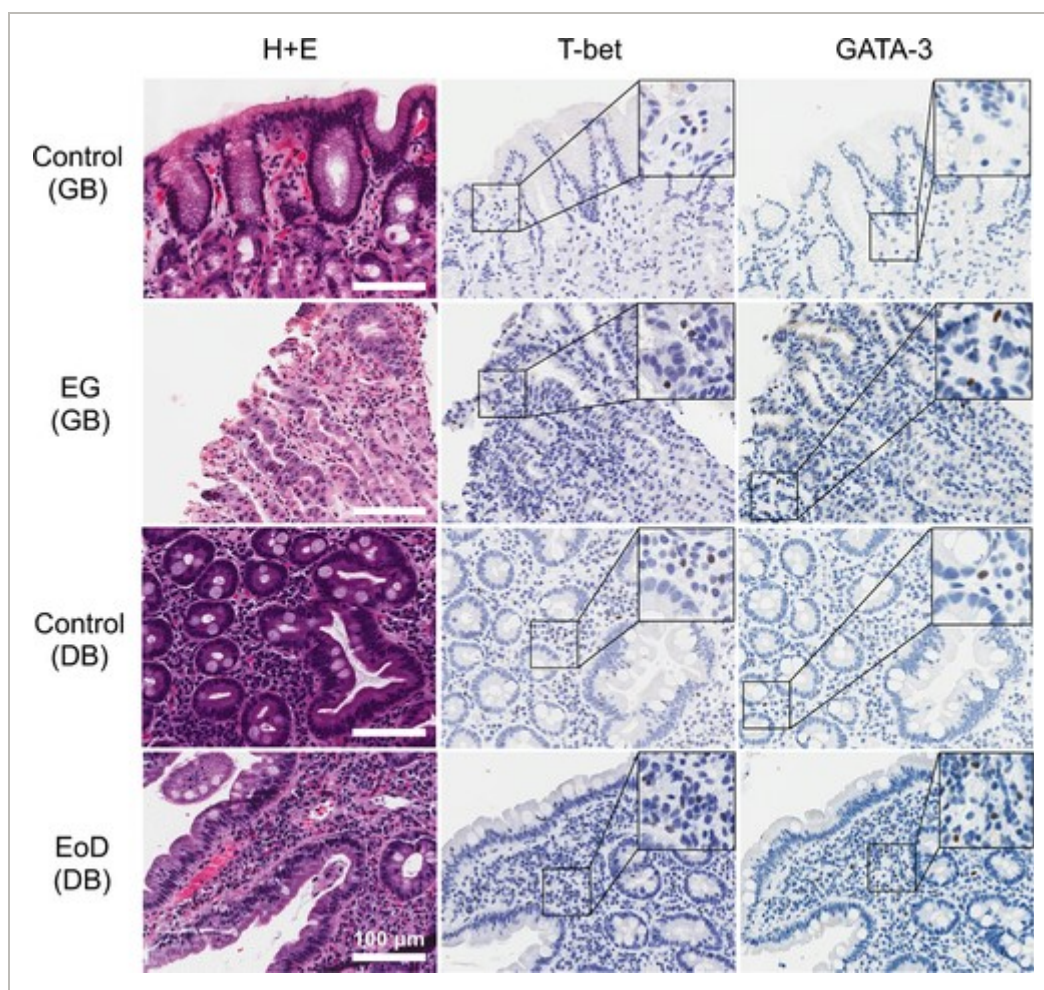


FIGURE 1

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Representative hematoxylin and eosin and immunohistochemistry stains for T-bet and GATA-3 in sections from the gastric body (GB) and duodenal bulb (DB) of cases and controls. Scale bar = 100 µm

T-bet expression did not differ among cases and controls (Figure 2A). EG/EoD cases demonstrated marked increases in the percentage of GATA-3+ nuclei in the gastric body, gastric antrum, and duodenal bulb (Figure 2B). The G:T ratio was elevated in EG/EoD

subjects and correlated with tissue eosinophil counts (Figure 2C, D). The G:T ratio accurately identified subjects with EG and/or EoD (Figure 2E, F).

