

Pediatric Eosinophilic Esophagitis Symptom Scores (PEESS v2.0) identify histologic and molecular correlates of the key clinical features of disease

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Background: The Pediatric Eosinophilic Esophagitis Symptom Score (PEESS v2.0) measures patient-relevant outcomes.

However, whether patient-identified domains (dysphagia, gastroesophageal reflux disease [GERD], nausea/vomiting, and pain) align with clinical symptomology and histopathologic and molecular features of eosinophilic esophagitis (EoE) is unclear. **Objective:** The purpose of this study was to determine whether clinical features of EoE, measured through PEESS v2.0, associate with histopathologic and molecular features of EoE. This represents a novel approach for analysis of allergic diseases, given the availability of allergic tissue biopsy specimens.

Methods: We systematically recruited treated and untreated pediatric patients with EoE (aged 2-18 years) and examined parent proxy-reported symptoms using the PEESS v2.0.

Clinical symptomology was collected by questionnaire.

Esophageal biopsy samples were quantified for levels of eosinophils, eosinophil peroxidase (EPX) immunohistochemical

staining, and mast cells. Molecular features were assessed by using the EoE Diagnostic Panel (94 EoE-related gene transcripts). Associations between domain scores and clinical symptoms and biological features were analyzed with Wilcoxon rank sum and Spearman correlation.

Results: The PEESS v2.0 domains correlated to specific parent-reported symptoms: dysphagia ($P = .0012$), GERD ($P = .0001$), and nausea/vomiting ($P < .0001$). Pain correlated with multiple symptoms ($P < .0005$). Dysphagia correlated most strongly with overall histopathology, particularly in the proximal esophagus ($P \leq .0049$). Markers of esophageal activity (EPX) were significantly associated with dysphagia (strongest $r = 0.37$, $P = .02$). Eosinophil levels were more associated with pain ($r = 0.27$, $P = .06$) than dysphagia ($r = 0.24$, $P = .13$). The dysphagia domain correlated most with esophageal gene transcript levels, predominantly with mast cell-specific genes.

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Conclusion: We have (1) established a validated, parent proxy–reported measure for pediatric EoE, the PEESS v2.0; (2) verified that the parent proxy effectively captures symptoms; (3) determined that the dysphagia domain most closely aligns with symptoms and tissue-based molecular biomarkers; (4) established that symptoms correlate with EPX staining; and (5) observed association between mast cells and dysphagia. (*J Allergy Clin Immunol* 2015;135:1519-28.)

Key words: Allergy, reflux, quality of life, surveys, mast cells, molecular genetics, pediatrics, microarray, patient-reported outcomes

Eosinophilic esophagitis (EoE) is a chronic allergic inflammatory disease driven by food antigen exposure. Although a variety of histologic features, including eosinophil and mast cell accumulation and deposition of their granule contents in tissue, as well as epithelial hyperplastic and remodeling changes, have been noted, the importance of each of these histologic findings for discrete symptoms has been unclear. Clinical outcome end points, such as disease-specific patient-reported outcomes (PROs), are increasingly being recognized as essential for linking disease processes with key effector mechanisms and developing treatments that effectively improve clinically relevant features.^{1,2} We recently developed the Pediatric EoE Symptom Score (PEESS v2.0) in an effort to identify and uniquely measure relevant outcomes that patients with EoE and their families identified as important.³ Input from patients and their families established that the 20 questions from the PEESS v2.0 could be consolidated into 4 major domains: dysphagia, gastroesophageal reflux disease (GERD), nausea/vomiting, and pain.³ To further validate these domains, it is important to demonstrate that the domains align with clinical symptomology and histopathologic features.

EoE is typified by eosinophil-predominant infiltration of the esophagus (≥ 15 eosinophils per high-power field [hpf] in at least 1 hpf in esophageal biopsy specimens) that is not responsive to prolonged, high-dose acid suppression with proton pump inhibitors.⁴ However, esophageal changes are not limited to the number of eosinophils and might also depend on the extracellular content of eosinophil granule proteins, such as eosinophil peroxidase (EPX), which might have a functional role in EoE.⁵ In addition, a unique esophageal gene expression profile exists in patients with EoE, and the magnitude of its expression is proportional to the quantity of inflammatory cells (eg, eosinophils and mast cells).^{6,7} This early work implicates T_H2 inflammatory responses (eg, IL-13 and eotaxin-3), as well as expression of mast cell-specific genes, in EoE pathogenesis.⁷⁻¹⁰ Importantly, the gene expression profiles of a specific set of 94 genes can discriminate EoE from non-EoE.¹¹ However, whether the underlying clinical symptomology, histology, and molecular profiles relate to specific clinical manifestations has not been established.

The purpose of this study was (1) to validate patient-defined domains of the parent proxy–reported PEESS v2.0 questionnaire, (2) to determine which histologic features correlate most strongly and specifically with distinct clinical symptoms, and (3) to gain insight into disease pathophysiology by deeply probing molecular transcript expression as a function of distinct clinical symptoms.

Abbreviations used

CCHMC:	Cincinnati Children's Hospital Medical Center
CLC:	Charcot-Leyden crystal galectin
CPA3:	Carboxypeptidase A3
EDP:	EoE diagnostic panel
EoE:	Eosinophilic esophagitis
EPX:	Eosinophil peroxidase
GERD:	Gastroesophageal reflux disease
GPR160:	G protein-coupled receptor 160
HPGDS:	Hematopoietic prostaglandin D synthase
hpf:	High-power field
IL5RA:	IL-5 receptor α
IQR:	Interquartile range
LRRC31:	Leucine-rich repeat containing 31
PEESS v2.0:	Pediatric EoE Symptom Score
PRO:	Patient-reported outcome
SAMSIN:	SAM domain SH3 domain and nuclear localization signals 1
SLC26A4:	Solute carrier family 26 (anion exchanger), member 4
VEGFA:	Vascular endothelial growth factor A

TABLE I. Characteristics of the pediatric EoE study cohort

Characteristic	Statistics
No.	46
Age $\pm$ SD (y)	8.2 $\pm$ 4.2 (median, 6.9; range, 2.4-17)
Age at diagnosis (y)	6.1 $\pm$ 4.4 (median, 4.4; range, 0.8-17)
Sex (% male)*	100
Race (% white)*	97.8
Duration since histologic diagnosis $\pm$ SD (y)	2.4 $\pm$ 1.6 (range, 0.2-7.3)
Treatment (%)	93.5
Diet only (%)	30.4
Swallowed steroids only (%)	28.3
Diet and swallowed steroids (%)	34.8
Peak eosinophil count (median)	35.5 (IQR, 7.8-88.8; range, 0-295)
Disease activity† (% of cohort)	
Active disease count (eosinophils ≥ 1 /hpf)	71.7
Intermediate count (15 > eosinophils ≥ 6 hpf)	6.5
Low count (6 > eosinophils > 0)	15.2
None (eosinophils = 0)‡	6.5
Distal eosinophil count (median)	27.5 (IQR, 7.8-77.3; range, 0-295)
Proximal eosinophil count (median)	4 (IQR, 0-35; range, 0-295)

*Inclusion of only male and primarily white subjects was an inclusion criterion for the analysis set to minimize heterogeneity.

†Activity was based on the peak eosinophil count (eg, maximum of the proximal and distal counts).

‡All subjects with no eosinophils were treated.

METHODS

Study subjects

Pediatric patients had a confirmed diagnosis of EoE, which was defined as the presence of upper gastrointestinal tract symptoms and an endoscopy with 15 or more eosinophils/hpf in proximal or distal esophageal tissue biopsy specimens per consensus recommendations.¹² Additional data collected included a parent-reported clinical symptom questionnaire and an endoscopic sample collection (see the [Methods](#) section in this article's Online Repository at www.jacionline.org). Consent was obtained by a study staff member and was completed either in same-day surgery or in the outpatient clinic. Assent was obtained for

TABLE II. Patient- and parent proxy-reported outcomes as a function of PEESS v2.0 and its domains

PEESS v2.0	Parent (n = 46)		Pediatric patient* (n = 28)		Spearman* (P value)
	Median (IQR)	Range	Median (IQR)	Range	
Total score	22.5 (6.7-31.4)	0-57.5	28.8 (13.8-41.3)	5-55.0	0.74 (.0001)
Domain scores					
Dysphagia	21.9 (9.4-33.6)	0-65.6	34.4 (10.9-46.9)	0-68.8	0.67 (.0008)
GERD	18.8 (0-26.6)	0-81.3	25.0 (3.1-28.1)	0-50.0	0.63 (.0019)
Nausea/vomiting	12.5 (0-37.5)	0-75.0	25.0 (3.1-43.8)	0-81.3	0.58 (.005)
Pain	25.0 (12.5-43.8)	0-100.0	37.5 (21.9-50.0)	0-56.3	0.78 (<.0001)

*Only reflects children age 8 years or older.

