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 2015;135:187-97.)

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

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,

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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 6[6]; 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 pathogeneses. 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 EDP[11] from RNA extracted from 60- to 80-μm 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 genes[11] 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 score[11] 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