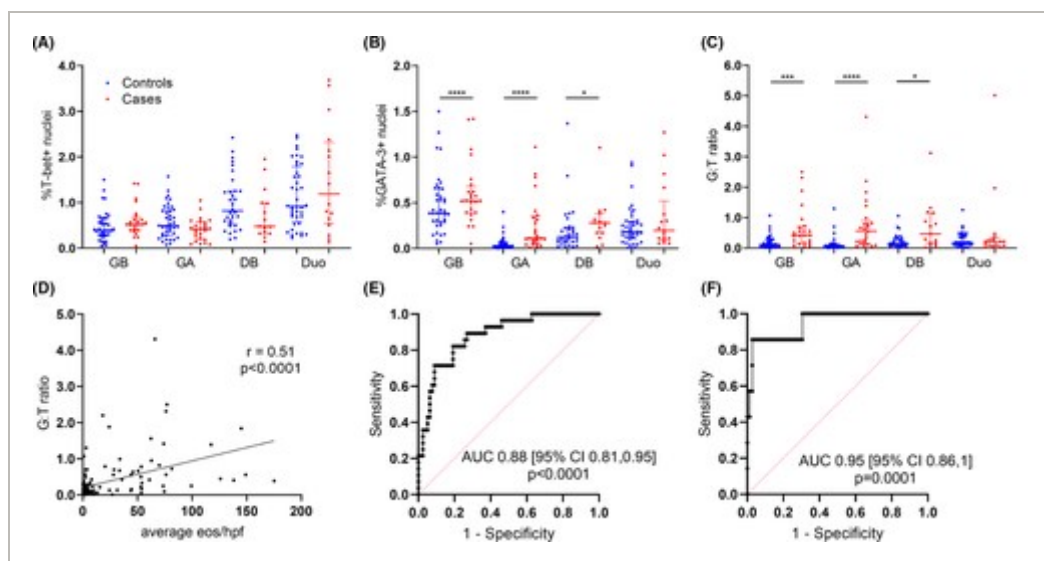


FIGURE 2

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Composite graphs comparing* percentages of T-bet+ (A) and GATA-3+ (B) nuclei. GATA-3+ nuclei were significantly increased in patients with biopsy-proven EGID compared with control biopsies, whereas no differences were seen for T-bet+ nuclei. The G:T ratio was increased in subjects with EGID (C) and correlated† with tissue eosinophil counts (average of 5 peak hpf) (D). In biopsies exceeding 30 eos/hpf in 5 hpf (stomach) and 3 hpf (duodenum), the G:T ratio accurately identified subjects with EG (E) and EoD (F), respectively. *Pairwise comparison performed using the Mann–Whitney *U* test. †Spearman's correlation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

In this study, we found the G:T ratio correlates with eosinophilic inflammation and accurately identified subjects with EG/EoD. In contrast to our previous study in EoE, we did not see significant differences in T-bet expression among EG/EoD subjects. The reason for this is unclear but may reflect a more profound Type 2 skewing of T-helper cell types in the stomach and duodenum of EG/EoD patients. We acknowledge that multiple cell types can express T-bet and GATA-3; notwithstanding, a majority of T-bet+ and GATA-3+ nuclei are likely T-cell nuclei (Data publicly accessible from the Immunological Genome Database at <http://www.immgen.org/databrowser>). We acknowledge this is a single-center study of children which may limit generalizability. We did not attempt to correlate the G:T ratio to clinical severity or treatment response. Lineage markers associated with other T-cell subsets such as RORC2 (Th17 cells) or FOXP3 (T regulatory cells) were not assessed, and validation of transcription factor staining using a second method was not performed due to limited tissue samples.

In summary, we have demonstrated subjects with EG/EoD have increased GATA-3+ cells within the gastric and duodenal mucosa and that GATA-3 and T-bet expression can be assessed by immunohistochemistry and image analysis. Future prospective longitudinal

studies are required to fully evaluate the G:T ratio as a potential histologic marker of non-esophageal EGIDs.

1 FUNDING INFORMATION

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CONFLICTS OF INTEREST

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Supporting Information



Filename	Description
all15171-sup-0001-Supinfo.docx Word document, 39.9 KB	Supplementary Material

Please note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing content) should be directed to the corresponding author for the article.

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Authors: Joseph L. Neely, BS¹, Annika Reimers², Steve Taylor, PA-C³, Shipra Garg, MD³, Mia Y. Masuda, BS², Shauna Schroeder, MD, MS⁴, Benjamin L. Wright, MD^{2, 5}, Alfred D. Doyle, PhD².