TABLE III. Parent-reported clinical symptoms* in patients with EoE and associations with PEESS v2.0 scores

Clinical symptom		No.	Frequency (%)	PEESS v2.0 score				
				Total	Dysphagia domain	GERD domain	Nausea/vomiting domain	Pain domain
Swallowing	Food impaction	46	8.7	—	—	—	—	—
	Choking/gagging	46	21.7	—	—	—	—	—
	Clinical dysphagia	46	28.3	—	25.0 (.0012)	—	—	—
	Difficulty swallowing	46	21.7	22.9 (.0006)	29.4 (.0017)	—	—	25.0 (.0005)
	Chest pain	46	19.6	—	—	—	—	—
Generalized gastrointestinal	Heartburn	46	28.3	21.2 (.0002)	27.6 (.0023)	12.5 (.0002)	37.5 (.0026)	25.0 (.0003)
	Reflux	45	33.3	—	—	14.6 (.0001)	—	—
	Poor appetite	46	43.5	12.5 (.0014)	—	—	—	—
	Food aversions	46	26.1	17 (.0011)	—	—	—	—
	Weight loss	46	15.2	—	—	—	—	—
	Abdominal pain	46	47.8	15.6 (.0004)	—	—	—	21.8 (.0002)
	Early satiety	46	50.0	—	—	—	—	—
	Poor weight gain	45	20.0	—	—	—	—	—
	Nausea	46	43.5	13.1 (.0027)	—	—	34.4 (<.0001)	21.8 (.0004)
	Emesis	46	23.9	—	—	—	43.8 (<.0001)	—
	Diarrhea	46	37.0	—	—	—	—	—
	Constipation	46	17.4	—	—	—	—	—
	Bloody stools†	46	4.3	—	—	—	—	—

—, Nonsignificant.

*Symptoms are separated by swallowing and generalized gastrointestinal symptoms. Those with significant differences ($P < .003$) are reported as the median difference between those with and without the condition (P value).

†The condition was too infrequent in the study cohort for comparative analyses.

participants aged 11 to 17 years. This study was approved by the Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Review Board.

Histologic and laboratory methods

Biopsy specimens obtained were evaluated for histologic features, including eosinophil measurement, tryptase- and chymase-positive cell measurement, and EPX-based immunohistochemistry (see the [Methods](#) section in this article's Online Repository). We performed PCR amplification of representative genes from total RNA extracted from distal esophageal biopsy specimens (see the [Methods](#) section in this article's Online Repository).¹¹

The PEESS v2.0 and domains

The PEESS v2.0 is a content-validated metric that seeks to capture EoE-specific symptoms directly from children with EoE (8-18 years of age) and from their parents (for children 2-18 years of age).¹² From parent/participant interviews, 4 domains were established: dysphagia, GERD, nausea/vomiting, and pain (see [Table E1](#) in this article's Online Repository at www.jacionline.org). The range for these PEESS v2.0 scores was 0 to 100, with a higher score being indicative of more frequent and/or severe symptoms for total scores and the dysphagia, GERD, nausea/vomiting, and pain domains. For further details, see the [Methods](#) section in this article's Online Repository.

Statistical analysis

To provide construct validation for the parent proxy-reported PEESS v2.0 domain scores, we sought to demonstrate that the domain scores associated in an anticipated manner with the presence of specific clinical features obtained by means of interview and to determine whether any of the domains were associated with expected EoE biological features. A correlation-corrected Bonferroni adjustment was made to establish the P value required for statistical significance (comparison between domains, $P \leq .005$; PEESS v2.0 and clinical symptoms, $P \leq .003$; eosinophil measures, $P \leq .03$; and mast cells, $P \leq .02$). All analyses were performed in JMP v9.0 (SAS Institute, Cary, NC). Detailed statistical analyses are described in the [Methods](#) section in this article's Online Repository.

RESULTS

Patient population

Overall, this pediatric cohort ($n = 46$) had a median age of 6.9 years and a median duration since initial diagnosis of 2.4 years ([Table I](#)). Only 3 participants were untreated at the time of sample collection. Therapies were swallowed steroids alone (either fluticasone propionate or budesonide), diet modification alone (elemental diet, elemental diet and food elimination diet, or food trial), or both swallowed steroids and diet modification ([Table I](#)).

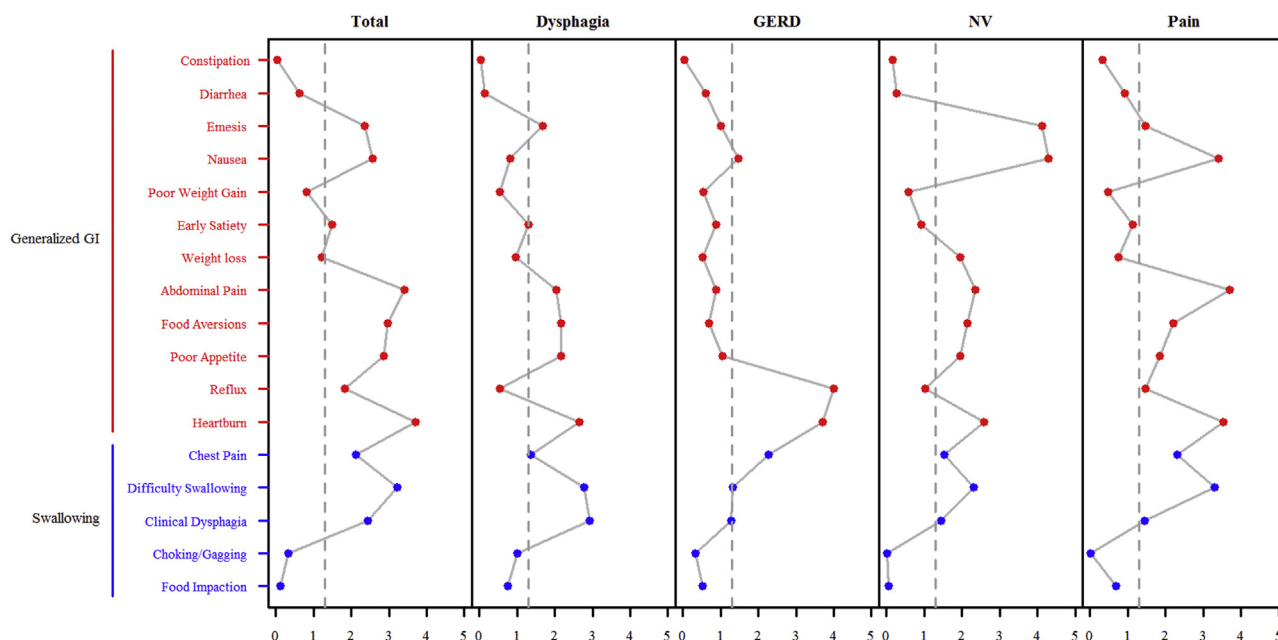


FIG 1. Relationships between gastrointestinal symptoms and PEESS v2.0 scores. The *x-axis* represents the negative $\log_{10}$ *P* value of the Wilcoxon rank sum test comparing subjects reporting the clinical symptom with those who do not report the clinical symptom. The *x-axis* columns in the figure are PEESS v2.0 scores, whereas the *y-axis* indicates clinical symptoms reported as yes/no. *GI*, Gastrointestinal; *NV*, nausea/vomiting domain.

Patients' characteristics and PEESS v2.0 scores

Parental reports correlated well with child reports, with correlations ranging from 0.58 to 0.78 (Table II). Overall, the PEESS v2.0 total and domain scores showed modest increases (median range, 12.5-25.0) compared with the expectation of a score close to zero for those not reporting symptoms and a maximum score of 100 (Table II). Of note, the pain domain score was significantly higher than the GERD domain, nausea/vomiting domain, and total scores ($P < .0025$ vs the pain domain for each).

PEESS v2.0 domains associate with clinical symptomatology

We sought to demonstrate the unique sensitivity of the domains in measuring disease-specific characteristics relative to patient symptomatology. Participants had a diverse set of symptoms, including a high proportion with upper gastrointestinal tract and allergic symptoms (Table III and see Table E2 in this article's Online Repository at www.jacionline.org). Wilcoxon rank sums were used to compare domain scores in subjects with and without specific symptoms to determine whether PEESS v2.0 domains were related to specific gastrointestinal symptoms often associated with EoE (Fig 1 and Table III). The total score was associated with symptoms of difficulty swallowing, heartburn, poor appetite, food aversion, abdominal pain, and nausea ($P \leq .0027$). However, because the total score is a composite measure of the 4 domains, it was important to examine associations with the individual domains. Of note, food impaction was not significantly associated with the dysphagia domain; however, this study was underpowered to detect an effect given the low frequency of food impaction in the study cohort (8.7%). In contrast, several symptoms were associated with multiple domains: difficulty swallowing was

associated with the dysphagia and pain domains ($P \leq .0017$, Table III), heartburn was significantly associated with each of the 4 domains ($P \leq .0026$), and nausea was associated with the nausea/vomiting and pain domains ($P \leq .0004$). Symptoms associated with a single domain included clinical dysphagia (dysphagia domain, $P = .0012$), reflux (GERD domain, $P = .0001$), and emesis (nausea/vomiting domain, $P < .0001$). The PEESS v2.0 domains were not associated with age, age at diagnosis, time since diagnosis, allergic status, asthma status, or eczema ($P > .2$).

