

Transcriptome analysis of proton pump inhibitor-responsive esophageal eosinophilia reveals proton pump inhibitor-reversible allergic inflammation

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Background: Esophageal eosinophilia can be proton pump inhibitor (PPI) resistant or responsive, representing 2 entities known as eosinophilic esophagitis (EoE) and PPI-responsive esophageal eosinophilia (PPI-REE), respectively. Although they present with similar clinical features, EoE is accepted to be an antigen-driven, T_H2-associated allergic disorder, whereas the cause of PPI-REE remains a mystery.

Objective: In this study, our aim was to investigate the pathogenesis of PPI-REE by using a recently described EoE diagnostic panel (EDP) composed of a set of 94 esophageal transcripts and to determine whether PPI therapy reverses any esophageal transcriptional abnormalities.

Methods: We evaluated the EDP signature in biopsy samples obtained from adult and pediatric patients with PPI-REE from

4 institutions and compared the pre- and post-PPI therapy expression profiles of these subjects with those of patients with active EoE.

Results: The EDP differentiated patients with EoE from control subjects with 100% accuracy among the 4 clinical sites.

Bioinformatics analysis revealed largely overlapping transcriptomes between patients with PPI-REE and those with EoE, including the genes for eosinophil chemotaxis (eotaxin 3, *CCL26*), barrier molecules (desmoglein 1, *DSG1*), tissue remodeling (periostin, *POSTN*), and mast cells (carboxypeptidase A, *CPA3*). PPI monotherapy alone almost completely reversed the allergic inflammatory transcriptome of patients with PPI-REE. Furthermore, we identified a set of candidate genes to differentiate patients with EoE from those with PPI-REE before treatment.

Conclusion: These findings provide definitive evidence that PPI-REE is a disease entity with significant molecular overlap with EoE, suggesting that many patients with PPI-REE represent a continuum of the same pathogenic allergic mechanisms that underlie EoE and thus might constitute a subphenotype of patients with EoE. The ability of PPI therapy to nearly entirely reverse gene expression associated with PPI-REE, particularly that associated with classic features of allergic inflammation, provides new insight into potential disease etiology and management strategies for patients with significant esophageal eosinophilia. (*J Allergy Clin Immunol* 2014;■■■:■■■-■■■.)

Key words: Eosinophil, glucocorticosteroid, molecular signature, eotaxin, reflux

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Esophageal eosinophilia occurs in patients with a number of disorders, including gastroesophageal reflux disease (GERD), Crohn disease, celiac disease, and eosinophilic esophagitis (EoE), a clinicopathologic chronic upper gastrointestinal tract disorder defined by esophageal dysfunction and eosinophil infiltration of 15 or more eosinophils per high-power field (hpf). Translational research in the past 10 years has uncovered a food allergen-driven, T_H2 cell immune-mediated disease pathogenesis.^{1,2} Because GERD can also elicit esophageal eosinophilia, a consensus recommendation for the diagnosis of EoE^{3,4} requires a proton pump inhibitor (PPI) trial to exclude the possibility of acid-induced esophageal eosinophilia. Although EoE is defined by a failed PPI trial, another form of esophageal eosinophilia that is frequently observed features tissue eosinophil levels as high as those in patients with EoE (in contrast to patients with GERD), diffuse infiltration along the esophageal length, and clinical characteristics representative of EoE, but PPI monotherapy is effective in reversing both histologic and clinical abnormalities.⁵ A number of explanations have been proposed,

Abbreviations used

CCHMC: Cincinnati Children's Hospital Medical Center
 EDP: EoE diagnostic panel
 EoE: Eosinophilic esophagitis
 FFPE: Formalin-fixed paraffin-embedded
 GERD: Gastroesophageal reflux disease
 hpf: High-power field
 PCA: Principal component analysis
 PPI-REE: Proton pump inhibitor-responsive esophageal eosinophilia

including (1) blockade of GERD-associated inflammation through the inhibition of acid by PPI; (2) the anti-inflammatory effects of PPI, such as inhibition of eotaxin-3 and signal transducer and activator of transcription 6⁶; and (3) the interaction of acid and food allergens. Because of the lack of a clear understanding of the natural history and pathogenesis, this enigmatic condition is currently termed PPI-responsive esophageal eosinophilia (PPI-REE). The frequency of PPI-REE among all patients with esophageal eosinophilia (≥ 15 eosinophils/hpf) is substantial, ranging from 10% to 50%.⁷⁻¹⁰

Defining the underlying mechanisms of this inflammation in patients with PPI-REE will help to guide appropriate therapeutic strategies. However, to date, there have been no molecular, cellular, endoscopic, or clinical markers or pH test results that clearly distinguish these entities from one another. EoE is treated with topical corticosteroids and/or dietary elimination, whereas PPI-REE is treated, at least transiently, with acid suppression.¹⁰ Currently, it remains to be determined whether these 2 entities involve the same or different molecular pathogenesises. An understanding of their molecular similarities and differences would provide diagnostic and therapeutic clarity for practitioners and patients because both patients with PPI-REE and those with EoE are clinically similar in terms of clinical symptoms, endoscopic findings, male predominance, and high rate of atopy.^{8,9}

Substantial progress has been made with regard to the molecular cause of EoE by using whole-genome transcript expression profiling of esophageal tissue.¹ Recently, a molecular EoE diagnostic panel (EDP) was identified that is composed of 94 EoE genes and distinguishes patients with EoE from control subjects without esophagitis or with GERD.¹¹ Although the EDP has been reported to have excellent accuracy ($>96\%$ sensitivity and specificity), it has only been applied to patients from one institution and has not been previously applied to patients with PPI-REE. In light of these points, this retrospective study with archived tissues aimed to answer the following crucial questions: (1) Does PPI-REE possess a typical EoE molecular signature that is characteristic of allergic inflammation, as defined by the EDP, or a unique gene expression profile? (2) Does remission induced by PPI monotherapy lead to transcript signature reversal? (3) Does the gene dysregulation in patients with PPI-REE correlate with eosinophilia at the molecular level, similar to that in EoE?¹¹ (4) Are there gene expression profiles that can differentiate patients with PPI-REE from those with EoE before a therapeutic PPI trial that are *a priori*? Herein we report that PPI-REE before PPI therapy has a molecular signature that is similar to that of EoE. Furthermore, this pretherapy PPI-REE gene expression profile is reversed in parallel with PPI-induced remission. Finally and of particular clinical

relevance, we identify a preliminary cluster of genes that is predictive for PPI-REE before intervention.

METHODS**Subject selection and study design**

Previously collected and archived paraffin-embedded samples from patients with PPI-REE, EoE, or GERD and healthy control subjects were obtained from 5 US institutions: University of California, San Diego/Rady Children's Hospital, San Diego; University of North Carolina—Chapel Hill; Walter Reed National Military Medical Center; Cincinnati Children's Hospital Medical Center (CCHMC); and Children's Hospital Colorado (see Table E1 in this article's Online Repository at www.jacionline.org). The inclusion criteria for patients with PPI-REE, as well as for patients with EoE, patients with GERD, and healthy control subjects (NL group), were standardized before the experiments and data analysis. Experts from each institution agreed on the definition and inclusion criteria and were directly involved in screening of patients with PPI-REE in their sites, identifying those with samples available before and after PPI therapy, as well as determining samples from patients with EoE, patients with GERD, and healthy control subjects. Specifically, control subjects were defined by normal endoscopic results, normal pathology with 0 eosinophils/hpf, and no known history of EoE. Patients with GERD were defined by clinical symptoms consistent with reflux (eg, heartburn and regurgitation), less than 15 peak eosinophils/hpf on biopsy, and no previous EoE history. A portion of the patients with GERD from CCHMC were confirmed to have reflux by means of concurrent pH/impedance testing.^{11,12} Patients with EoE were defined as having symptomatic esophageal dysfunction and 15 or more peak esophageal eosinophils/hpf, even after an 8- to 12-week PPI trial, as per consensus guidelines.^{3,4} Patients with PPI-REE were defined as having symptoms consistent with esophageal dysfunction and initial esophageal eosinophilia of 15 or more eosinophils/hpf on index endoscopy that resolved (<15 eosinophils/hpf) after an 8-week course of PPI therapy (20–40 mg of available agents twice daily for adults or 10–30 mg of available agents twice daily for pediatric subjects). All patients with PPI-REE exhibited symptomatic (improvement of symptoms by means of self-report at the time of the repeat endoscopy) and endoscopic improvements after monotherapy with PPI. Both adult (≥ 18 years) and pediatric (<18 years) subjects were included in the study. All secondary causes of gastrointestinal tract eosinophilia, including concomitant eosinophilic gastroenteritis, were excluded before confirming the diagnosis of EoE. Atopy was defined by clinical diagnosis and a documented history of food allergies determined by means of either clinical reactions or skin testing. This study was approved by the institutional review boards of the participating institutions.