Affiliations:

¹University of Arizona College of Medicine – Phoenix, Phoenix, AZ

²Division of Allergy, Asthma, and Clinical Immunology, Department of Medicine, Mayo Clinic Arizona, Scottsdale, AZ

³Division of Laboratory Medicine and Pathology, Phoenix Children's Hospital, Phoenix, AZ

⁴Division of Gastroenterology, Phoenix Children's Hospital, Phoenix, AZ

⁵Section of Allergy and Immunology, Division of Pulmonology, Phoenix Children's Hospital, Phoenix, AZ

Corresponding author: Alfred D. Doyle, PhD, Doyle.Alfred@mayo.edu

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Supplemental Methods:

Study Population, Case Definitions, and Clinical Data

The inclusion and exclusion criteria for this study were published previously.¹ Briefly, we conducted a retrospective analysis of gastrointestinal biopsies from pediatric subjects who met clinical and histologic criteria for eosinophilic gastritis and/or duodenitis. Subjects were identified using ICD-9 and ICD-10 codes for eosinophilic gastritis and eosinophilic gastroenteritis. Clinical symptoms of abdominal pain, vomiting, or failure to thrive were confirmed by chart review. Eosinophil counts were confirmed by a pediatric gastrointestinal pathologist. Histopathologic diagnosis required ≥ 30 eos/hpf in the gastric body or gastric antrum in at least 5 hpf for EG² and ≥ 30 eos/hpf in at least 3 hpf in the duodenal bulb or duodenal body for EoD.³ Archived gastrointestinal biopsies from control subjects were obtained from individuals undergoing upper endoscopy without a pathologic diagnosis. A review of the electronic health record was performed and demographics, medical history, other allergic disorders, clinical symptoms, and final diagnoses were recorded. Endoscopic findings were evaluated retrospectively based on images and descriptions provided in the clinical endoscopy report. Because we analyzed existing clinical pathology specimens this study posed minimal risk to human subjects and a waiver of consent was obtained.

Immunohistochemical Staining and Image Analysis

Tissue sectioning and IHC staining was performed at the Pathology Research Core (Mayo Clinic, Rochester, MN) using the Leica Bond RX stainer (Leica Biosystems, Nussloch, Germany). IHC staining was performed using GATA-3 (clone L50-823, Biocare Medical, Pacheco, CA), and T-bet (SC-21003, Santa Cruz Biotech, Dallas, TX) as previously described.⁴ Tissue sections were digitized (Aperio AT Turbo, Leica Biosystems, Buffalo Grove, IL) and analyzed using Aperio ImageScope software (version 12.4.3, Aperio Technologies, Vista, CA).

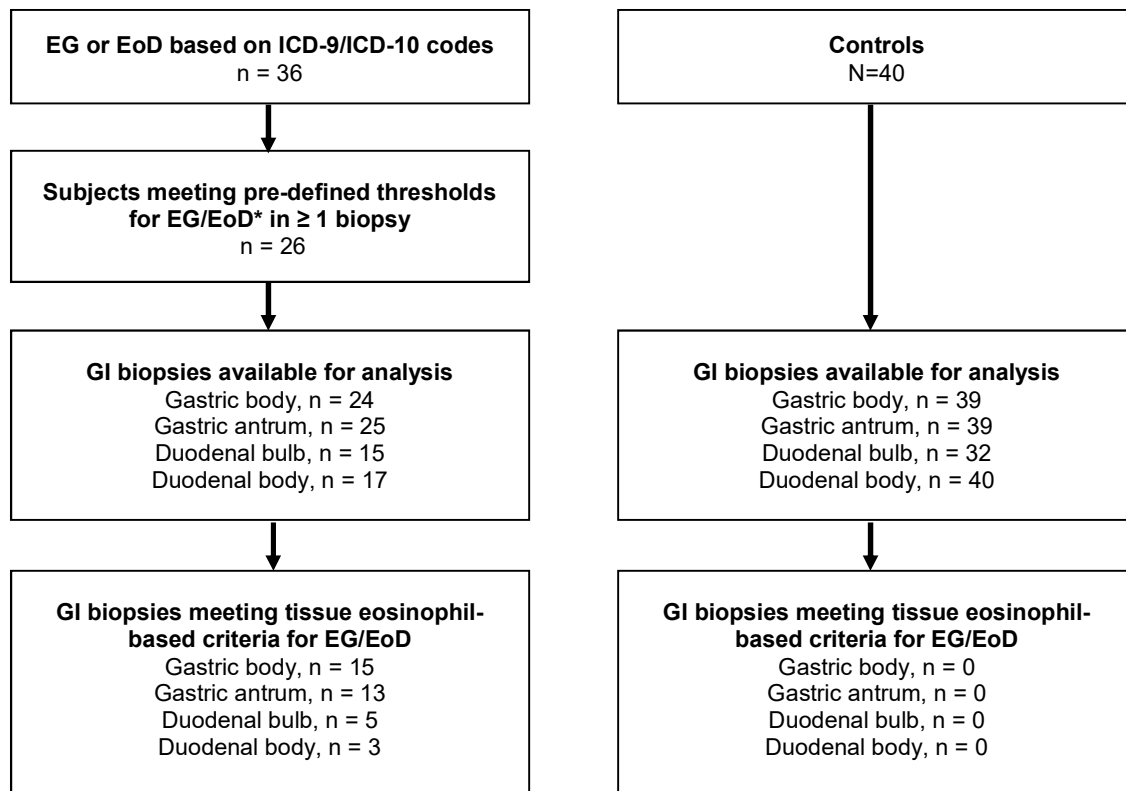
58 Image analysis using a nuclear algorithm was used to quantify the cells staining positive for T-
59 bet and GATA-3. Only nuclei that stained strongly or moderately positive according to the
60 algorithm were considered positive.