PEESS v2.0 domains associate with EoE biological features

Eosinophil measures most strongly correlate with the dysphagia domain. Most subjects ($n = 33$) had active EoE, and very few ($n = 3$) had biopsy specimens without eosinophils. Peak eosinophil counts ranged from 0 to 295 eosinophils/hpf, with a median count of 35.5 eosinophils/hpf (Table I). Counts from the distal esophagus tended to be higher than those from the proximal esophagus (Table I). Extracellular EPX staining demonstrated that most patients had some level of eosinophil activation and that this eosinophil activation was much more prominent in the distal than the proximal esophagus (see Table E3 in this article's Online Repository at www.jacionline.org).

Esophageal mucosal eosinophil counts did not exhibit statistically significant ($P > .05$) associations with the PEESS v2.0 domain scores, but suggestive trends were identified (Fig 2). The strongest association with the distal eosinophil count was with the pain domain ($r = 0.28$, $P = .06$), whereas the strongest association with the proximal eosinophil count was with the dysphagia domain ($r = 0.24$, $P = .13$). Although patients with histologically active disease (≥ 15 eosinophils/hpf)

		Total	Dysphagia	GERD	NV	Pain
Eos Count	Peak	-0.1	0.18	0.09	-0.03	0.24
	Distal	-0.08	0.21	0.13	-0.04	0.28
	Proximal	-0.03	0.24	0.003	0.13	0.16
Distal EPX	Total	0.15	0.28	0.15	-0.06	0.19
	Reproducibility	0.14	0.27	0.15	-0.07	0.15
	Patchiness	0.07	0.27	0.08	-0.16	0.16
	Degranulation	0.17	0.25	0.14	-0.23	0.15
	Intact	0.18	0.26	0.15	-0.15	0.17
	Random	0.17	0.23	0.17	-0.07	0.15
Proximal EPX	Total	0.12	0.3	-0.06	-0.09	-0.0002
	Reproducibility	0.14	0.33	-0.05	-0.1	-0.04
	Patchiness	0.15	0.29	-0.06	-0.09	-0.07
	Degranulation	0.02	0.37	-0.02	-0.04	0.12
	Intact	0.17	0.29	-0.08	-0.14	-0.07
	Random	0.16	0.27	-0.06	-0.12	-0.11
Distal Epithelium	Tryptase	0.09	0.2	0.02	-0.01	0.1
	Chymase	0.01	0.28	0.17	0.15	0.14
Proximal Epithelium	Tryptase	0.11	0.32	0.1	0.13	0.2
	Chymase	-0.04	0.34	0.08	0.22	0.12
Gene Expression	Tryptase	0.03	0.28	0.14	-0.08	-0.004
	Chymase	0.05	0.19	-0.04	0.05	0.01
	<i>CPA3</i>	-0.08	0.36	0.14	-0.004	0.12

FIG 2. Spearman correlations between PEESS v2.0 scores and disease parameters. The strength of the association with a diagnostic subset of the EoE transcriptome is measured by using Spearman ρ (text within cell). Darker red shades indicate stronger negative correlations, whereas darker blue shades indicate stronger positive correlations. Boldface values indicate correlations that are significant at a P value of .05 or less. *Eos*, Eosinophil; *NV*, nausea/vomiting domain.

had higher domain scores than those with normal histology (0 eosinophils/hpf) with respect to PEESS v2.0 domain or total scores (see Table E4 in this article's Online Repository at www.jacionline.org), these differences were not statistically significant, likely because of the small sample size in the normal histology group ($n = 3$). Furthermore, when we examined all possible comparisons between eosinophil classifications, no difference

reached significance ($P > .05$). However, subjects in remission had the lowest PEESS v2.0 scores (see Table E4).

Among all the PEESS v2.0 scores, the dysphagia domain score consistently exhibited the strongest correlation with EPX values for both distal and proximal biopsy specimens ($\rho = 0.23$ - 0.37 , $P = .019$ -. 17 ; Fig 2). The only correlations that reached nominal ($P \leq .05$) significance were between EPX parameters of the

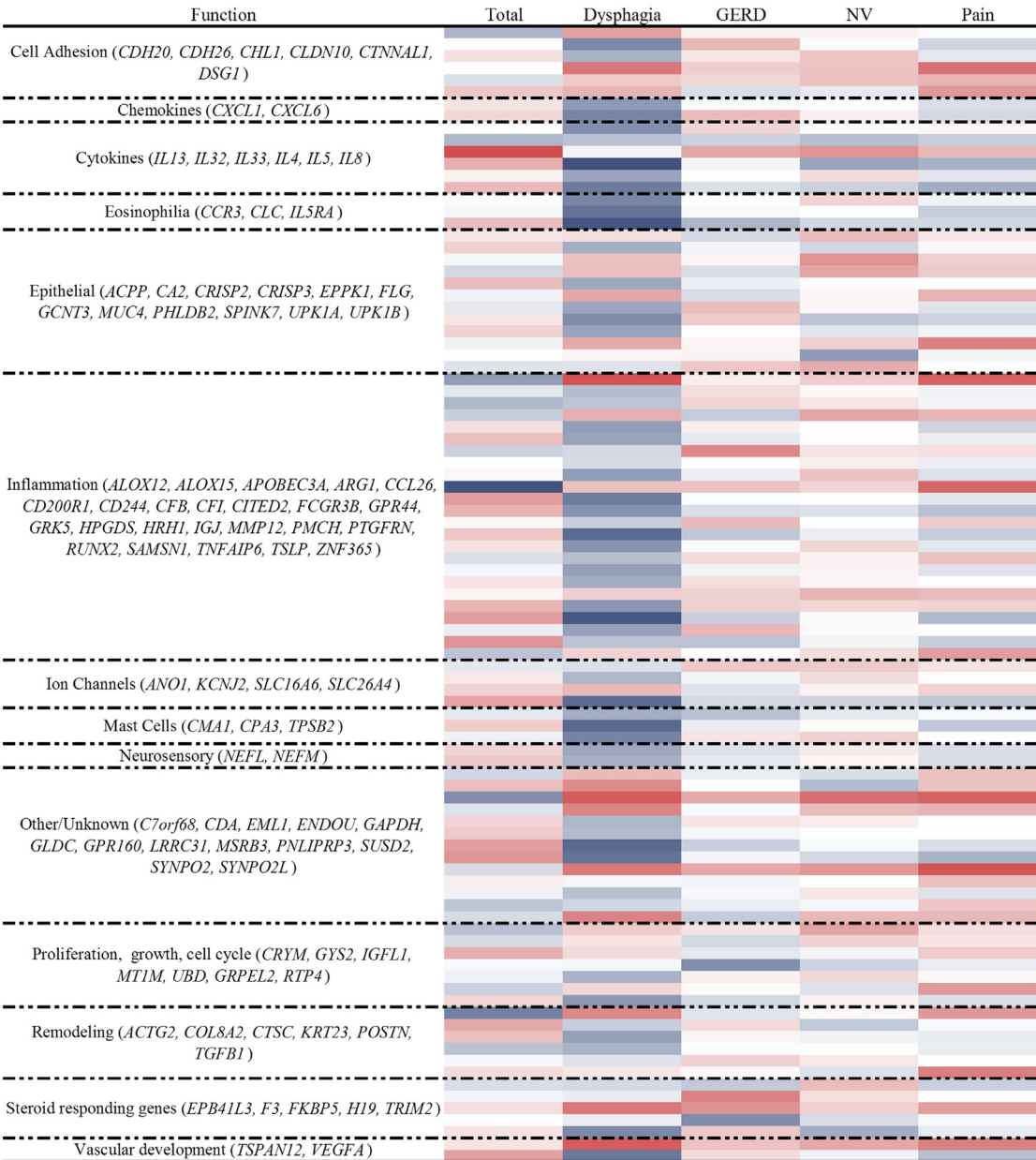


FIG 3. Spearman correlations between PEESS v2.0 scores and a diagnostic subset of the EoE transcriptome. Darker red shades indicate stronger negative correlations, whereas darker blue shades indicate stronger positive correlations. Correlations are by functional groupings of genes. NV, Nausea/vomiting domain.

proximal esophagus (degranulation and reproducibility) and the dysphagia domain. To determine whether the dysphagia domain correlates more strongly with eosinophil markers than the other domains, we compared the median correlation coefficient of eosinophil counts and EPX of the dysphagia domain with those of the other domains. The dysphagia domain had higher median correlation than the total and domain scores for both distal ($P \leq .022$) and proximal ($P \leq .0022$) measures (see Fig E1 in this article's Online Repository at www.jacionline.org).

