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Proton Pump Inhibitors Modulate Gene Expression Profile in Esophageal Mucosa and Microbiome

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OBJECTIVE Proton pump inhibitors (PPIs) are commonly used to manage children with upper gastrointestinal symptoms and without a formal diagnosis. We investigated the effect of PPIs on esophageal mucosal transcriptome and active microbiota in children with normal esophagi. Furthermore, we examined whether the differences in host esophageal mucosal gene expression were driven by an underlying esophageal epithelial cell type composition.

METHODS Using metatranscriptomics, the host transcriptional and active microbial profiles were captured from 17 esophageal biopsy samples (PPI naïve [PPI−], n = 7; PPI exposed [PPI+], n = 10) collected from children without any endoscopic and histologic abnormalities in their esophagus (normal esophagus). Deconvolution computational analysis was performed with xCell to assess if the observed epithelial gene expression changes were related to the cell type composition in the esophageal samples.

RESULTS The median (IQR) age of our cohort was 14 years (12–16) with female (63%) preponderance. Both groups were similar in terms of their demographics and clinical features. Compared with PPI−, the PPI+ had upregulation of 27 genes including the *MUC* genes. The cell type composition was similar between the PPI− and PPI+ groups. *Prevotella* sp and *Streptococcus* sp were abundant in PPI+ group.

CONCLUSIONS In children with normal esophagus, PPI exposure can be associated with upregulation of esophageal mucosal homeostasis and epithelial cell function genes in a cell-type independent manner, and an altered esophageal microbiome. Additional studies are warranted to validate our findings and to investigate the causal effect of PPIs on the normal esophageal epithelium and microbial communities.

ABBREVIATIONS DBMT1, deleted in malignant brain tumors 1; EGD, esophagogastroduodenoscopy; EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease; GI, gastrointestinal; mOTU, marker gene–based operational taxonomic unit; MUC, mucin glycoprotein genes; PCSK9, proprotein convertase subtilisin/kexin type 9; PHGR1, proline-, histidine-, and glycine-rich protein 1; PIGR, polymeric immunoglobulin receptor; PPI, proton pump inhibitor

KEYWORDS deconvolution analysis; esophagus; metatranscriptomics; microbiome; pediatrics; proton pump inhibitors

Introduction

Proton pump inhibitors (PPIs) are frequently used in children with symptomatic gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EoE).[1] The effect of PPIs on esophageal mucosal transcription and microbiome in diseases such as GERD and EoE are being studied.[2,3] PPIs can be used to empirically manage upper gastrointestinal (GI) symptoms in children with normal esophageal anatomy and histology (normal esophagus).[4,5] However, the effect of PPIs on the esophageal mucosal transcription and local microbiome in normal pediatric esophagus remains to be understood.

We investigated the association between PPI exposure and the esophageal mucosal gene expression and microbiota in children with a normal esophagus, using metatranscriptomic approaches optimized for low-bacterial-load human samples. Additionally, we investigated whether differences in the host mucosal gene expression were driven by composition of the esophageal cell types. We hypothesized that PPI exposure alters the normal pediatric esophageal mucosal transcriptional profile in a cell-type–dependent manner and affects the local microbiome.

Materials and Methods

The distal esophageal mucosal biopsy samples collected by using conventional biopsy forceps and corresponding metadata collected as part of previously

published studies were used for this research.[6,7] In brief, children 6 to 18 years of age with upper GI symptoms and undergoing an esophagogastroduodenoscopy (EGD) at Vanderbilt Children's Hospital were enrolled in the original studies. In addition to 4 to 6 esophageal mucosal biopsy samples (from proximal and distal esophagus combined) collected for clinical care, distal (≤5 cm from the lower esophageal sphincter) esophageal biopsy samples were collected for research. The demographic (age at EGD, sex, ethnicity) and clinical (presenting symptoms, allergic comorbidities, PPI exposure [name, dose, duration], and the number of esophageal biopsies) information were gathered from electronic medical records. Those with known GERD; EoE; prior esophageal injury or surgery; celiac disease; inflammatory bowel disease; exposure to antibiotics, topical steroids, or systemic steroids within the past 30 days; and/or on concurrent histamine-2 receptor blockers (i.e., famotidine) were excluded to minimize confounding. Children with endoscopically normal-appearing esophagus and with no histologic alterations in their esophageal biopsy samples were considered as having a normal esophagus.