EoE transcriptome PCR amplification by means of EDP

The EoE transcriptome was determined, as reported previously, by using the EDP¹¹ from RNA extracted from 60- to 80- μ m tissue sections from formalin-fixed, paraffin-embedded (FFPE) blocks. Briefly, 500 to 1000 ng of RNA was reverse transcribed to cDNA and subjected to EDP amplification by using the ABI 7900HT qPCR system (Applied Biosystems, Foster City, Calif). The data were then imported into GeneSpring (GX 12.5) software for implementation of the dual algorithm, namely cluster analysis and EoE score calculation. To compensate for the long archiving time for some of the FFPE samples, a 50% call rate filter was applied to the 77 definitive diagnostic genes¹¹ to focus on informative genes, resulting in a cluster of 59 genes (F59) that formed the basis of all of the following analyses.

Statistical and bioinformatics analysis

The transcriptomes of the entire cohort of 114 samples (from 96 independent subjects) were compared by using clustering (the signature analysis), EoE score¹¹ calculation, ANOVA, and principal component analysis (PCA). Most of our algorithm tools were previously reported.¹¹ Briefly, an EoE score (F59) was derived from entities that passed a greater than 50% call rate filter, resulting in 59 of the 77 diagnostic genes of the EDP. With the individual

TABLE I. Subjects' characteristics by disease type*

	Overall (n = 96)	Control group (n = 16)	EoE group (n = 33)	Pre-PPI-REE group (n = 28)	Post-PPI-REE group (n = 21)	GERD group (n = 13)
Male sex, no. (%)	64 (67)	8 (50)	24 (73)	21 (75)	17 (81)	6 (46)
White, no. (%)	81 (84)	12 (75)	29 (88)	23 (82)	18 (86)	12 (92)
Age (y), mean $\pm$ SD	25.4 $\pm$ 17.9	31.5 $\pm$ 22.2	26.6 $\pm$ 15.3	31.5 $\pm$ 17.4	21.3 $\pm$ 19.1	9.5 $\pm$ 5.5
Symptoms, no. (%)						
Dysphagia	36 (38)	5 (31)	29 (88)	21 (75)	9 (43)	4 (31)
Food impaction	19 (20)	0 (0)	14 (42)	4 (14)	2 (10)	1 (8)
Heartburn	27 (28)	5 (31)	9 (27)	9 (32)	6 (29)	2 (15)
Chest pain	3 (3)	0 (0)	1 (3)	0 (0)	1 (5)	1 (8)
Abdominal pain	26 (27)	7 (44)	6 (18)	3 (11)	5 (24)	8 (62)
Nausea	10 (10)	5 (31)	1 (3)	1 (4)	3 (14)	2 (15)
Vomiting	18 (19)	3 (19)	3 (9)	2 (7)	7 (33)	5 (38)
Failure to thrive	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15)
Atopic diseases, no. (%)						
Allergic rhinitis/sinusitis	34 (35)	4 (25)	14 (42)	13 (46)	8 (40)	1 (8)
Eczema	16 (17)	1 (6)	8 (24)	4 (14)	2 (11)	3 (23)
Asthma	19 (20)	3 (19)	7 (21)	5 (28)	4 (20)	2 (15)
Food allergy	20 (21)	1 (6)	9 (27)	9 (32)	4 (20)	0 (0)
Endoscopic appearance, no. (%)						
Normal	27 (28)	14 (88)	1 (3)	5 (18)	6 (29)	3 (50)
Rings	36 (38)	0 (0)	22 (67)	14 (50)	5 (24)	0 (0)
Stricture	16 (17)	0 (0)	13 (39)	3 (11)	1 (5)	0 (0)
Narrowing	8 (8)	0 (0)	8 (24)	0 (0)	0 (0)	0 (0)
Linear furrows	47 (49)	0 (0)	29 (88)	17 (61)	10 (48)	0 (0)
Crêpe-paper	2 (2)	0 (0)	2 (6)	0 (0)	0 (0)	0 (0)
White plaques/exudates	20 (21)	0 (0)	2 (6)	0 (0)	0 (0)	0 (0)
Decreased vascularity/pallor	28 (29)	1 (6)	17 (52)	7 (25)	8 (38)	2 (15)
Erosive esophagitis	8 (8)	0 (0)	1 (3)	4 (14)	1 (5)	3 (23)
Hiatal hernia	8 (8)	1 (6)	2 (6)	5 (18)	3 (14)	0 (0)
Schatzki ring	8 (8)	0 (0)	3 (9)	5 (18)	1 (5)	0 (0)
Eosinophil counts (maximum eosinophils/hpf $\pm$ SD)						
Pre-PPI trial†	45.8 $\pm$ 60.4	0 $\pm$ 0	114.1 $\pm$ 77.6	55.7 $\pm$ 40.2	63.8 $\pm$ 57.2	2.0 $\pm$ 2.5
Post-PPI trial†	40.5 $\pm$ 43.1	—	71.6 $\pm$ 36.0	4.3 $\pm$ 3.9	2.9 $\pm$ 3.4	—

*This is a per-subject analysis. In the overall column, subjects' demographic characteristics are only represented once, even if paired pre/post-PPI samples were analyzed. However, the pre- and post-PPI-REE groups have data from University of North Carolina–Chapel Hill and University of California, San Diego, subjects who are in each category.

†The pre-PPI trial includes patients with EoE if data were available for values off PPIs. The post-PPI trial includes all patients with EoE during their confirmatory esophagogastroduodenoscopy.

EoE scores (F59) from the 5 subject groups (healthy control subjects and patients with GERD, EoE, PPI-REE before PPI treatment [PPI-REE-pre], or PPI-REE after PPI treatment [PPI-REE-post]), 1-way ANOVA with the Bonferroni multiple comparison posttest was used to identify all significant pairs. PCA was also used to generate a 3-dimensional plot of the top 3 variance contributors between the PPI-REE-pre and EoE cohorts by using the NL and PPI-REE-post cohorts as references. The paired *t* test was used to compare the treatment effect of paired samples before and after PPI. A 2-tailed *P* value of less than .05 was deemed statistically significant. For correlation analysis between eosinophil counts and gene dysregulation (EoE score [F59]), Spearman correlation was used to derive *r* and *P* values.

RESULTS

Subjects' characteristics

A total of 114 FFPE samples from 96 subjects were analyzed. Age, demographics, clinical symptoms, endoscopic findings, and esophageal eosinophil levels of all groups are detailed in [Table I](#), and subject numbers stratified by group and center are provided in [Table E1](#). The age range for all subjects was 10 months to 72 years, with 40 pediatric subjects (mean age, 8.7 $\pm$ 5.1 years) and 56 adults (mean age, 37.4 $\pm$ 13.5 years). The EoE and pretherapy PPI-REE (PPI-REE-pre) groups both had a male predominance (73% and 75%, respectively), and the majority of

clinical features between the EoE and PPI-REE-pre groups were similar. Some clinical and endoscopic findings differed significantly, with food impactions, esophageal strictures, luminal narrowing, linear furrows, and decreased vascularity being more common and a normal endoscopic appearance being less common in the EoE group than the PPI-REE-pre group (*P* < .05 for all subjects, [Table I](#)). Compared with the NL and GERD cohorts, the EoE and PPI-REE groups have higher rates of atopic disease (*P* = .01 for both allergic rhinitis and food allergy, *P* = .50 for eczema, and *P* = .78 for asthma; [Table I](#)). At baseline, before PPI treatment, the maximum eosinophil count was higher in the EoE group than in the PPI-REE-pre group (114 vs 56 eosinophils/hpf, *P* = .002). After the PPI trial, the eosinophil count remained increased in the EoE group (72 eosinophils/hpf) but markedly decreased in the PPI-REE group (4 eosinophils/hpf).