Mast cell markers and CPA3 gene expression associate with the dysphagia domain. Tryptase- and chymase-positive mast cells were identified in proximal and distal esophageal biopsy specimens (see Table E3). In the proximal esophagus, both tryptase- and chymase-positive intraepithelial

mast cells were significantly associated with the PEESS v2.0 dysphagia domain ($\rho = 0.34$, $P = .036$ and $\rho = 0.32$, $P = .041$, respectively; Fig 1). The distal measures did not reach statistical significance. Notably, we found a strong correlation between dysphagia and carboxypeptidase A3 (CPA3) gene expression ($\rho = 0.36$, $P = .02$). The dysphagia domain also exhibited a correlative tendency with tryptase gene expression ($\rho = 0.28$) that did not reach statistical significance ($P = .076$), perhaps because of the small cohort size.

The diagnostic subset of the EoE transcriptome most strongly associates with the dysphagia domain. Overall, there was weak correlation between the domains and genes (absolute median value $\rho = 0.08$; interquartile range [IQR], 0.04-0.14; range, 0-0.42; Fig 3 and see Table E5 in this article's

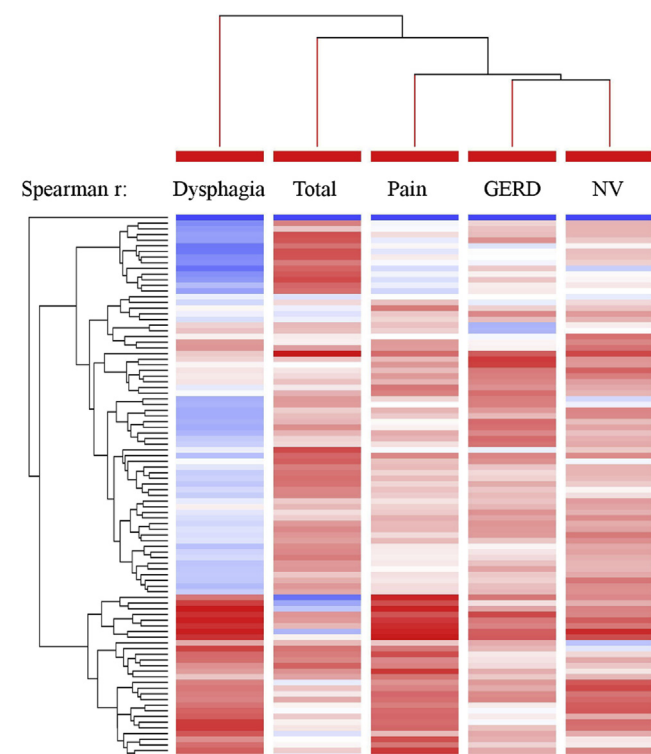


FIG 4. Hierarchic relationships between domains based on gene expression profile correlations. Using Spearman r for the correlation between the EDP gene expression and PEES v2.0 domain scores, we created a clustering tree representing the hierarchic order of the domain's representativeness and plotted it with the Spearman r -based heat diagram for the correlation at gene level. Darker red shades indicate stronger negative correlations, whereas darker blue shades indicate stronger positive correlations. The shorter the distance (tree-branch length), the more similar the expression correlation for each domain. NV, Nausea/vomiting domain.

Online Repository at www.jacionline.org). The dysphagia domain exhibited a significantly ($P < .0001$) higher magnitude of correlation with the EoE diagnostic panel (EDP) than the other domains and the total score (absolute median value $\rho = 0.18$ [IQR, 0.10-0.25] for the dysphagia domain; $\rho = 0.07$ [IQR, 0.04-0.12] for the total score; $\rho = 0.06$ [IQR, 0.02-0.10] for the GERD domain; $\rho = 0.06$ [IQR, 0.02-0.10] for the nausea/vomiting domain; and $\rho = 0.08$ [IQR, 0.04-0.12] for the pain domain). Focusing on the dysphagia domain and gene categories, we observed that genes related to eosinophilia (IQR, 0.28-0.41), chemokines (IQR, 0.23-0.28), mast cells (IQR, 0.10-0.36), neurosensory features (IQR, 0.18-0.20), cytokines (IQR, 0.11-0.33), and inflammation (IQR, 0.08-0.24) had positive Spearman correlation IQRs that did not overlap zero, suggesting a positive relationship with the dysphagia domain. To determine how the domains relate to each other with respect to gene expression patterns, we created a hierarchic tree using Spearman correlation values (Fig 4), as indicated by tree-branch hierarchy. The PEES v2.0 dysphagia domain differed from the other domains but was most similar to the total score. These data suggest that the dysphagia domain is more effective at capturing biological processes underlying the EDP than the other PEES v2.0 domains or the total score.

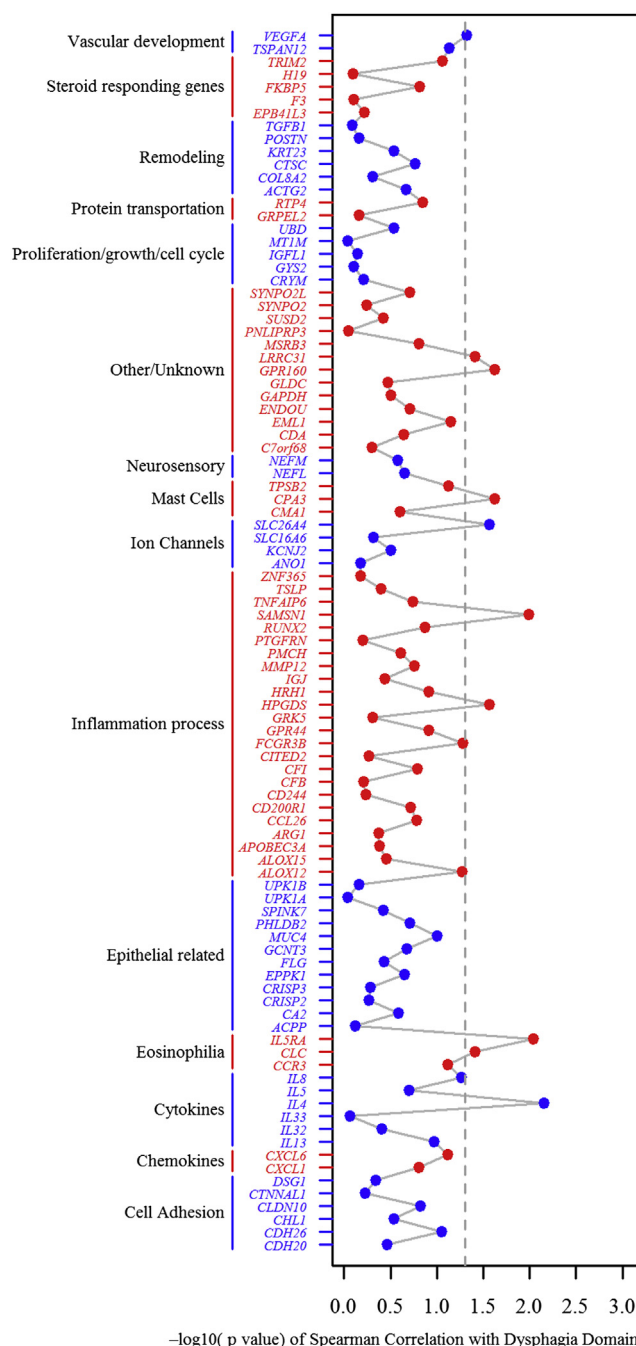


FIG 5. Associations between the PEES v2.0 dysphagia domain and a diagnostic subset of the EoE transcriptome. The x-axis represents the negative $\log_{10} P$ value of the Spearman correlation between the dysphagia domain and a diagnostic subset of genes from the EoE transcriptome (EDP). The y-axis is organized by genes within functional groupings; color shading is for ease of interpretation only. The dashed line indicates a P value of .05.