Total RNA was extracted as previously described.[8] After rRNA depletion, Illumina sequencing libraries were made, and the libraries were sequenced on an Illumina NovaSeq6000 platform (San Diego, California, USA) see Supplemental Material for details). A threshold of log2 fold change >1 and adjusted p < 0.05 was used to identify significantly upregulated or downregulated genes. Following quality control and initial data-processing steps, the sequence reads mapped to bacteria were used to profile the esophageal microbiome. The reads mapped to human transcripts were used to analyze the esophageal mucosal transcriptome. The microbial reads were subjected to marker gene–based operational taxonomic unit (mOTU) analysis to obtain the relative abundance for each mOTU.[9] The statistical significance of the differences in proportions (or relative abundance) of bacteria of interest was tested by using a Kruskal-Wallis test.[10] The Phyloseq R package version 1.30.0 was used to analyze microbial richness and alpha diversity metrics.[11] Shannon and Chao1 diversity indices were calculated from the mOTU counts in the samples to assess the alpha diversity of the microbial communities they represent. Wilcoxon rank sum test was used to test for significant differences in microbial richness or alpha diversity. To assess whether observed gene expression changes could be related to the cell type composition in the biopsy samples, deconvolution analysis was performed by using xCell.[12,13] Briefly, the raw gene counts were converted to log counts per million with a prior count of 3 using limma (version 3.50.3). Raw enrichment scores were then calculated and normalized. Subsequently, a beta distribution was used to exclude cell types not in the gene count mixture to improve accuracy, using an arbitrary threshold of

cell types present in at least 3 samples. Finally, spill-over was calculated with the default alpha of 0.5, and Z-scores of enrichment score were graphed by using Complex Heatmap (version 2.10.0).[14] A Wilcoxon signed rank test with correction for multiple testing according to Benjamin-Hochberg was executed to assess the statistical relation between the cell type scores and treatment status.

Results

The median (IQR) age of our cohort was 14 years (12–16) with female (63%) predominance. Seven (37%) were PPI naïve (PPI−) and 10 (63%) were on a PPI (PPI+) at the time of EGD. Both groups were similar in terms of their demographics and clinical features. In the PPI+ group, most (80%) were on omeprazole for a median (minimum–maximum) duration of 53 days (3–224) and dose of 0.6 mg/kg/day (0.4–0.9) (Supplemental Table S1).

As compared with the PPI− group, 27 genes were significantly upregulated in the PPI+ group. Proline-, histidine-, and glycine-rich protein 1 (*PHGR1*), mucin glycoprotein genes (*MUC2, MUC3A*), deleted in malignant brain tumors 1 (*DMBT1*), and polymeric immunoglobulin receptor (*PIGR*) were among the most highly upregulated genes. Proprotein convertase subtilisin/kexin type 9 (*PCSK9*), which is a regulator of low-density lipoprotein cholesterol clearance, was slightly but not statistically significantly upregulated in the patients receiving PPI. No genes were significantly downregulated (Figure 1 and Supplemental Table S2). The deconvolution analysis revealed that cell type composition was not significantly different between the PPI− and PPI+ groups, indicating that the observed gene expression changes could not be readily explained by a difference in proportion of cell types between the 2 groups of patients (Supplemental Figure).

The esophageal microbiome of both PPI+ and PPI− groups was dominated by *Cutibacterium acnes* and members of *Streptococcus* and *Haemophilus* species. Interestingly, the mean abundance of *Streptococcus* sp was significantly higher in the PPI+ group than the PPI− group (20% vs 0%; p = 0.053, Kruskal-Wallis). At the species level, there were no significant differences between PPI+ and PPI− for the alpha (Shannon and Chao1 indices) and beta (Bray-Curtis dissimilarities) diversity as well as for the overall community composition (Figure 2).