Untreated PPI-REE has an EDP signature that overlaps with that of EoE and reveals T_H2-associated allergic inflammation as the fundamental molecular pathogenesis

To address whether PPI-REE has an EoE-like genetic signature, we evaluated the transcriptome of PPI-REE-pre

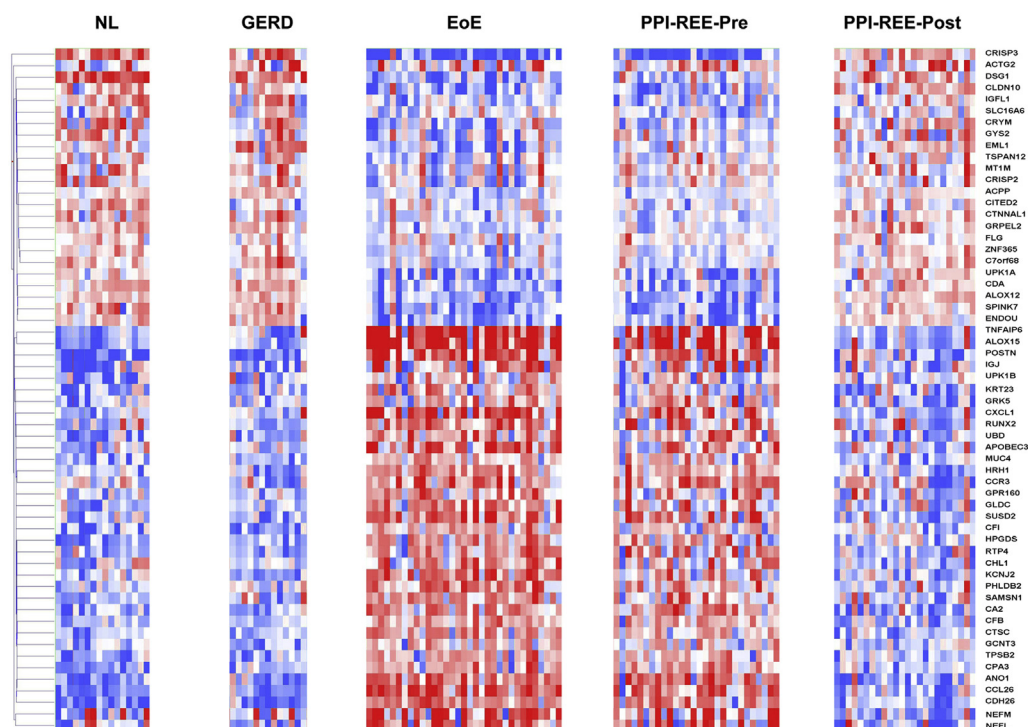


FIG 1. Comparison of esophageal transcriptomes of study cohorts. A total of 114 samples from 5 centers were analyzed by using the EDP. Heat maps were generated on the basis of the 59 EoE genes that passed a greater than 50% call rate of the EDP's 77 significant genes (F59). *Red* indicates higher expression (upregulation), and *blue* represents lower expression (downregulation). NL, Healthy control subjects; PPI-REE-post, posttherapy PPI-responsive esophageal eosinophilia; PPI-REE-pre, pretherapy PPI-responsive esophageal eosinophilia.

samples within the scope of representative EoE genes embedded on the EDP. As illustrated by the heat map, the gene upregulation and downregulation pattern comprising the EoE hallmark gene signature was present in PPI-REE-pre samples (Fig 1), including increased *CCL26* (eotaxin 3) levels for eosinophil chemotaxis, *CPA3* (carboxypeptidase A) levels for mastocytosis, *MUC4* (mucin 4) levels for IL-13 response, and *POSTN* (periostin) levels for tissue remodeling. There was a remarkable conservation of the EoE esophageal transcriptome in the PPI-REE-pre group. In contrast, this pattern was not present in the NL, GERD, and treated PPI-REE (PPI-REE-post) groups.

For statistical comparison, individual signature quantification was performed by using EoE scoring, which has been shown to directly reflect EoE disease activity (Fig 2, A).¹¹ Of note, when comparing the NL and EoE cohorts, a sensitivity of 100% and specificity of 100% were achieved from these independent, non-CCHMC FFPE samples, demonstrating high merit for the EDP with external FFPE samples. Receiver operating characteristic analysis resulted in an area under the receiver operating characteristic curve of 1.0 (Fig 2, B). After comparison by using ANOVA with the Bonferroni multiple comparison posttest (Fig 2, A, lower panel), the NL and GERD cohorts were significantly different from the EoE cohort. Despite their comparable signature pattern (Fig 1), there was a small but significant difference in EoE scores between the PPI-REE-pre and EoE groups, with EoE being molecularly more severe (more pronounced signature [Fig 1] and lower EoE score [Fig 2, A]), and there was a small group of heterogeneous subjects in the PPI-REE-pre cohort (Fig 2, A). This observed magnitude difference between the EoE and PPI-REE-pre groups

also held true after the EoE score was normalized to eosinophils/hpf (Fig 2, C and D), suggesting that although untreated PPI-REE appears to be within the same molecular spectrum as EoE, untreated PPI-REE is not as altered as classical EoE in terms of its transcriptome. When the expression profiles of the NL, EoE, PPI-REE-pre, and PPI-REE-post groups were plotted in 3 dimensions by using PCA (used as a dimensionality reduction method), with each axis representing one of the top 3 variance contributors, the PPI-REE-pre group was geometrically close to the EoE group (Fig 2, E). In contrast, the NL and PPI-REE-post groups clustered together (Fig 2, E). Correlation of EoE scores and eosinophils/hpf values for all FFPE samples indicated a robust molecular-histologic linkage ($r = -0.81$, $P < .0001$; Fig 2, F). There was a mild but significant correlation between the EoE score and eosinophils/hpf in EoE samples but not in PPI-REE samples (Fig 2, F). Notably, patients with EoE and those with PPI-REE have a retained signature regardless of the age group, as indicated by comparable EoE scores between pediatric and adult subjects (see Fig E1 in this article's Online Repository at www.jacionline.org). Treatment with PPIs did not modify the molecular signature of EoE in the NL and EoE cohorts, as shown in Fig E2 in this article's Online Repository at www.jacionline.org and a previous publication from our group.¹

In addition to the heat map (signature) and EoE score (disease severity) comparisons, we performed additional analyses to address whether the transcriptomes of patients with EoE and those with PPI-REE are similar at the single-gene level. First, when compared with the NL reference, the EoE and PPI-REE-pre groups yielded a similar number of significant genes with

