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Original article

Molecular Analysis of Duodenal Eosinophilia

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Conflicts of Interest

M.E.R. is a consultant for Pulm One, Spoon Guru, ClostraBio, Serpin Pharm, Allakos, Celldex, Nexstone One, Bristol Myers Squibb, Astra Zeneca, Ellodi Pharma, GlaxoSmith Kline, Regeneron/Sanofi, Revolo Biotherapeutics, and Guidepoint and has an equity interest in the first seven listed and royalties from reslizumab (Teva Pharmaceuticals), PEESV2 (Mapi Research Trust), and UpToDate. M.E.R. is an inventor of patents owned by Cincinnati Children's Hospital. M.H.C. has received research funding from AstraZeneca, Meritage Pharma Inc., Receptos/Celgene, Regeneron Pharmaceuticals, and Shire, a Takeda company, and is a consultant for Allakos, Arena Pharmaceuticals, AstraZeneca, Calypso Biotech, EsoCap Biotech, GlaxoSmithKline, Receptos/Celgene/BMS, Regeneron Pharmaceuticals, Robarts Clinical Trials Inc./Alimentiv, Inc.,

and Shire, a Takeda company. V.A.M. is a consultant for Shire/Takeda, Allakos, and Sanofi; has received research funding from Shire/Takeda; and serves on an adjudication board for Alladapt. All other authors declare that they have no competing interests.

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Author Contributions

T.S. and M.E.R. conceived the study and design. T.S. performed the laboratory work. M.R. and L.E.M. provided analytical and bioinformatic support. J.M.C. and G.A.O. provided technical support. T.S. contributed to the statistical analysis. M.H.C. performed the pathologic assessments. V.A.M. and P.E.P. provided clinical support through the Cincinnati Center for Eosinophilic Disorders (CCED). T.S. and M.E.R. drafted the manuscript. M.E.R. obtained funding and led the study. All of the authors discussed the results and reviewed and commented on the manuscript.

Abbreviations Used

APFED, American Partnership for Eosinophilic Disorders; CCED, Cincinnati Center for Eosinophilic Disorders; CCHMC, Cincinnati Children's Hospital Medical Center; CURED, Campaign Urging Research for Eosinophilic Disease; DE, duodenal eosinophilia; EFC, Eosinophilic Family Coalition; EoE, eosinophilic esophagitis; EoG, eosinophilic gastritis; EoD,

67 eosinophilic duodenitis; EoC, eosinophilic colitis; EGID, eosinophilic gastrointestinal disorders;
68 EMT, epithelial-mesenchymal transition; FDR, false-discovery rate; GO, gene ontology; GAPDH,
69 glyceraldehyde-3-phosphate dehydrogenase; GI, gastrointestinal; GOI, gene of interest; HPF,
70 high-power field; IPA, Ingenuity Pathway Analysis; IQR, interquartile range; NK, natural killer
71 (NK); PPI, proton pump inhibitor.

ABSTRACT

Background: Eosinophilic duodenitis (EoD), characterized by non-specific gastrointestinal symptoms and increased duodenal eosinophils, may be in the eosinophilic gastrointestinal disease (EGID) spectrum. However, diagnostic thresholds and pathogenic processes of duodenal tissue eosinophilia are inadequately characterized.

Objective: We aimed to define an EoD transcriptome and pathologic pathways.

Methods: RNA sequencing and histologic features of human duodenal biopsies were analyzed as a function of duodenal eosinophils levels. For analyses, we defined EoD as >52 peak eosinophils per high-power field (eosinophils/HPF) (n=8), duodenal eosinophilia (DE) as 30-52 eosinophils/HPF (n=11), and normal controls (NL) as <30 eosinophils/HPF (n=8). Associations between gene expression and histologic features were analyzed with Spearman correlation.

Results: We identified 382 differentially expressed genes (EoD transcriptome) between EoD and NL (>2-fold change, adjusted $P < .05$). The EoD transcriptome distinguished EoD from controls (DE and NL). The duodenal eosinophil count correlated with a distinct EoD transcriptome when 50-60 peak eosinophils/HPF were present. The EoD transcriptome was enriched in genes involved in IL-4/IL-13 signaling, mast cells, and myeloid progenitor cells. Among duodenal histologic features, lamina propria eosinophil sheets was most associated with transcriptomic changes ($r = 0.66$, $P < .01$). EoD gene signatures were shared with eosinophilic esophagitis and eosinophilic gastritis, but not eosinophilic colitis or celiac disease.

Conclusion: We have identified an EoD transcriptomic signature that emerges at 50-60 peak eosinophils/HPF and establishes EoD as part of a spectrum of upper EGID associated with type 2

immunity and distinct from eosinophilic colitis and celiac disease. These findings provide a basis for improving diagnosis and treatment.