Discussion

Using metatranscriptomics and a gene-signature–based method to enumerate cell subsets from transcriptomes, we found that in normal pediatric esophagus PPI exposure can be associated with esophageal mucosal changes in a cell-type–independent manner and with altered local microbiome. This study provides original insights into the association

Figure 1. Volcano plot showing differentially expressed host genes between children on PPI and children not on PPI. We used a threshold of log2 fold change >1 and adjusted p < 0.05 to call the genes that are upregulated or downregulated. The upregulated genes that satisfy the threshold are shown in red dots.
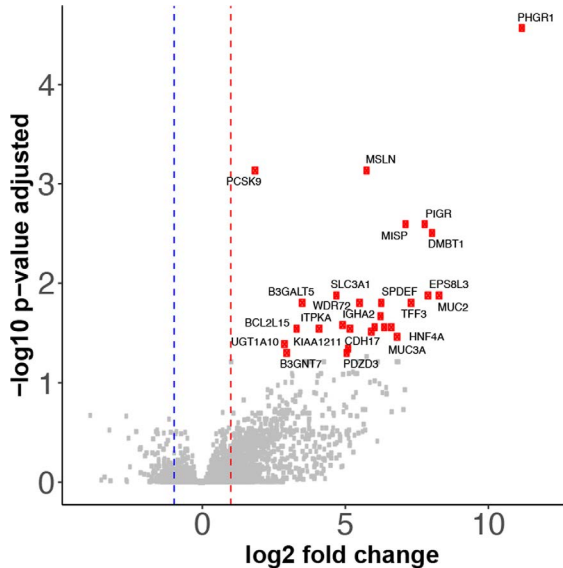
BCL2L15, BCL2 like 15; B3GALT5, beta-1:3-galactosyltransferase 5; B3GNT7, UDP-GlcNAc:betaGal beta-1:3-N-acetylglucosaminyltransferase 7; CDH17, cadherin 17; DMBT1, deleted in malignant brain tumors 1; EPS8L3, EPS8 like 3; HNF4A, hepatocyte nuclear factor 4 alpha; IGHA2, immunoglobulin heavy constant alpha 2; ITPKA, inositol-trisphosphate 3-kinase A; KIAA1211, KIAA1211; MISP, mitotic spindle positioning; MSLN, mesothelin; MUC2, mucin 2; MUC3A, mucin 3A; PCSK9, proprotein convertase subtilisin/Kexin type 9; PDZD3, PDZ domain containing 3; PHGR1, proline-, histidine-, and glycine-rich 1; PIGR, polymeric immunoglobulin receptor; PPI, proton pump inhibitor; SLC3A1, solute carrier family 3 member 1; SPDEF, SAM pointed domain containing ETS transcription factor; TFF3, trefoil factor 3; UGT1A10, UDP glucuronosyltransferase family 1 member A10; WDR72, WD repeat domain 72.

between PPI use and the esophageal mucosal molecular mechanisms and local microbiome. Taken together our results hold promise to enhance our understanding of the complex community behavior of the microbiome in esophagus and can also inform the use of PPIs in pediatric practice.

Previously, PPIs have been shown to curtail transcriptomic processes involved in cellular proliferation and interleukin (IL)-13–induced responses in esophageal cell culture experiments.[15] In GERD and EoE, omeprazole has been shown to inhibit the expression of eotaxin-3 (an eosinophil chemoattractant, also known as C-C motif chemokine ligand or CCL26) in the esophageal epithelium.[16] Our results indicate that in endoscopically and histologically normal pediatric esophagus, PPIs induce genes involved in O-linked glycosylation

of mucins (MUC2, MUC3A) and B3GNT7. Transcription of mucin genes is important in attenuating effects of noxious stimuli in the GI tract.[17] A recent study using human-derived enteroids demonstrated that an increased expression of B3GNT7 alone is sufficient to promote the augmented display of Lex-decorated carbohydrate glycan structures primarily on O-glycosylated intestinal epithelial glycoproteins.[18] Taken together, these genes play an important role in enhancing protective mucus secretion and epithelial cell differentiation.[19,20] Genes involved in vesicular transport and metabolic processes (PHGR1),[21] regulation of beta-cell development (HNF4A), and cell-cell junction organization (CDH17) are also upregulated in children exposed to PPIs.

In a setting of altered transcriptional landscape, studying the contribution of individual cell types to the gene expression patterns is pivotal because cells may behave differently depending on microenvironment.[22] We used deconvolution of bulk RNA-sequencing data and assessed the proportion of 64 immune and stromal cell types and found no differences between PPI+ and PPI− subjects. These results suggest that the differences in esophageal epithelial gene expression upon exposure to PPI is unlikely to be driven by changes in the proportion of specific cell types. To investigate this further, a more focused approach, such as single cell–RNA sequencing, might be required.