Genes that correlated with the dysphagia domain (Fig 5) include the vascular development gene vascular endothelial growth factor A (*VEGFA*; $\rho = 0.32$, $P = .048$); the other/unknown category genes G protein-coupled receptor 160 (*GPR160*; $\rho = 0.36$, $P = .024$) and leucine-rich repeat containing 31 (*LRRC31*; $\rho = 0.33$, $P = .039$); the mast cell gene *CPA3* ($\rho = 0.36$, $P = .024$); the ion channel gene solute carrier

family 26 (anion exchanger), member 4 (*SLC26A4*; $\rho = 0.35$, $P = .027$); the inflammation genes SAM domain, SH3 domain and nuclear localization signals 1 (*SAMSNI*; $\rho = 0.40$, $P = .01$) and hematopoietic prostaglandin D synthase (*HPGDS*; $\rho = 0.35$, $P = .027$); the eosinophil genes IL-5 receptor α (*IL5RA*; $\rho = 0.42$, $P = .0092$) and Charcot-Leyden crystal galectin (*CLC*; $\rho = 0.33$, $P = .039$); and the cytokine gene *IL4* ($\rho = 0.42$, $P = .0070$).

DISCUSSION

EoE has been well characterized biologically, but the severity and prevalence of signs and symptoms of this disease are less well documented. The development of therapeutics that improve the biological and clinical underpinnings of EoE in children are dependent on the availability of validated metrics for measuring clinical symptoms in pediatric patients with EoE.^{1,13,14} This study demonstrated that parent proxy-reported PEESS v2.0 domains are consistent with both clinical symptomology obtained by parent interview and EoE biological features, thus providing construct validity for these domains. Specifically, using a well-characterized prospective cohort of treated and untreated pediatric patients with EoE, we were able to examine associations between parent proxy-reported PEESS v2.0 domains and parent-reported clinical symptoms, EoE-specific gene expression, and histology, thereby testing the validity of the domains. We found that parent proxy-reported PEESS v2.0 domains correlated with parent-reported patient symptomology. Additionally, the EDP most strongly correlated with the PEESS v2.0 dysphagia domain. Furthermore, the PEESS v2.0 dysphagia domain significantly correlated with esophageal histology (both EPX staining and mast cell levels) in the proximal esophagus. Notably, EPX staining, but not eosinophil levels, significantly correlated in the proximal esophagus, suggesting that it is the activity of the eosinophils rather than their absolute levels that correspond most as a marker of dysphagia. Collectively, these results support the value of the parent proxy-reported PEESS v2.0 and its domains, especially dysphagia, and suggest a key role of eosinophil activity and mast cells in eliciting symptoms.

Most (93.5%) of our patient population was undergoing treatment, but they still exhibited signs of disease based on both clinical symptoms and histology. The most common clinical symptoms included early satiety, abdominal pain, and nausea. On the basis of these clinical symptoms, our patient population seems representative of EoE pediatric populations because abdominal pain is one of the most commonly reported symptoms.¹⁵⁻²⁰ The lower frequency of dysphagia, food impaction, and emesis compared with other studies might be due to the high frequency of treated subjects.^{21,22} We also found that most of our patients had detectable eosinophils in their esophageal biopsy specimens; only 6.5% were in remission (no eosinophils), and 71% had active disease (≥ 15 eosinophils/hpf). Although both dietary and swallowed steroid therapies have been noted to have high success rates,^{23,24} patients whose EoE has periodic flares and relapses might require multiple courses of treatment.^{25,26} Of note, given the study design, we were unable to explore the effect of different treatments on patient symptomology. Longitudinal studies will be required to capture the full symptomology of disease and response to treatment.

The parent proxy-reported PEESS v2.0 demonstrated substantial disease burden across various domains. The PEESS v2.0²⁷ addresses the concerns most pressing to patients with EoE and their families. Patients had previously identified 4 domains (dysphagia, GERD, nausea/vomiting, and pain). Importantly, our patients exhibited moderate impairment across all domains, which is consistent with the reported symptoms of EoE.^{16,17,19,28,29} Reported clinical symptoms aligned well with related domains designed to capture the different aspects of disease, thus validating the domains. This is important because the effect of EoE clearly extends beyond dysphagia³⁰ but might not be captured by generic PROs.²⁷ These results provide tools that can be used to evaluate different aspects of EoE and determine the efficacy of different treatments on the quality of life of pediatric patients. This work will complement the recently developed symptom-based activity index for adults by Schoepfer et al,³¹ which aligns well with endoscopic changes seen less frequently in children (rings, furrows, and strictures), as well as the recently developed Pediatric Quality of Life Inventory EoE Module.^{13,14}

Beyond clinical symptoms, we also found that one specific domain of the PEESS v2.0, the dysphagia domain, correlated with a newly developed molecular diagnostic test for EoE, the EDP,¹¹ to a stronger degree than any of the other domains or the total score. Previous studies of EoE have identified sets of genes (approximately 500) whose esophageal expression can discriminate EoE from non-EoE.^{6,10,32,33} This work led to the development of a smaller set of genes that provides strong discriminatory value (the EDP) for subjects who have 15 eosinophils/hpf or more in the esophagus compared with those with 2 eosinophils/hpf or less in the esophagus.¹¹ Thus the PEESS v2.0 dysphagia domain captures information associated with alterations in esophageal gene expression. Specifically, genes exhibiting association with dysphagia include *IL4*, *IL5RA*, *CLC*, *SAMSNI*, *HPGDS*, *CPA3*, *GPR160*, *LRRC31*, *SLC26A4*, and *VEGFA*. Although little is understood about the biology of *LRRC31*, *SLC26A4*, and *GPR160*, the other genes have strong ties to eosinophils (*CLC*, *IL4*, *IL5RA*, and *HPGDS*) and mast cells (*VEGFA*, *HPGDS*, *CPA3*, *SAMSNI*, *IL4*, and *IL5RA*).^{7,34-38} These genes have deep and diverse implications because *CLC* is a marker that is now being used in a new minimally invasive test, the EoE string test³⁹; *CPA3* is a specific marker of mast cells^{9,40,41}; *IL4* and *IL5* are targets of relevant therapeutic humanized antibody therapies being developed (eg, anti-IL-5/anti-IL-5 receptor, and anti-IL-4 receptor)⁴²⁻⁴⁴; *HPGDS* can be blocked by a variety of drugs (eg, cyclooxygenase inhibitors)⁴²; and *VEGFR* highlights consideration of anti-*VEGF* and related angiogenesis-based therapeutics, particularly for treatment of the cardinal EoE symptom dysphagia. Furthermore, one gene (*CPA3*) has been associated with chronic pelvic pain,⁴⁵ thereby supporting a role in patient symptomology. Notably, only the PEESS v2.0 dysphagia domain was associated with these tissue-based molecular biomarkers. It is interesting to speculate that dysphagia might be more reflective of pathophysiologic mucosal responses, whereas pain and nausea/vomiting might reflect processes primarily outside of the mucosa. The failure to associate EoE gene transcripts with the other PEESS v2.0 domains (pain, nausea/vomiting, and GERD) could also explain why current treatments that alter the transcriptome do not resolve all symptoms.⁴⁶

Importantly, we found that histologic markers of EoE related to both eosinophil activity and mast cells exhibited the strongest associations with the dysphagia domain. Although an increased esophageal eosinophil level is a hallmark of EoE, recent work also supports a role for mast cells.^{7,47-50} The association between dysphagia and mast cells is consistent with recent findings from adult studies.⁵¹ In addition, both eosinophil activity (EPX staining) and mast cell levels from the proximal esophagus showed stronger associations than those from the distal esophagus with the PEESS v2.0 dysphagia domain. The reason for these relationships is not known but perhaps could be related to acid exposure in the esophagus, which also contributes to distal esophageal eosinophilia. These data support the value of collecting proximal esophageal biopsy specimens because these might be more reflective of patient symptomology.

Lastly, unlike EPX staining, the absolute measures of eosinophils in the proximal esophagus were not significantly correlated with patient symptomology, suggesting that the presence of eosinophils alone is not the primary contributor for patient symptomology. The lack of association between dysphagia and eosinophil numbers is notable because the eosinophil level in the esophageal epithelium is considered the gold standard for the diagnosis of EoE. It is important to highlight that there was a trend between eosinophil levels and symptom scores, indicating that eosinophil levels alone might have a clinical correlation, but it is likely less contributory compared with the levels of their activation and levels of mast cells. It should also be noted that within the dysphagia domain, subjects with low eosinophil counts had a wide range of dysphagia scores, suggesting that an esophageal count of less than 15 eosinophils/hpf might still be clinically significant. These results are consistent with previous reports demonstrating that subjects with low eosinophil counts (eg, 5 eosinophils/hpf) were at risk for multiple endoscopies and chronic symptoms.^{52,53} Thus other factors, such as eosinophil and mast cell activity, as well as other histologic alterations, might also contribute to disease. These data suggest that additional evaluation of esophageal histologic changes might be valuable in understanding clinical symptoms.