Keywords: duodenitis; eosinophil; eosinophilic duodenitis; celiac disease; transcriptome

99 Clinical Implications

100 We defined a transcriptome of eosinophilic duodenitis (EoD) that emerges at 50-60 peak
101 eosinophils/HPF and established EoD as part of a spectrum of upper EGID associated with type 2
102 immunity.

103

104

105 Capsule Summary

106 We identified the molecular signature for eosinophilic duodenitis, its association with duodenal
107 eosinophils, and its clinical significance by concurrent analysis of histologic findings and other
108 gastrointestinal diseases, providing insights into disease pathogenesis and improved diagnosis.

INTRODUCTION

Eosinophilic gastrointestinal diseases (EGID) are chronic inflammatory conditions characterized by persistent gastrointestinal (GI) symptoms and increased eosinophil counts in the GI tract without known causes of eosinophilia.¹ These disorders are classified by the presence and location of tissue eosinophils. Although most studies in the EGID field have focused on eosinophilic esophagitis (EoE), nonesophageal EGIDs have become increasingly recognized globally.²⁻⁴ These nonesophageal EGID, such as eosinophilic duodenitis (EoD), are less understood.^{5, 6}

EoD is not well defined, in part due to the lack of diagnostic guidelines and non-specific symptoms that are shared with other GI disorders (e.g., celiac disease).^{7, 8} Eosinophils are normal residents of the GI tract, except for the esophagus,⁹ and also are increased in the duodenum in other GI disorders (e.g., celiac disease, functional dyspepsia).^{10, 11} There is uncertainty about the thresholds for abnormal duodenal eosinophil levels, in contrast to the detailed threshold and guidelines for diagnosis of EoE.¹² Although the United States Food and Drug Administration has accepted 30 eosinophils per microscopic high-power field (eosinophils/HPF) in 3 HPFs in duodenum biopsies as the threshold criteria for clinical trials of EoD,¹³ it has not issued guidance statements including a threshold value for diagnosis, as it has for EoE;¹⁴ notably, others have proposed >52 eosinophils/HPF in 1 HPF,¹⁵ resulting in a broad range of duodenal eosinophilia (30–52 eosinophils/HPF in 1-3 HPF) for diagnostic purposes.

The understanding of EGID molecular pathogenesis has been advanced by elucidating the transcriptome of tissue biopsies. At present, transcriptomes for EGID have been identified, such

as the EoE transcriptome,¹⁶ EoG transcriptome,¹⁷ and EoC transcriptome,¹⁸ providing insight into disease pathogenesis and potential clinical application. These molecular findings have linked EoE and EoG on the basis of strong type 2 immunity characterized by IL-13–driven transcriptional responses involving CCL26 and other protein products of STAT6-induced genes;^{16, 17} in contrast, EoC shows relatively weak type 2 immunity.¹⁸ In this context, we aimed to determine whether an EoD transcriptome could be derived using a cut-off of 52 eosinophils/HPF, the functional enrichment of the duodenal transcriptome, and its potential utility for molecular diagnosis by answering the following fundamental questions: (1) Does EoD have a distinct molecular profile compared with control tissue?; (2) Will specific molecular elements correlate with disease?; (3) Will specific histologic features correlate with the EoD transcriptome?; and (4) What is the molecular relationship an EoD-associated transcriptome to the transcriptome of other EGID and other GI diseases involving the duodenum? Accordingly, we examined duodenal biopsies from patients that exhibited duodenal eosinophilia by genome-wide transcriptomic profiling, parallel histologic analysis, and pathway interrogation and compared the derived findings relative to other EGID and celiac disease.

METHODS

Study Design and Participants

Subjects with duodenal eosinophilia and controls in the Cincinnati Center for Eosinophilic Disorders (CCED) EGID database from 2008–2020, which included children and adults who had undergone endoscopy as a part of clinical care, were surveyed for this study. We included subjects having fresh duodenal biopsy specimens stored in RNAlater. The clinical features of subjects were determined during routine clinic visits using standardized intake forms. Dichotomous yes/no answers regarding gastrointestinal symptoms and information on diet therapy, medication therapy, and atopic history were collected at the time of biopsy.

EoD was defined by peak duodenal biopsy eosinophil counts based on a threshold value for diagnosis that is 2X the upper limit of normal for each anatomic site in normal biopsies;^{9, 15, 19} using the reported peak eosinophil count in duodenal biopsies of 26 eosinophils/HPF, the threshold used for this study is >52 eosinophils/HPF in 1 HPF (0.27 mm²).⁹ In each case, alternative causes of mucosal eosinophilia were ruled out; negative tests typically included stool culture for pathogenic bacteria or parasites, viral and parasitic antibody titers and/or PCR, and celiac and inflammatory bowel disease (IBD) serology screens. Recent studies have defined EoD based on peak duodenal eosinophil counts of at least 30 eosinophils/HPF in 3 HPFs.¹³ As such, in slides showing potentially greater than expected eosinophil counts, the counts for at least 3 HPF were recorded and the highest count was considered the peak eosinophil count.