61 **Supplemental Table E1. Study Population Characteristics**

	Controls (n=40)	EGID cases (n=26)	p value*
Males (n, %)	11 (27.5)	12 (46.2)	NS
Race (n, %)			
White	24 (60)	22 (84.6)	NS
Black/African American	1 (2.5)	3 (11.5)	NS
Native American	1 (2.5)	0	NS
Other	7 (17.5)	1 (3.8)	NS
Ethnicity (n, %)			
Hispanic	6 (15)	1 (3.8)	NS
Age (median yrs, (IQR))	9.5 (5-15.75)	11.5 (7-16)	NS
Atopy (any) (n, %)	6 (15)	25 (96.2)	p < 0.0001
Allergic rhinitis	4 (10)	16 (61.5)	p < 0.0001
Asthma	2 (5)	8 (30.8)	NS
Food allergy	1 (2.5)	12 (46.2)	p < 0.0001
Atopic dermatitis	0	3 (11.5)	NS
Symptoms (n, %)			
Abdominal pain	25 (62.5)	17 (65.4)	NS
Nausea	12 (30)	10 (38.5)	NS
Vomiting	12 (30)	14 (53.8)	NS
Weight loss	8 (20)	7 (26.9)	NS
Dysphagia	5 (12.5)	7 (26.9)	NS
Reflux	5 (12.5)	5 (19.2)	NS
Constipation	9 (22.5)	4 (15.4)	NS
Diarrhea	5 (12.5)	3 (11.5)	NS
Feeding problems	5 (12.5)	4 (15.5)	NS
Diagnosis (n, %)			
EG	0	17 (65.4)	p < 0.0001
EoD	0	5 (19.2)	p = 0.007
EG + EoD	0	4 (15.4)	p < 0.02
EoE + EG/EoD	0	13 (50)	p < 0.0001
Functional GI disorder	25 (62.5)	0	p < 0.0001
Feeding problem	4 (10)	0	NS
Other (FPIES, med related, infection)	6 (15)	0	NS
Lactose intolerance	5 (12.5)	0	NS
Gastrointestinal endoscopic findings (n, %)			
Ulcers (any)	0	14 (53.8)	p < 0.0001
Gastric ulcer	0	5 (19.2)	p = 0.007
Duodenal bulb ulcer	0	7 (26.9)	p = 0.0008
Duodenal body ulcer	0	2 (7.7)	NS
Superficial hemorrhage/erythema	4 (10)	13 (50)	p = 0.0005
Hyperplastic gastric folds	0	13 (50)	p < 0.0001
Gastric nodularity	0	6 (23.1)	p = 0.0025
Esophageal endoscopic findings (n, %)			
Erosions	0	0	NS
Edema	2 (5)	12 (46.2)	p = 0.0001
Linear furrows	2 (5)	7 (26.9)	p = 0.02
Exudates/micro-abscesses	0	3 (11.5)	NS
Rings	0	2 (7.7)	NS
Stricture	0	0	NS

62
63 *Categorical variables are compared using the Fisher's exact test. Median age (continuous variable) is
64 compared using a Mann-Whitney U test. Reprinted with permission.

Supplemental Figure E1. Flow diagram



*≥30 eos/hpf in at least 5 hpf in the gastric body or gastric antrum and ≥30 eos/hpf in at least 3 hpf in the duodenal bulb or duodenal body