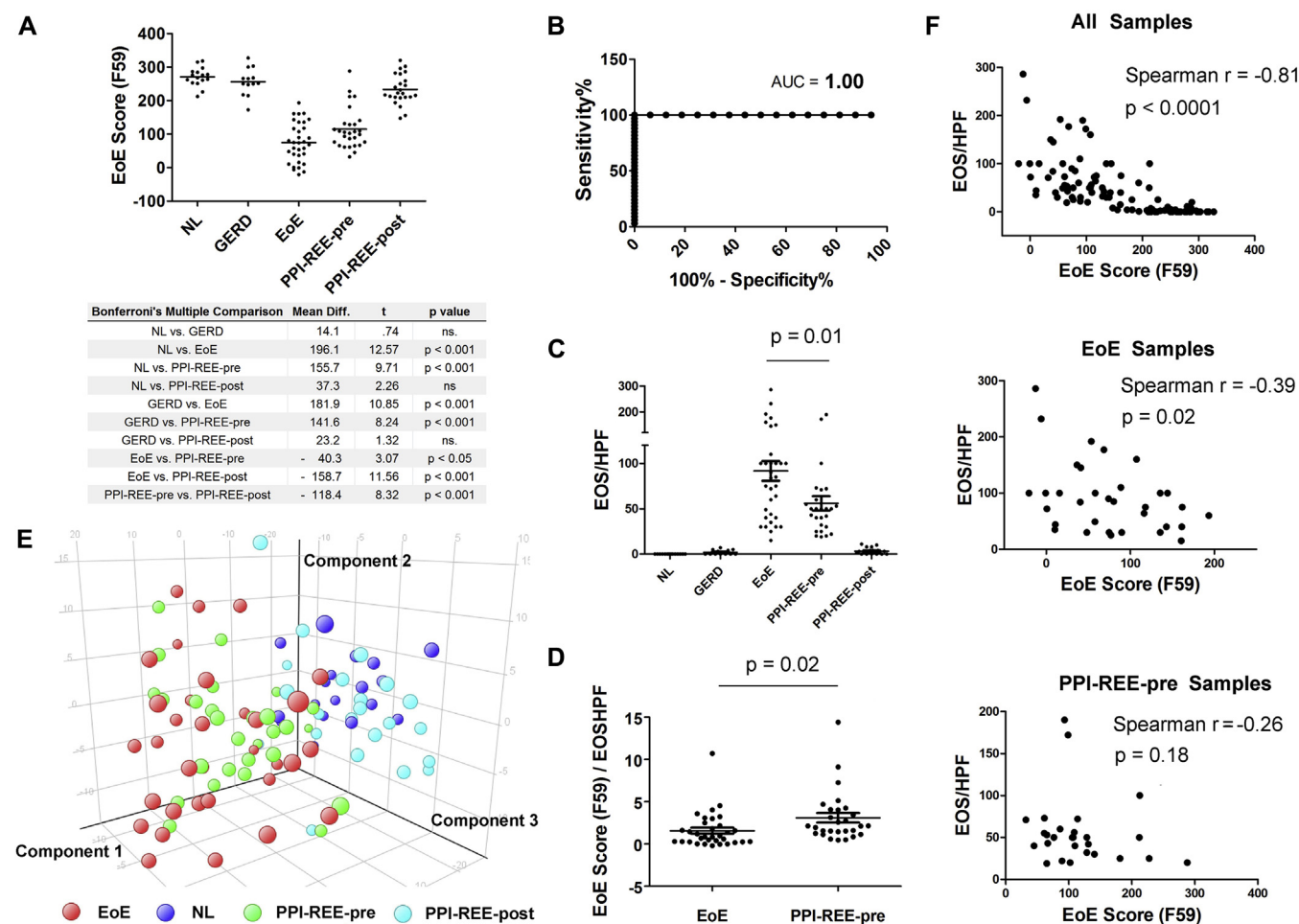


FIG 2. Quantitative analysis of esophageal transcriptome and relationship to esophageal eosinophilia. **A**, EoE scores (F59) were calculated for all 5 cohorts with the EDP algorithm reported previously.¹¹ Circles represent individual subjects with an average line superimposed. One-way ANOVA with the Bonferroni multiple comparison posttest was used, with results summarized in the table on the lower panel. *ns*, Not significant. **B**, Receiver operating characteristic (ROC) curve resulting from the comparison between mixed NL and EoE cohorts from multiple centers. An optimal diagnostic cutoff EoE score (F59) of 203 was derived herein. *AUC*, Area under the curve. **C**, Eosinophil/hpf (EOS/HPF) counts were demonstrated for all FFPE samples studied as peak esophageal biopsy count. **D**, In addition to the significant differences found in EoE scores (F59) and eosinophils/hpf for the EoE versus PPI-REE-pre groups, a statistical difference was also identified when the EoE score (F59) was normalized to peak eosinophils/hpf. **E**, A 3-dimensional plot containing sample points from the NL, EoE, PPI-REE-pre, and PPI-REE-post cohorts was derived from PCA on the entitles demonstrated in the heat map to visualize the geometric distance between any given cohort pair. **F**, *Top panel*, An overall correlation between EoE score and eosinophils/hpf for all FFPE samples included in this study. *Two lower panels*, Breakdown graphs showing the correlation of EoE score (F59) of EoE samples and PPI-REE-pre samples with eosinophils/hpf separately. Scatterplot data were presented as mean $\pm$ SEM.

comparable bidirectional distribution patterns, as shown by the volcano plots of fold change and *P* value (Fig 3, A). A large percentage of the significant genes (46 genes vs the NL group) overlapped between the EoE and PPI-REE-pre groups (Fig 3, B, and see Table E2 in this article's Online Repository at www.jacionline.org). There was a high correlation of the expression levels of these 46 common genes between patients with EoE and those with PPI-REE ($R^2 = 0.85$, $P < .0001$; Fig 3, C). Moreover, an ontologic analysis on biological functions on these 46 common genes revealed an overall image of T_H2 allergic inflammation with high significance (Fig 3, D), indicating a shared allergic inflammatory response between patients with EoE and those with PPI-REE.

Molecular signature and mastocytosis of untreated PPI-REE are reversible by using PPI monotherapy

The histologic eosinophilia associated with PPI-REE is responsive to PPI therapy, but it is not known whether the PPI-REE gene expression pattern normalizes after PPI therapy. Among the 15 paired PPI-REE-pre and PPI-REE-post samples, 13 of the PPI-REE-pre samples had molecular similarity to EoE samples, as measured by EoE scores (EoE score [F59] < 203 ; Fig 2, A). On the basis of these 13 pairs, the significant EoE score (F59) reversal ($P < .001$, paired *t* test, PPI-REE-pre vs PPI-REE-post; Fig 4, A) after PPI is consistent with the notion that PPIs are sufficient to induce histologic and symptomatic remission in patients with PPI-REE. Mastocytosis is a hallmark for allergic