With regard to microbiome, PPI exposure has been shown to decrease Comamondadaceae and increase Clostridiaceae and Micrococcaceae in esophageal biopsy samples obtained from healthy adults.[23,24] We found that PPI exposure was associated with increased abundance of Streptococcus sp without any notable difference in the richness, diversity, or overall community composition when compared with those who were PPI naïve. While the significance of these differences remains to be investigated, we postulate that these differences may be related to the pediatric age and the type, duration, and dose of PPI.[25]

The small sample size, wide range for PPI exposure, and cross-sectional design are major limitations of our study and limit the generalizability of our results and our ability to make any causal inferences. Next, we did not have dietary modification data, which could confound our findings. Despite these limitations our study has multiple strengths. Given how often PPIs are used to manage upper GI symptoms in children,[26] understanding their effect on the esophagus is an important scientific pursuit. By including children, we were able to eliminate several confounders that can be relatively more common in adults (e.g., multiple medications). To the best of our knowledge, this is the first study to apply metatranscriptomics optimized for low-bacterial-load human samples and a validated gene-signature–based method to enumerate cell subsets from transcriptomes to interrogate the contribution of individual cell types to the gene expression patterns.

Figure 2. (A) A color-coded bar plot shows the average relative abundance of all microbial taxa that could be identified in the esophagus in PPI− and PPI+ groups. (B) Richness and alpha diversity of the esophagus microbiome. Alpha diversity and richness (measured by Shannon and S.Chao1 index) are compared between the PPI− and PPI+ groups. Differences in alpha diversity between the groups were not significant. (C) A principal coordinate analysis plot of Bray-Curtis dissimilarities (beta diversity) over the first two-axis is shown. Dots represent individual data points, and the color represents PPI+ and PPI− groups. Overall, the microbiome community composition was not significantly dissimilar among the PPI+ and PPI− groups.
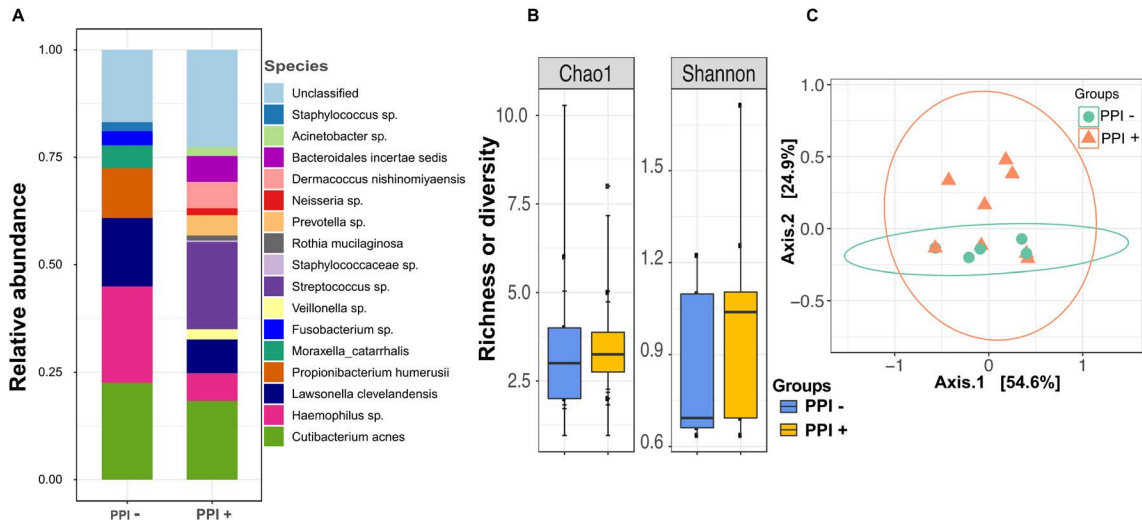
PPI, proton pump inhibitor.

Adequately designed studies in the future can allow validation of our findings and investigate the causal effect of PPIs on the normal esophageal epithelium and microbial communities, and examine if these vary by PPI type, duration of exposure, or dose.

Article Information

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