In summary, PROs are increasingly recognized as important for the management of chronic conditions, but validated questionnaires for pediatric EoE are just now emerging. Previous work had validated 20 questions comprising the PEESS v2.0, and pediatric patients with EoE and their parents indicated that these questions reflected 4 major domains (dysphagia, GERD, nausea/vomiting, and pain). Herein, we have 1) established a validated report measure for pediatric EoE, the PEESS v2.0. Although this metric could be considered limited by parent report by proxy, we view this as a potential strength because it allows capture of symptoms in this key age range, particularly focused on an age window at which approved drugs are needed. Then, 2) despite its potential limitations, we show that parent proxy report is effective in capturing symptoms using this instrument; 3) demonstrate the value of the 4 domains of dysphagia, GERD, pain and nausea/vomiting; 4) determine that the dysphagia domain most closely aligns with parent-reported symptoms and tissue-based molecular biomarkers; 5) establish that symptoms correlate with the eosinophil activity marker of EPX staining; and 6) highlight a relatively strong association between mast cells (and their markers) and dysphagia. Collectively, these results demonstrate that the parent proxy-reported PEESS v2.0 is an objective measure of patient symptomology and can be used to

help evaluate treatment response and better understand how biological changes associate with patients' and parents' perceptions of wellness.

Key messages

- Parent proxy-reported PEESS v2.0 domains correlate with symptomology.
- The dysphagia domain correlates with EoE-specific gene expression and esophageal histology, most notably identifying a link between markers of mast cell and eosinophil activity with symptomology.
- Eosinophil activity, rather than absolute levels, associate with dysphagia.

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METHODS

EoE cohort identification

Patients with EoE and their parents were systematically recruited for this study from August 2010 through May 2011 at CCHMC. These pediatric patients were restricted to those with a confirmed diagnosis of EoE, which was defined as the presence of upper gastrointestinal tract symptoms and an endoscopy with 15 or more eosinophils/hpf in the proximal or distal esophageal tissue biopsy specimens; 87% had proton pump inhibitor therapy before the diagnostic endoscopy. Patients were systematically recruited with all degrees of treatment (*de novo* untreated patients and prior patients who were untreated or treated); 51 patients were screened, and 3 did not have a biopsy specimen. Given the typical EoE demographic and our relatively small sample cohort, patients in this analysis were restricted to males (2 female patients were excluded) to minimize heterogeneity. On initial review, all participants self-reported race as white. However, a single patient reported his race as mixed, which was noted during our data cleaning process, and was retained to maximize sample size.

Clinical symptom questionnaire

In addition to the use of validated metrics to capture symptoms and health-related quality of life that are described below, we also captured general clinical information relevant to EoE during research interviews at the time of endoscopy. Dichotomous yes/no answers regarding gastrointestinal symptoms (food impaction, choking/gagging, clinical dysphagia, difficulty swallowing, heartburn, reflux, poor appetite, food aversions, weight loss, abdominal pain, early satiety, poor weight gain, chest pain, nausea, emesis, diarrhea, and bloody stools) were gathered from parents about their children. Information on diet therapy, medication therapy, atopic history (allergies, asthma, eczema, and urticaria) and general clinical parameters (cough, irritability, headache, and migraines) at the time of the study was also collected.

Endoscopic sample collection

Patients undergoing diagnostic endoscopy for ongoing clinical care consented to provide additional esophageal biopsy specimens for research in addition to our standard clinical practice of obtaining 3 proximal and 3 distal esophageal endoscopic biopsy specimens. Research biopsy specimens were obtained after specimens obtained for clinical purposes. The tissues obtained for research were then placed in RNeasy lysis buffer (76104; Qiagen, Valencia, Calif) and later processed for RNA extraction.

Histopathologic staining and analysis

Biopsy specimens obtained for clinical purposes were fixed in 10% formalin and routinely processed. Sections were cut at 5 μ m and stained with hematoxylin and eosin. All clinical biopsy specimens were reviewed in a blinded fashion by a pathologist at CCHMC (M.H.C.) who has extensive experience evaluating EoE esophageal biopsy specimens. Eosinophil counts were expressed as eosinophils/hpf ($\times 400$ magnification, 0.3 mm²) and recorded as peak eosinophil counts in the proximal and distal biopsy specimens.

Additional sections were stained with antibody to tryptase (predilute, EDTA with 8 minutes of antigen retrieval; Cell Marque, Rocklin, Calif) or chymase (1:50, EDTA with 36 minutes of antigen retrieval; AbD Serotec, Raleigh, NC). The most densely inflamed areas were identified, and peak counts of tryptase-positive and chymase-positive cells in the most inflamed hpf were recorded.

EPX-based immunohistochemistry

EPX-based immunohistochemistry of esophageal tissues sections from formalin-fixed, paraffin-embedded biopsy specimens was performed, as previously described.^{E1} Briefly, infiltrating intact eosinophils and evidence of eosinophil degranulation (ie, the presence of free cytoplasmic granules, extracellular matrix deposition of EPX, or both) were assessed by means of

immunohistochemistry with an eosinophil-specific mouse mAb (clone MM25-82.2.1). Immunocytochemical staining was performed with Dako detection/visualization reagents purchased from Dako Cytomation (Carpinteria, Calif). Eosinophil-containing sections from patients identified by using traditional pathologic assessments were used as positive control slides. Negative control slides (ie, antibody isotype controls and negative tissue control sections) were also included as part of the processing of each group of slides examined. Permanent Red Substrate-Chromogen (catalog no. K0695, Dako Cytomation) was used to visualize EPX-specific staining. Slides were counterstained with methyl green before being mounted on coverslips and photomicroscopy with a Zeiss Axiophot microscope equipped with an AxioCam MRC5 digital camera (Zeiss, Oberkochen, Germany).

RNA extraction and reverse transcription

Total RNA was extracted from single distal esophageal biopsy specimens collected in RNeasy lysis buffer (76104, Qiagen) by using the miRNeasy RNA Extraction Kit (217004, Qiagen), according to the manufacturer's manual protocol. After RNA quantity and quality analysis with a NanoDrop spectrometer, an aliquot of 500 ng of RNA was acquired for reverse transcription by using the iScript cDNA Synthesis Kit (170-8891; Bio-Rad Laboratories, Hercules, Calif), according to the manufacturer's manual protocol. Briefly, 500 ng of RNA was mixed with reaction mix and the reverse transcriptase enzyme in a total volume of 20 μ L; incubated at 25°C for 5 minutes, 42°C for 30 minutes, and 85°C for 5 minutes; and then kept at 4°C and later at -20°C for storage.

PCR amplification of representative EoE genes

A selection of representative EoE genes was amplified from cDNA stock generated as mentioned above. The TaqMan reagents for amplification of major EoE signature genes^{E2,E3} were obtained from Applied Biosystems (Foster City, Calif), and TaqMan real-time PCR amplification was performed on an ABI 7900HT System (Applied Biosystems). The amplification protocol consisted of a hot start of 95°C for 10 minutes, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. After the quantitative PCR was complete, raw cycle threshold values for each sample/each gene were exported into GeneSpring GX 11.5 (Agilent Technologies, Santa Clara, Calif) for statistical analysis and heat map generation. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an expression control for all of the genes analyzed.

PEESS v2.0 and domains

The PEES v2.0 has 20 questions: 11 ask the participants to rank the frequency of symptoms, and 9 ask the participants to rank the severity of symptoms. Both frequency and severity are scored from 0 to 4, with 4 being the worst (Table 1). In the validation phase of the PEES v2.0, the parents of patients with EoE were asked what the questions were about and how the items were related. From these parent participant interviews, 4 domains for these 20 questions were established: dysphagia, GERD, nausea/vomiting, and pain. We created total and domain scores by summing the individual elements and dividing by the maximum total score. Because only a subset of children were eligible to fill out the PEES v2.0 (because of age restrictions), all validation of the PEES v2.0 scores were conducted by using the parent proxy-report scores.

Data management

All clinical data were collected on a paper form and entered into one of 2 electronic data sets. Basic research data were collected through the use of a Structured Query Language Server database developed and maintained in the Cincinnati Center for Eosinophilic Disorders. These data, as was all clinical laboratory data, were captured with DocFlowSheets in an EPIC electronic medical record and were later extracted for analysis. Once the forms were all entered, the electronic record was compared with the paper forms to ensure

that there were no discrepancies from data entry. Data extracted from the separate databases were then joined by using statistical software.

Statistical analysis

Before analyses, all variables were examined for distributional issues and data plausibility. Descriptive statistics were reported as means and SDs or medians, IQRs, and frequencies, depending on the variable distributions.

Consistency between parent proxy reports and child self-report scores. Self-reported outcomes are considered the standard for PROs, yet children of less than a certain age might not have the developmental capacity to answer such questionnaires.^{E4} Thus we examined the subset of participants with both parent proxy-reported and child self-reported PEESS v2.0 scores and used Spearman correlations (because of the nonnormal distributions).