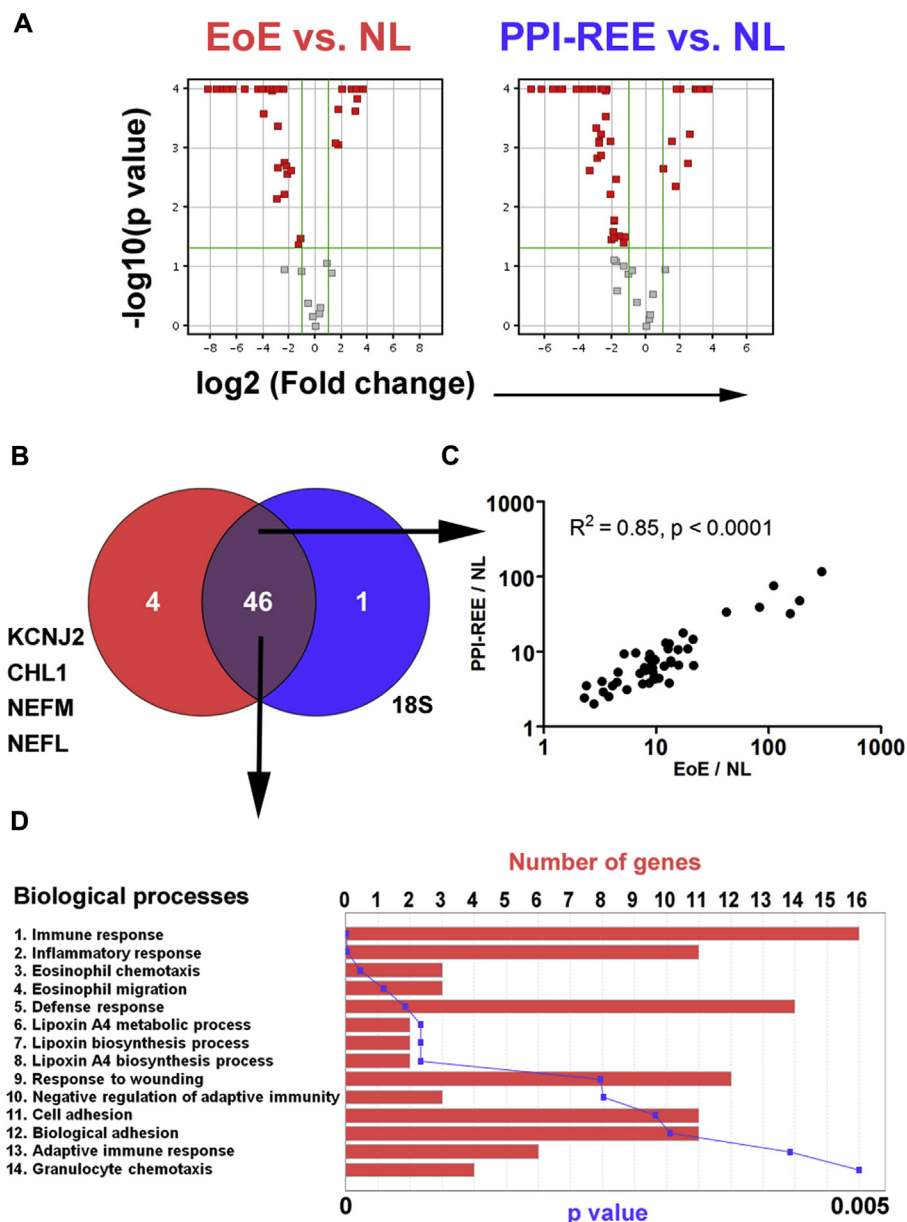


FIG 3. PPI-REE exhibits a continuum of EoE's allergic inflammation signatures. **A** and **B**, Within the scope of EDP F59, bioinformatics comparison ($P < .05$, fold change > 2.0 , 2-tailed unpaired t test) yielded 50 and 47 significant genes between the EoE and NL groups and the PPI-REE-pre and NL groups, respectively. A pair of volcano plots ($\log_2$ fold change as x -axis and $-\log_{10} P$ value as y -axis) demonstrates the similarity of bidirectional dysregulation in the EoE and PPI-REE groups when compared with the NL reference group. **C**, A dysregulation (based on fold change over NLs) linear correlation analysis between the EoE (EoE/NL) and PPI-REE (PPI-REE/NL) groups was shown on the basis of the 46 overlapping EoE genes dysregulated in both the EoE and PPI-REE groups. **D**, On the basis of these 46 common genes, a gene ontology analysis focusing on biological function was performed with the number of genes and corresponding P values shown, revealing a pathologic basis for an adaptive T_H2 allergic inflammation ($P < .05$, Bonferroni correction).

inflammation; accordingly, we found that histologic remission of PPI-REE after PPI therapy was accompanied by normalization of mastocytosis from paired samples (tryptase immunohistochemical staining: $P < .01$, paired t test; $n = 10$; Fig 4, B and C), which is consistent with the normalization of mast cell genes (*CPA3* and *TPSB2* [tryptase]) by using PPI therapy (Fig 4, D and E). Although the overall signature normalized after PPI therapy,

when individual expression of the EDP genes was compared between all PPI-REE-post and NL samples, 8 EoE genes, including *CCL26*, *POSTN*, and *DSG1* (desmoglein 1), had significantly different expressions ($P < .05$, fold change > 2.0 ; Fig 4, F). These data indicate a pronounced and specific effect of PPIs on the gene expression pattern of patients with PPI-REE, corroborating the histologic remission.

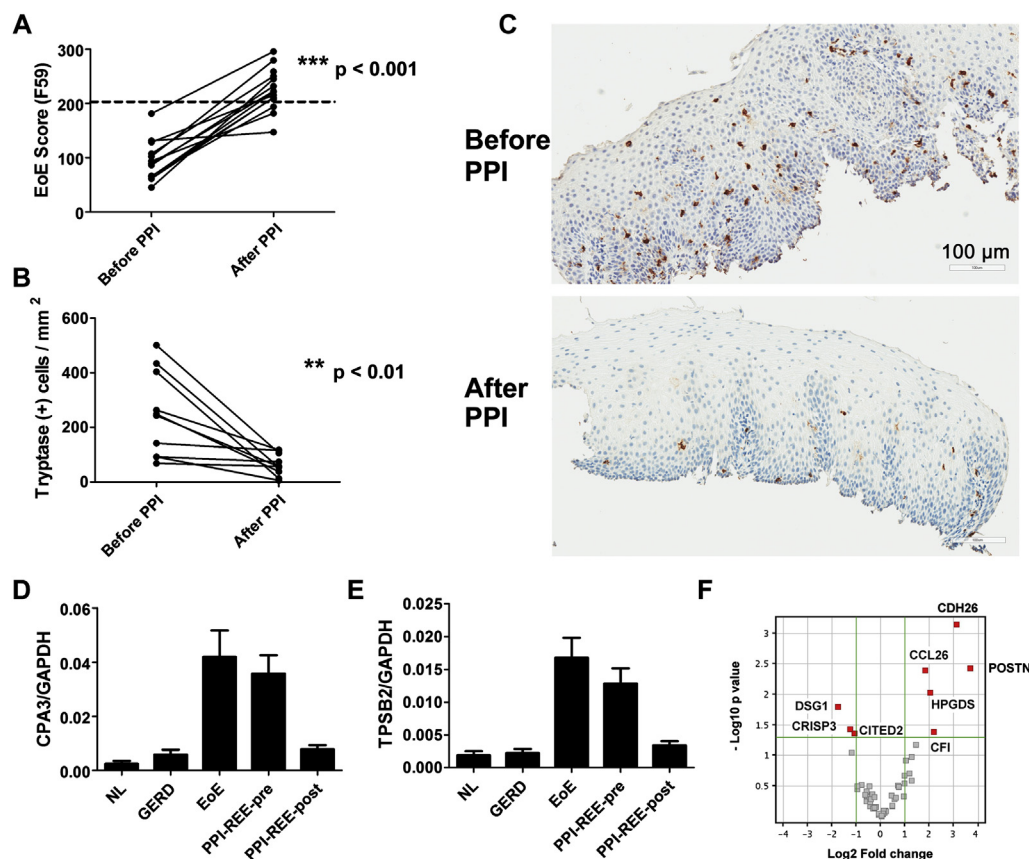


FIG 4. Effect of PPI therapy on the esophageal transcriptome of patients with PPI-REE. **A**, EoE scores before and after PPI monotherapy for 13 paired samples (7 from the University of California, San Diego, and 6 from University of North Carolina–Chapel Hill). A diagnostic cutoff EoE score of 203 was derived from receiver operating characteristic analysis between the normal and EoE cohorts (dashed line). **B**, EoE score amelioration is accompanied by mastocytosis remission shown by means of tryptase staining before and after PPI therapy ($n = 10$, $P < .01$, paired t test). **C**, Representative pair of micrographs showing tryptase staining before and after PPI in patients with PPI-REE. **D**, mRNA expression of the mast cell gene *CPA3* among all cohorts. **E**, mRNA expression of the mast cell gene *TPSB2* (tryptase) among all cohorts. **F**, Although the overall signature normalizes in the PPI-REE-post group (vs the NL group), there are 8 genes that remain statistically dysregulated. The volcano plot depicts bidirectional fold change ($\log_2$) on the x -axis and negative $\log_{10}$ P value (NL vs PPI-REE-post groups) on the y -axis. Significance (red square genes) was defined by a P value of less than .05 and fold change of greater than 2.0. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase. Scatterplot data were presented as mean $\pm$ SEM.

Distinguishing untreated PPI-REE and EoE

Considering that EoE and PPI-REE-pre are clinically and histologically indistinguishable but different in their therapeutic response to PPI treatment, a molecular approach to identify PPI-REE before pharmaceutical intervention would be a transformative advance for patient care. To further molecularly differentiate these diseases, we collectively compared the EDP molecular signature of the EoE and PPI-REE-pre cohorts at the individual gene level. We identified a cluster of 10 genes whose expression was significantly different in the PPI-REE-pre group compared with the EoE group ($P < .05$, fold change > 2.0 ; Fig 5, A). On the basis of these 10 differentially expressed genes, a proposed protein-protein interaction network highlighted the potential involvement of chemotaxis systems, ion channel activities, and the ubiquitin/proteasome system in differentiating molecular pathogenesis between the EoE and PPI-REE-pre groups (Fig 5, B). With a more stringent false-detection filter (Westfall-Young) applied, *KCNJ2* (potassium inwardly-rectifying channel, subfamily J, member 2/Kir2.1)

became the only gene with significant differential expression ($P < .01$, false-corrected $P = .04$; Fig 5, C). It is notable that *KCNJ2* was among the 4 genes that are specifically differentially expressed in the EoE group compared with the NL group but not in the PPI-REE group versus the NL group (Fig 3, B) and that it is expressed in the esophagus.¹³ For diagnostic merit, using *KCNJ2* resulted in 72% sensitivity and 72% specificity to predict PPI-REE-pre versus EoE. *KCNJ2* encodes the potassium channel Kir2.1, which is abundant in gastrointestinal mucosa and colocalizes with the $H^+-K^+-ATPase/proton$ pump^{14,15}; therefore, we propose a potential interaction between this potassium channel and proton pump in the upper gastrointestinal epithelium (Fig 5, D).

DISCUSSION

An emerging body of data suggests that PPI therapy is effective at reducing and/or eliminating esophageal eosinophilic inflammation and has led to the emergence of a new disease

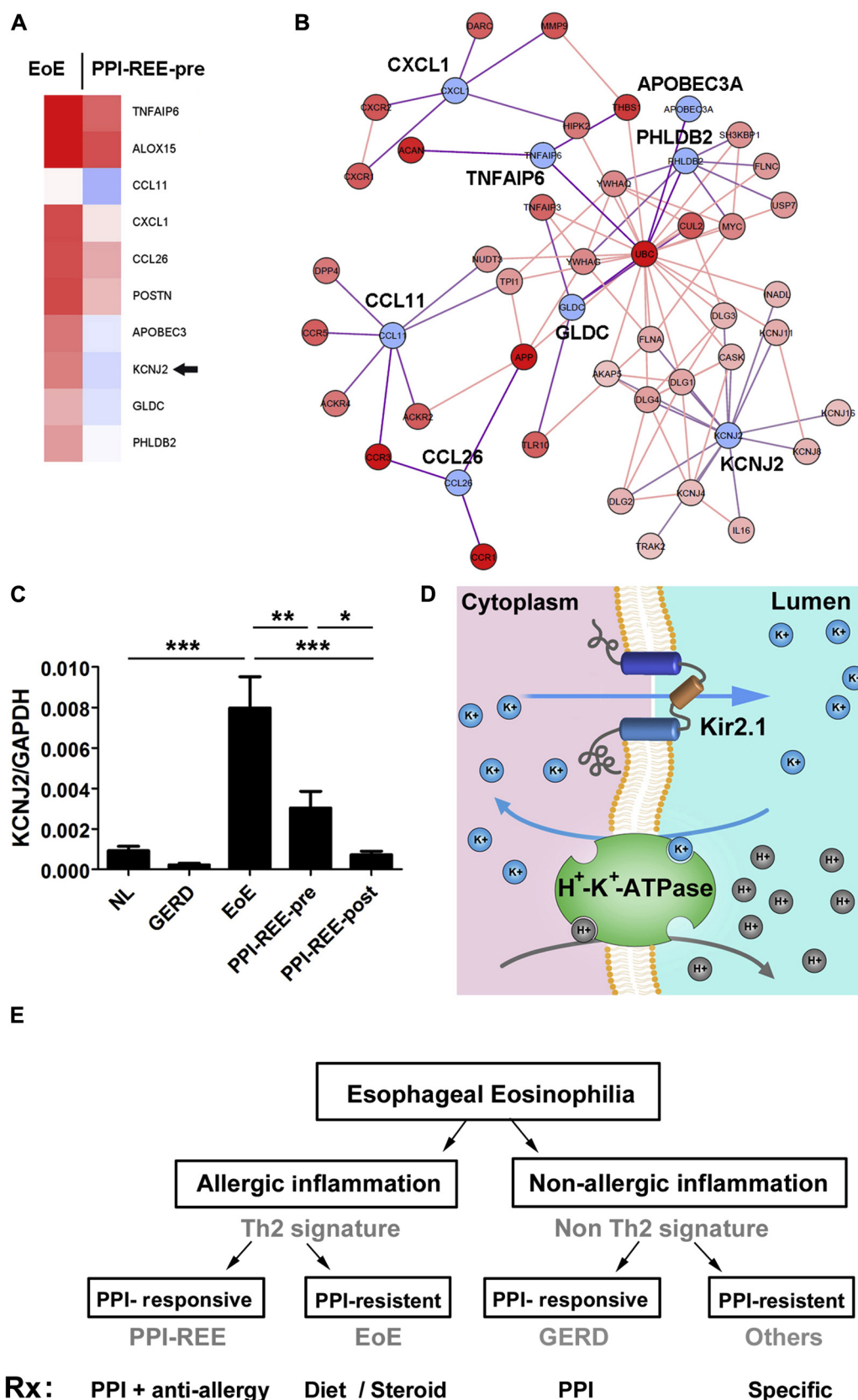


FIG 5. Gene cluster differentially expressed between the EoE and PPI-REE-pre groups. **A**, List of 10 EoE genes (within the EDP) whose expression is significantly different between the EoE and PPI-REE-pre groups (2-tailed Student *t* test, *P* < .05, fold change > 2.0, *n* = 33 for the EoE group and *n* = 28 for the PPI-REE-pre group). Arrowhead, *KCNJ2*. **B**, A predictive protein-protein interaction derived from the pathway analysis

entity, PPI-REE, yet this disease is not well understood. The relationship between PPI-REE and EoE remains enigmatic. Clinically, PPI-REE resembles EoE more than GERD, as evidenced by the findings that (1) tissue eosinophilia of patients with PPI-REE are usually higher than levels typically seen in patients with GERD; (2) a high percentage of patients with PPI-REE have atopy, food allergy, or both, similar to patients with EoE; and (3) both patients with EoE and those with PPI-REE show a male predominance and susceptibility in white subjects.⁹ It can be difficult to distinguish PPI-REE from EoE on the basis of clinical, endoscopic, and histologic features,^{8,9} thus leading to the question of whether similar mechanisms might govern their clinicopathologic features. In this retrospective study, we show that untreated PPI-REE has a molecular signature that overlaps with that of EoE, providing compelling evidence that the 2 diseases are part of the same spectrum of a T_H2-associated disease process in nearly all patients. It is notable that we identified a subset (approximately 14%) of patients with PPI-REE who did not have an EoE-like signature before PPI therapy. Whether this finding represents heterogeneity in the biopsy samples or distinct pathogenesis will require future studies. One of the strengths of this study is the inclusion of both adult and pediatric subjects from multiple centers; we did not observe major heterogeneity between adult and pediatric subjects in each cohort, which is consistent with our previously reported similar EoE signature between adult and pediatric patients with EoE.¹¹

Moreover, the molecular signature of PPI-REE is almost completely reversible by PPI treatment, which does not modify the signature in the EoE and NL cohorts. This is similar to what happens to the EoE signature after diet elimination and/or glucocorticoid therapy.^{11,16} It is notable that we found that expression of 8 of the EoE genes did not normalize to that found in the NL group; perhaps these refractory genes might explain the clinically observed relapsing propensity of PPI-REE.¹⁷ Given the ability of PPIs to modulate multiple components of the inflammatory axis, such as chemokines,^{6,18,19} interleukins,²⁰ and vascular cell adhesion molecules,²¹ as well as mucosal barrier function,²² the effects of high-dose, twice-daily PPI might have an anti-inflammatory effect in addition to its effect on acid reduction.¹⁹ Our study supports this hypothesis.