Interrelationship of PEESS v2.0 domains. To gain an understanding of how different instruments relate to each other in a pediatric EoE cohort, we first performed pairwise analysis between the PEESS v2.0 domains by using Wilcoxon signed-rank paired test to determine whether the EoE pediatric cohort had specific domains that they perceived as worse than others. This resulted in 10 statistical tests. To account for the possibility of false-positive results in these tests, we used a standard Bonferroni correction ($0.05/10 = 0.005$). Additionally, we used Spearman correlations to understand how these different measures covaried with each other. In total, 10 correlations were performed; the *P* value for significance of the correlation in this analysis is an α value of .005.

Validation of PEESS v2.0 domains with respect to clinical features. To determine whether PEESS v2.0 domains are associated with clinical symptoms in an anticipated manner, we tested whether subjects who reported a symptom had worse domain scores than those who did not by using Wilcoxon rank sum tests. Because many tests were performed, a multiple testing adjustment was included. After accounting for the 17 gastrointestinal symptoms with frequencies of greater than 5%, a *P* value of .003 was required (Bonferroni correction $0.05/17 = 0.003$) to reach statistical significance. Given the high level of correlation between the PEESS v2.0 domains, no additional multiple testing corrections were applied. We also examined the relationship of the domains to allergic symptoms as a negative control because we would not expect the PEESS v2.0 domains to differ in subjects with and without allergic symptoms. Because this was a negative control, the significance threshold was .05.

Validation of PEESS v2.0 domains with EoE biological features. To determine whether the PEESS v2.0 domains aligned with the biological features associated with EoE, we examined several aspects of EoE biology, including a diagnostic subset of the EoE transcriptome (EDP) and histologic features (eosinophil and mast cell measures). Because previous work identified a gene expression panel highly specific to active EoE (EDP),^{E3} we tested the hypothesis that the EDP (either as a whole or individual genes)

would be associated with the PEESS v2.0 domains. For this analysis, we performed Spearman correlation analysis between the gene levels on the EDP and the PEESS v2.0 domains. In addition, we tested whether the median of the correlation (using the absolute value to account for differences in the direction of the effect across genes) was enriched for any of the domains by using Wilcoxon rank sum tests.

To test the hypothesis that the PEESS v2.0 domains are related to eosinophil measures, we considered both the quantitative measure of peak eosinophil count and a disease activity classification schema defined as none (0 eosinophils/hpf), low (1-5 eosinophils/hpf), intermediate (6-14 eosinophils/hpf), and active (≥ 15 eosinophils/hpf) by using the peak eosinophil count (maximum of the proximal and distal counts). Additionally, we examined eosinophil activation as measured based on EPX staining from both proximal and distal samples. Analyses with continuous eosinophil counts (proximal, distal, and peak counts) were evaluated by using Spearman correlations. For analyses using categorical eosinophil counts, the Wilcoxon rank sum test was used. For analysis of EPX staining, Spearman rank correlations were used because of the nonnormal distribution of the EPX staining values. To account for multiple testing within eosinophil measures, we used a Bonferroni correction adjusting for the overall correlation between these 15 measures ($p = 0.76$), yielding an α value of .03.

To test the hypothesis that PEESS v2.0 domains are associated with mast cells, we tested for correlation between PEESS v2.0 domains and levels of tryptase, chymase, and *CPA3* mRNA. To quantify mast cell markers, we first examined gene expression from the distal esophagus for tryptase, chymase, and *CPA3*. We also examined the tryptase and chymase quantitative staining from both distal and proximal esophagus biopsy specimens, with the amounts varying because of the number of samples ($n = 30-40$) collected from each esophageal location; mast cell levels were expressed as cell numbers per hpf. To account for the multiple testing within mast cell parameters, we used a Bonferroni correction adjusting for the overall correlation between these 7 measures ($p = 0.56$), yielding an α value of .02.

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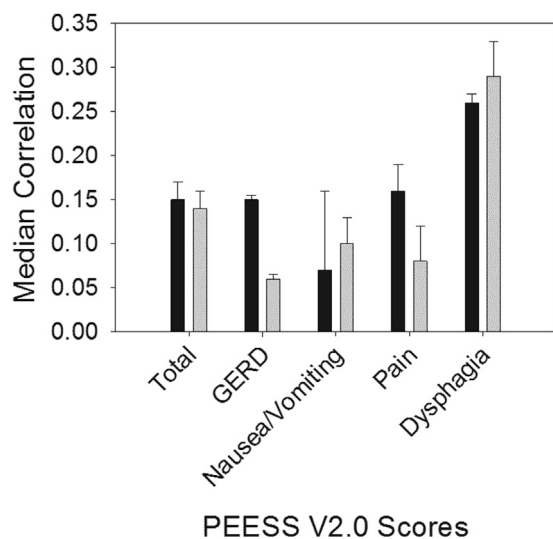


FIG E1. Median correlation (and upper 75% quartile) between eosinophil measures (eosinophil count and EPX staining) and PEESS v2.0 scores demonstrates specific association with the dysphagia domain. *Black bars* represent the distal esophagus, and *gray bars* represent the proximal esophagus. The median dysphagia domain is statistically higher than the total and domain scores for both the proximal and distal esophagus ($P \leq .0049$ for all except distal pain [$P = .022$]).

TABLE E1. Question composition of parent proxy–reported PEESS v2.0 domains

Domain	Question
Dysphagia	How often does your child have trouble swallowing?
	How bad is your child's trouble swallowing?
	How often does your child feel like food gets stuck in his/her throat or chest?
	How bad is it when your child gets food stuck in his/her throat or chest?
	How often does your child need to drink a lot to help swallow food?
	How bad is it when your child needs to drink a lot to help swallow food?
GERD	How often does your child eat less than others?
	How often does your child need more time to eat than others?
	How often does your child have heartburn (burning in the chest, mouth, or throat)?
	How bad is your child's heartburn (burning in the chest, mouth, or throat)?
	How often does your child have food come back up in his/her throat when eating?
	How bad is it when food comes back up in your child's throat?
Nausea/ vomiting	How often does your child vomit (throw up)?
	How bad is your child's vomiting (throwing up)?
	How often does your child feel nauseous (feel like throwing up but does not)?
	How bad is your child's nausea (feeling like throwing up, but does not)?
Pain	How often does your child have chest pain, ache, or hurt?
	How bad is your child's chest pain, ache, or hurt?
	How often does your child have stomach aches or belly aches?
	How bad are your child's stomach aches or belly aches?

TABLE E2. Frequency of allergic conditions

	No. with data available	Frequency
Drug allergy	45	31.1
Environmental allergy	43	67.4
Food allergy	46	84.8
Urticaria	46	65.2
Eczema	46	58.7
Asthma	45	51.1

TABLE E3. EPX staining and mast cell measures*

Measurement	Distal	Proximal
EPX staining values		
Total	12.5 (0-37.8; 0-50)	0 (0-32; 0-48)
Reproducibility	1.5 (0-4; 0-4)	0 (0-3; 0-4)
Patchiness	0 (0-6; 0-8)	0 (0-2; 0-8)
Degranulation	0 (0-3; 0-10)	0 (0-4; 0-8)
Intact	4.5 (0-12; 0-12)	0 (0-12; 0-12)
Randomness	2 (0-9; 0-16)	0 (0-8; 0-16)
Tryptase		
Epithelium	10.5 (6-24.8; 0-49)	9 (6-22; 1-65)
Chymase		
Epithelium	4.5 (2-10.8; 0-28)	5 (2-8.5; 0-43)
Gene expression†		
Tryptase	−0.07 (−1.00 to 1.78; −3.8 to 3.23)	—
Chymase	−0.04 (−7.8 to 1.03; −11.10 to 4.26)	—
<i>CPA3</i>	−0.05 (−1.5 to 2.07; −4.19 to 5.01)	—

*All values are reported as medians (IQRs; ranges).

†Gene expression levels were normalized to the housekeeping gene *GAPDH*.

TABLE E4. Relationship of PEESS v2.0 scores in patients with active EoE (≥ 15 eosinophils) and who are in remission (no eosinophils)

PEESS scores	Active (n = 33)	Remission (n = 3)	Non active (n = 13)	<i>P</i> value, active vs remission	<i>P</i> value, active vs non active
Total score	25.0 (10.0-37.5)	22.5 (8.8-23.4)	18.75 (8.8-41.8)	.37	.16
Dysphagia	25.0 (12.5-39.7)	25.0 (9.4-33.3)	12.5 (9.4-26.6)	.89	.14
GERD	18.8 (0-28.1)	6.3 (6.3-8.3)	18.75 (3.1-28.1)	.23	.62
Nausea/vomiting	12.5 (0-40.6)	31.3 (12.5-37.5)	12.5 (0-34.4)	.45	.77
Pain	31.25 (12.5-43.8)	8.3 (6.3-18.8)	18.75 (10.4-31.3)	.13	.21

Data are presented as medians (IQRs). *P* values are from Wilcoxon rank sum tests.

TABLE E5. Spearman correlations between PEESS v2.0 scores and a diagnostic subset of the EoE transcriptome

Function	Gene	Total	Dysphagia	GERD	NV	Pain
Cell adhesion	<i>CDH20</i>	0.17	-0.15	-0.02	-0.02	0.01
	<i>CDH26</i>	0.00	0.27	-0.12	0.01	0.10
	<i>CHL1</i>	-0.05	0.17	-0.04	-0.09	0.05
	<i>CLDN10</i>	0.00	-0.23	-0.08	-0.10	-0.25
	<i>CTNNAL1</i>	0.07	-0.09	-0.06	-0.10	-0.12
Chemokines	<i>DSG1</i>	-0.09	-0.12	0.07	0.05	-0.17
	<i>CXCL1</i>	-0.04	0.23	0.02	0.00	0.07
	<i>CXCL6</i>	-0.06	0.28	-0.11	-0.03	0.09
Cytokines	<i>IL13</i>	0.00	0.26	-0.07	0.01	-0.01
	<i>IL32</i>	0.16	0.14	0.10	0.14	0.11
	<i>IL33</i>	-0.32	0.03	-0.15	-0.18	-0.12
	<i>IL4</i>	-0.13	0.42	0.03	0.21	0.18
	<i>IL5</i>	-0.02	0.21	0.00	-0.06	0.05
Eosinophilia	<i>IL8</i>	-0.11	0.31	0.07	0.10	0.19
	<i>CCR3</i>	0.01	0.28	0.01	-0.07	0.03
	<i>CLC</i>	0.02	0.33	0.02	0.00	0.11
Epithelial	<i>IL5RA</i>	-0.11	0.41	0.16	0.09	0.09
	<i>ACPP</i>	-0.04	-0.05	0.08	-0.11	-0.04
	<i>CA2</i>	-0.07	0.18	0.02	0.09	0.01
	<i>CRISP2</i>	0.02	-0.10	-0.02	-0.18	-0.08
	<i>CRISP3</i>	0.09	-0.10	0.08	-0.15	-0.09
	<i>EPPK1</i>	-0.11	0.20	0.04	0.01	0.01
	<i>FLG</i>	0.03	-0.14	0.08	-0.02	-0.13
	<i>GCNT3</i>	0.05	0.20	-0.10	-0.02	0.06
	<i>MUC4</i>	-0.04	0.26	-0.08	0.13	0.10
	<i>PHLDB2</i>	-0.07	0.21	0.01	0.06	0.03
	<i>SPINK7</i>	0.03	-0.14	-0.02	-0.08	-0.21
	<i>UPK1A</i>	0.00	-0.02	-0.02	0.23	0.02
	<i>UPK1B</i>	0.06	0.06	-0.09	-0.13	0.01
	<i>ALOX12</i>	0.23	-0.31	-0.03	-0.08	-0.27
	<i>ALOX15</i>	0.05	0.15	-0.05	-0.01	0.03
Inflammation	<i>APOBEC3A</i>	0.16	0.13	-0.07	-0.04	0.02
	<i>ARG1</i>	0.11	-0.13	0.12	-0.15	-0.12
	<i>CCL26</i>	-0.05	0.22	-0.03	0.00	0.09
	<i>CD200R1</i>	-0.10	0.21	0.05	0.00	0.04
	<i>CD244</i>	0.10	0.09	-0.20	-0.05	-0.05
	<i>CFB</i>	0.00	0.08	0.00	-0.03	0.04
	<i>CFI</i>	-0.01	0.22	0.04	-0.10	0.07
	<i>CITED2</i>	0.42	-0.10	-0.10	-0.07	-0.27
	<i>FCGR3B</i>	-0.16	0.31	0.01	0.00	0.05
	<i>GPR44</i>	-0.13	0.25	0.08	0.07	0.14
	<i>GRK5</i>	-0.01	0.11	-0.11	0.00	-0.08
	<i>HPGDS</i>	-0.09	0.35	0.12	0.04	0.12
	<i>HRH1</i>	-0.05	0.25	0.01	-0.06	0.05
	<i>IGJ</i>	0.07	0.15	-0.06	-0.02	-0.10
	<i>MMP12</i>	0.02	0.22	0.03	-0.02	0.06
Ion channels	<i>PMCH</i>	-0.04	0.19	-0.05	-0.01	0.01
	<i>PTGFRN</i>	-0.02	-0.08	-0.07	-0.12	-0.11
	<i>RUNX2</i>	-0.12	0.24	-0.07	-0.06	-0.07
	<i>SAMSN1</i>	-0.16	0.40	0.11	0.01	0.16
	<i>TNFAIP6</i>	0.04	0.22	-0.12	-0.01	0.00
	<i>TSLP</i>	-0.18	0.14	0.14	0.03	0.11
	<i>ZNF365</i>	0.13	-0.07	-0.01	-0.05	-0.16
	<i>ANO1</i>	0.05	0.07	-0.09	-0.08	-0.04
	<i>KCNJ2</i>	-0.04	0.16	0.02	-0.06	0.01
	<i>SLC16A6</i>	-0.07	-0.11	0.06	-0.02	-0.07
	<i>SLC26A4</i>	-0.15	0.35	0.08	0.09	0.13
Mast cells	<i>CMA1</i>	0.05	0.19	0.14	0.05	0.01
	<i>CPA3</i>	-0.08	0.36	0.04	0.00	0.12
	<i>TPSB2</i>	0.03	0.28	-0.04	-0.07	0.00
Neurosensory	<i>NEFL</i>	-0.07	0.20	0.06	-0.03	0.07

(Continued)

TABLE E5. (Continued)

Function	Gene	Total	Dysphagia	GERD	NV	Pain
Other/unknown	<i>NEFM</i>	-0.09	0.18	0.05	-0.01	0.08
	<i>C7orf68</i>	0.09	-0.11	0.04	0.07	-0.10
	<i>CDA</i>	-0.10	-0.19	0.00	0.15	-0.10
	<i>EML1</i>	0.26	-0.29	-0.14	-0.25	-0.27
	<i>ENDOU</i>	0.06	-0.21	0.02	-0.11	-0.13
	<i>GAPDH</i>	-0.07	0.16	-0.04	-0.03	0.00
	<i>GLDC</i>	-0.08	0.16	0.02	0.00	0.01
	<i>GPR160</i>	-0.16	0.36	0.11	0.02	0.08
	<i>LRRC31</i>	-0.18	0.33	0.02	0.09	0.17
	<i>MSRB3</i>	0.08	-0.23	-0.14	-0.17	-0.31
Proliferation, growth, cell cycle	<i>PNLIPRP3</i>	-0.03	0.02	0.02	0.00	-0.10
	<i>SUSD2</i>	0.03	0.14	0.02	-0.04	0.06
	<i>SYNPO2</i>	0.13	0.09	0.04	0.02	-0.09
	<i>SYNPO2L</i>	0.08	-0.21	0.12	-0.12	-0.12
	<i>CRYM</i>	0.14	-0.08	-0.04	-0.15	-0.05
	<i>GYS2</i>	0.08	-0.04	0.09	-0.07	-0.04
	<i>IGFL1</i>	-0.13	-0.06	0.05	0.02	-0.08
	<i>MTIM</i>	0.01	0.02	0.26	0.10	0.04
	<i>UBD</i>	0.03	0.17	-0.03	-0.06	-0.01
	<i>GRPEL2</i>	0.10	-0.06	-0.01	0.06	-0.17
Remodeling	<i>RTP4</i>	-0.06	0.24	0.09	-0.02	0.07
	<i>ACTG2</i>	0.29	-0.20	0.06	-0.01	-0.17
	<i>COL8A2</i>	-0.14	0.11	-0.06	0.12	0.01
	<i>CTSC</i>	-0.10	0.22	-0.02	0.03	0.05
	<i>KRT23</i>	0.13	0.17	0.01	0.00	0.04
	<i>POSTN</i>	0.02	0.06	-0.07	-0.04	0.00
	<i>TGFB1</i>	-0.06	-0.04	-0.01	0.07	-0.22
	<i>EPB41L3</i>	0.07	0.08	0.12	-0.11	0.10
	<i>F3</i>	0.02	0.04	-0.21	-0.05	0.00
	<i>FKBP5</i>	-0.05	-0.23	-0.18	-0.07	-0.16
Steroid-responding genes	<i>H19</i>	0.00	0.04	0.27	0.08	0.02
	<i>TRIM2</i>	-0.04	0.27	-0.09	0.18	0.05
	<i>TSPAN12</i>	-0.04	-0.29	-0.10	-0.14	-0.22
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
Vascular development	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14
	<i>VEGFA</i>	-0.16	0.31	-0.06	0.03	0.14

Correlations by functional groupings of genes are shown. The text within the cell indicates the Spearman *r* value. Values in boldface indicate nominal statistical significance (*P* < .05).

NV, Nausea/vomiting.