The observed EoE score was significantly more severe in the EoE than PPI-REE-pre groups. Although the difference in eosinophils/hpf (EoE vs PPI-REE-pre group) might explain part of the variation, this difference was still significant after normalizing to eosinophils/hpf; however, this difference does not influence the general interpretation that EoE and PPI-REE have overlapping molecular signatures in addition to their similar clinicopathologic features. Importantly, the *CCL26* level in patients with PPI-REE was robustly reduced by PPI therapy, which is consistent with the histologic remission and the finding that PPI has an anti-inflammatory role by regulating *CCL26* transcription activity⁶ and protein expression.¹⁸ Concurrent with the decrease in *CCL26* levels, there was normalization of the mast cell genes (*CPA3* and *TPSAB2*), T_H2 inflammation

indicators (*TNFAIP6* [tumor necrosis factor, alpha-induced protein 6] and *ALOX15* [arachidonate 15-lipoxygenase]), epithelial barrier genes (*DSG1*, *CDH26* [cadherin 26], and *FLG* [filaggrin]), tissue fibrosis markers (eg, *KRT13* [keratin 13]), and IL-13/IL-4-induced genes (*POSTN* and *MUC4*) after PPI treatment, suggesting that PPI-REE and EoE share a common pathogenesis. Mastocytosis remission at both the protein and mRNA levels supports the theory that PPI-REE is an allergic T_H2-driven disorder. These findings align with previous data demonstrating that patients with PPI-REE have decreased mast cell and CD45RO⁺ cell numbers after PPI monotherapy.¹⁷ Moreover, the similar signature of PPI-REE-pre versus EoE also raises the questions of whether PPI-REE shares a comparable natural history with EoE and whether, if left unmanaged, PPI-REE can lead to adverse prognosis, such as tissue fibrosis, as recently reported.²³⁻²⁷

Histologic severity, as measured by esophageal eosinophils/hpf, correlated well with EoE score at an overall level (all cohorts); however, when the EoE and PPI-REE-pre groups were analyzed individually, the correlation between histologic severity (eosinophils/hpf) and EoE score was not significant in the PPI-REE-pre group yet was significant in patients with EoE, despite the gene dysregulation signature similarity between patients with EoE and those with PPI-REE. These differences in correlation and the distribution of EoE scores in the PPI-REE-pre cohort suggest potential heterogeneity in the patient populations. Alternatively, PPI-REE might involve a combinatorial trigger of food and acid, which could potentially lead to differences in gene expression. Our current study is not powered to evaluate this potential heterogeneity. Interestingly, the EoE score was statistically different in the EoE versus PPI-REE-pre cohorts, with the EoE group showing a larger difference than the PPI-REE-pre group compared to control subjects. Thus, PPI-REE might be less severe, at least in terms of the molecular dysregulation, as defined by the EDP. The finding that PPI-REE might be potentially less severe is consistent with the modestly lower level of esophageal eosinophils and the lower incidence of endoscopic abnormalities in the PPI-REE group compared with the EoE group. This study is restricted by the EDP scope without genome-wide coverage; therefore, we cannot rule out additional genome-wide mechanisms that might differentiate the 2 entities or explain the differential eosinophilia. Accordingly, using a genome-wide approach to query the *ex vivo* esophageal transcriptome in the presence and absence of PPI would be a promising approach as well.

Our results suggest that PPI-REE is allergic in nature and that its clinical management might be more similar to EoE rather than GERD. Moreover, being able to differentiate patients with EoE and those with PPI-REE before the PPI trial would be a meaningful advance for patients, possibly shortening time to diagnosis and effective treatment. Having predictor genes makes untreated PPI-REE and EoE potentially distinguishable before PPI treatment, leading to a new conceptual classification of esophageal eosinophilia. Moreover, being able to predict the PPI response might significantly enhance the patient's quality of life and will likely reduce medical costs because of the distinct

of the 10 significant genes, EoE versus PPI-REE-pre groups (<http://toppgenes.cchmc.org>). **C**, With a false-discovery correction filter (Westfall-Young permutation), *KCNJ2* (Kir2.1) is the only significant gene within the scope of the EDP (EoE vs PPI-REE-pre groups, corrected $P = .04$). * $P < .05$, ** $P < .01$, and *** $P < .001$ (mean $\pm$ SEM). *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase. **D**, A hypothetical illustration suggesting the interaction of the proton pump ($H^+-K^+-ATPase$) and Kir2.1 in the gastrointestinal mucosa. **E**, A proposed schematic illustration of the classification and treatment of esophageal eosinophilia. Scatter-plot data were presented as mean $\pm$ SEM.

therapies for PPI-REE and EoE. We initially identified a cluster of 10 genes capable of differentiating EoE and PPI-REE-pre. After a subsequent false-detection filter, differential *KCNJ2* expression between the EoE and PPI-REE-pre groups remained significant. Interestingly, Kir2.1 has a unique biological significance because this potassium channel was found to be coupled to the proton pump in the gastrointestinal epithelium.^{14,15} Moreover, Kir2.1 conductance property is subjected to pH change and is involved in acid secretion from parietal cells.¹⁴ Kir2.1 ensures that the potassium ion produced by the proton pump as a byproduct can be readily removed from the intracellular space. Therefore, a lower *KCNJ2* expression level, as in the case of PPI-REE, could result in lower efficiency of the proton pump and help explain the efficacy of PPI.

In conclusion, in this multicenter study using EDP signature analysis, we demonstrated that untreated PPI-REE shares a largely similar molecular transcriptome with EoE, suggesting that PPI-REE and EoE are highly related and have a common T_H2 /allergy pathogenesis. It is remarkable that the hallmark features of allergic inflammation are present in patients with PPI-REE, showing that PPI therapy can reverse classic allergic inflammation at the molecular level. Clearly understanding the mechanism responsible for this is now a paramount question because this has implications for diseases associated with allergic inflammation. On the basis of our collective findings, we propose that many subjects with PPI-REE represent a continuum of the same marked allergic inflammatory response that underlies EoE and thus might constitute a subphenotype of EoE (Fig 5, E). The EoE-like signature of untreated PPI-REE is reversible by using PPI therapy, resulting in a remission signature close to that of healthy control subjects. The molecular similarity of untreated PPI-REE and EoE mirrors the clinical, endoscopic, and histologic similarities between the 2 conditions. Interrogating the molecular signatures at an individual gene level might distinguish PPI-REE from EoE before the PPI trial and reveal potentially different molecular pathways. Because the debate regarding the nature of PPI-REE is ongoing, these findings are timely and clinically important because understanding the molecular etiology and pathogenesis of PPI-REE might help to better define the subform of esophageal eosinophilia, unveil the pathogenesis of PPI-REE, and ultimately lead to improvement of diagnosis and therapeutic management of both PPI-REE and EoE.

We thank Shawna Hottinger for editorial assistance.

Key messages

- PPI-REE shares a largely overlapping molecular signature with EoE.
- PPI treatment alone is sufficient to reverse the allergic T_H2 inflammatory signature of patients with PPI-REE almost to baseline levels. This includes hallmark molecular features of allergic inflammation, including eosinophilia, mastocytosis, tissue remodeling (including periostin), and impaired barrier function.
- A preliminary cluster of genes capable of differentiating PPI-REE and EoE before PPI therapy has been identified.

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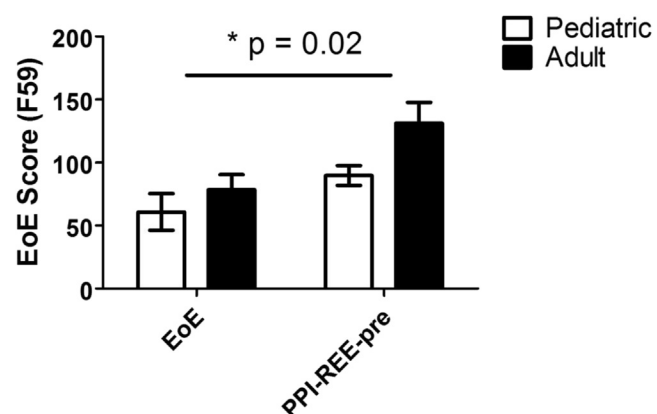


FIG E1. Effect of age on the molecular severity of the EoE and PPI-REE cohorts. The EoE and PPI-REE (-pre) subjects were stratified by age (<18 years = pediatric; ≥ 18 years = adult). A total of 33 patients with EoE and 28 patients with PPI-REE were analyzed by using 2-way ANOVA, and a significant difference was observed for disease factor (EoE vs PPI-REE groups, $*P = .02$) but not for age factor ($P = .08$) or the interaction of the two ($P = .47$). Scatterplot data were presented as mean $\pm$ SEM.

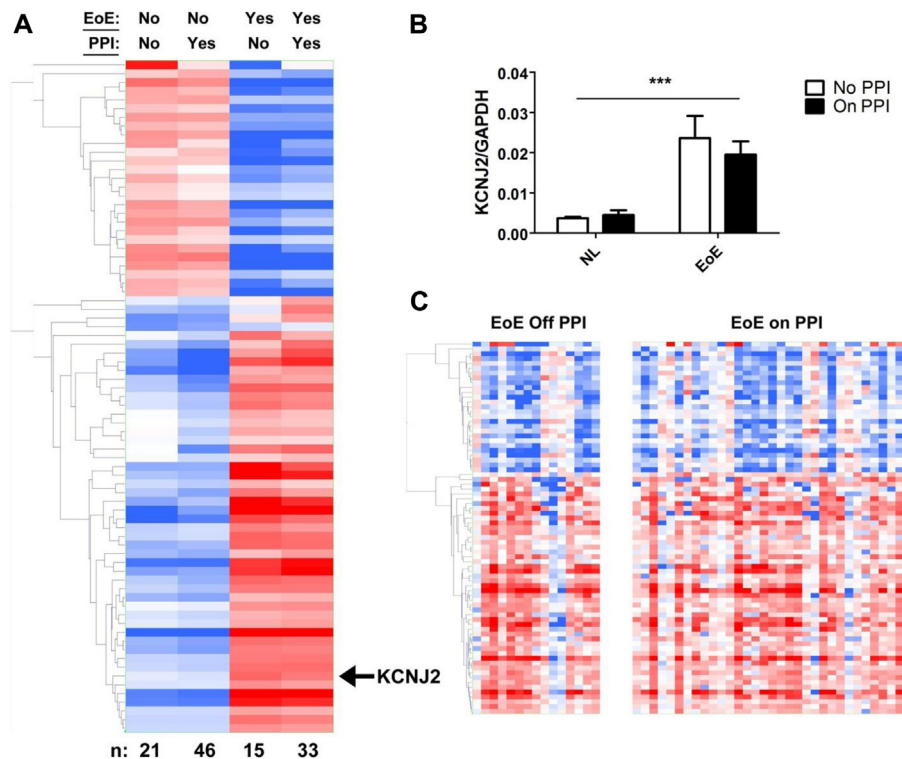


FIG E2. The effect of PPI on EoE transcriptome, including *KCNJ2* expression. **A**, Subjects in the NL ($n = 67$) and EoE ($n = 48$) groups were stratified into on-PPI and off-PPI groups and shown on the collective (averaging) heat maps. Numbers are displayed at the bottom. **B**, Expression level of *KCNJ2* in the NL and EoE groups on and off PPI. *** $P < .001$ for EoE phenotype effect, nonsignificant for PPI effect and interaction, 2-way ANOVA. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase. Scatterplot data were presented as mean $\pm$ SEM. **C**, EoE cohort was substratified into on- and off-PPI subgroups, and the individual signatures were compared (each column represents 1 subject).

TABLE E1. Subject numbers by group and center

Study site	UCSD* (n = 36)	UNC* (n = 31)	Walter Reed (n = 32)	CCHMC (n = 7)	Colorado (n = 6)
Normal, no. (%)	7 (19)	4 (13)	5 (16)	0 (0)	0 (0)
EoE, no. (%)	8 (22)	10 (32)	15 (47)	0 (0)	0 (0)
Pre-PPI-REE, no. (%)	7 (19)	9 (29)	12 (38)	0 (0)	0 (0)
Post-PPI-REE, no. (%)	8 (22)	8 (26)	0 (0)	0 (0)	6 (100)
GERD, n (%)	6 (17)	0 (0)	0 (0)	7 (100)	0 (0)

Colorado, Children's Hospital Colorado; *UCSD*, University of California, San Diego; *UNC*, University of North Carolina–Chapel Hill; *Walter Reed*, Walter Reed National Military Medical Center.

*These overall numbers include the paired pre/post-PPI PPI-REE groups at UCSD (n = 7) and UNC (n = 8).

TABLE E2. The common set of 46 genes dysregulated in patients with EoE and those with PPI-REE

Gene	Regulation	NL vs EoE groups		NL vs PPI-REE groups	
		P value	Fold change	P value	Fold change
<i>UPK1B</i>	Up	1.10E-04	9.81	.035175	4.26
<i>IGJ</i>	Up	2.69E-04	15.70	.002381	10.56
<i>TNFAIP6</i>	Up	5.87E-10	156.18	2.37E-06	31.96
<i>SUSD2</i>	Up	5.81E-07	13.60	7.73E-04	7.08
<i>GPR160</i>	Up	7.68E-05	5.54	.030939	3.11
<i>HRH1</i>	Up	2.01E-07	7.15	2.67E-05	5.09
<i>CFI</i>	Up	6.26E-06	13.53	.001499	7.50
<i>APOBEC3A</i>	Up	2.13E-05	13.10	.016765	3.78
<i>CCR3</i>	Up	.0020266	4.62	2.95E-04	5.31
<i>FKBP5</i>	Up	.0426904	2.44	.003383	3.49
<i>UBD</i>	Up	1.47E-07	21.30	1.97E-05	14.61
<i>CTSC</i>	Up	2.48E-06	9.35	1.08E-04	5.28
<i>NCAM1</i>	Up	.0060031	5.23	8.57E-05	9.28
<i>TPSB2</i>	Up	3.03E-06	12.84	3.96E-06	10.93
<i>HPGDS</i>	Up	7.81E-09	19.20	1.53E-05	10.95
<i>CCL26</i>	Up	1.16E-15	83.25	1.98E-10	38.96
<i>RTP4</i>	Up	5.14E-06	9.19	8.22E-04	7.00
<i>POSTN</i>	Up	2.52E-15	297.61	1.02E-10	116.17
<i>RUNX2</i>	Up	7.65E-06	8.66	.032632	3.80
<i>ANO1</i>	Up	2.67E-14	42.23	2.56E-08	33.59
<i>CXCL1</i>	Up	2.84E-06	21.63	.001359	6.47
<i>GLDC</i>	Up	4.21E-08	15.92	5.91E-04	6.61
<i>CA2</i>	Up	2.02E-07	13.11	2.01E-06	12.77
<i>CCL11</i>	Up	2.01E-05	10.63	.006125	4.39
<i>ALOX15</i>	Up	3.18E-11	189.74	5.25E-07	47.63
<i>SYNPO2</i>	Up	.0338341	2.28	.032077	2.38
<i>KRT23</i>	Up	4.38E-04	7.57	.016584	3.73
<i>GRK5</i>	Up	.0027821	4.46	.02564	3.87
<i>PHLDB2</i>	Up	8.40E-08	9.51	7.85E-04	4.33
<i>CPA3</i>	Up	5.43E-08	17.40	2.96E-09	17.71
<i>SAMSN1</i>	Up	2.65E-09	11.83	4.41E-05	6.43
<i>MUC4</i>	Up	2.74E-06	9.62	4.66E-04	7.90
<i>GCNT3</i>	Up	.0024152	3.77	.040205	2.52
<i>CDH26</i>	Up	8.38E-16	111.18	1.09E-10	75.65
<i>CFB</i>	Up	6.42E-10	7.91	6.16E-06	6.10
<i>CITED2</i>	Down	8.82E-04	3.27	7.48E-05	4.03
<i>UPK1A</i>	Down	2.39E-04	8.10	.001833	5.56
<i>SPINK7</i>	Down	5.16E-06	12.21	3.87E-06	13.03
<i>ENDOU</i>	Down	1.02E-06	9.81	3.36E-05	7.73
<i>CDA</i>	Down	9.34E-08	8.72	1.10E-05	8.06
<i>IGFL1</i>	Down	5.68E-06	6.57	1.79E-06	9.57
<i>CLDN10</i>	Down	1.48E-04	9.27	5.86E-04	6.03
<i>GRPEL2</i>	Down	5.03E-05	4.13	4.02E-05	3.50
<i>C7orf68</i>	Down	2.24E-04	3.40	7.73E-04	2.88
<i>ALOX12</i>	Down	1.29E-06	8.77	8.70E-07	9.22
<i>ACPP</i>	Down	8.31E-04	2.81	.002245	2.01