In contrast, duodenal eosinophilia (DE) was defined by duodenal eosinophil counts more than the normal level (≥ 30 eosinophils/HPF) but less than the threshold level (≤ 52 eosinophils/HPF) (30

eosinophils/HPF $\leq$ DE $\leq$ 52 eosinophils/HPF). Subjects with EoD or DE who had concomitant EGID involving other GI segments (esophagus: ≥ 15 eosinophils/HPF in 1 HPF,¹² stomach: ≥ 30 eosinophils/HPF in 5 HPF,¹⁵ colon: ≥ 65 eosinophils/HPF in 1 HPF¹⁵) were not excluded on the basis of the nomenclature consensus recommendations.²⁰

Non-EoD control subjects (normal [NL]) had no history of EoD or abnormal pathologic evidence surveyed during the index endoscopy and had duodenal biopsies collected for research purposes during the index endoscopy. NL were patients who underwent endoscopic examination due to digestive symptoms but did not show pathology. As a relevant disease control, publicly available duodenal transcriptome datasets from patients with celiac disease were extracted, as described later. This study was approved by the institutional review board of Cincinnati Children's Hospital Medical Center (CCHMC) (#2008-0090). An informed consent/assent form was signed by the subjects and/or their legal guardians per institutional guidelines prior to inclusion in the study.

Molecular Evaluation

RNA sequencing was performed using the QuantSeq 3' mRNA Seq Library Prep Kit FWD for Illumina (Lexogen) as previously described.²¹ Briefly, fresh biopsy specimens collected from subjects (EoD, DE, and NL) were stored in RNAlater until they were subjected to RNA isolation. Total RNA was extracted with the miRNeasy kit (Qiagen, Valencia, Calif) and evaluated with an Agilent Bioanalyzer by the CCHMC Gene Expression Core. Data analyses were performed using DESeq2 in CLC Genomics Workbench software (CLC bio, Waltham, MA, USA) and GeneSpring software ver. 14.9 (Agilent Technologies). Transcripts per kilobase million (TPM) were assessed for statistical significance. Subjects with EoD were compared separately with subjects with NL

and DE. Data are available at EGIDExpress (<https://egidexpress.research.cchmc.org/data/>) and listed as EoD RNAseq; all other data supporting the findings of this study are available from the corresponding author upon request. Functional enrichment analyses were performed with the ToppGene suite and CluGO.^{22, 23} Upstream regulator analysis was done with Ingenuity Pathway Analysis (IPA) (Qiagen Inc., Valencia, CA, USA). Cell type enrichment analysis was performed with xCell.²⁴ EoD score was calculated by summing the normalized expression values of genes dysregulated in the EoD transcriptome. A real-time, reverse transcription–quantitative polymerase chain reaction (RT-qPCR) was performed to determine mucosal expression of inflammatory cytokines/chemokines in subjects with EGID and NL.¹⁸ Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an expression control for all analyzed genes. Lists of esophagus-, stomach-, duodenum-, and colon-specific genes were obtained from the Human Protein Atlas (<http://www.proteinatlas.org>).²⁵

Histologic Features

Duodenal biopsies were assessed for the peak eosinophil counts and other histologic features of EoD.^{19, 26} Hematoxylin and eosin (H&E)–stained biopsy slides were reviewed by an expert pathologist (M.H.C.) blinded to the diagnosis. Histologic features assessed were lamina propria eosinophil sheets, pericryptal circumferential collars, eosinophils in surface epithelium, eosinophil cryptitis, eosinophils in muscularis mucosa/submucosa, villous atrophy, elongated crypts, subcryptal eosinophilic inflammation, and reactive epithelial changes. To evaluate the potential presence of other diseases and to determine the potential frequency of features believed to be uncommon in or absent from EoD, subcryptal lymphoplasmacytic inflammation and acute inflammatory cells were scored. Each feature was scored using a 3-point scale (0 = absent, 1 =

mild/moderate, 2 = marked) (see Table E1 in this article's Online Repository at www.jacionline.org).

Statistical Analysis

Data are presented as n (%) or median (interquartile range [IQR]) unless otherwise stated. Missing data were excluded from all formal statistical analyses. For gene expression analysis, we estimated a sample size of 8 in order to have 80% power to detect a 2-fold difference in expression levels with a false-discovery rate (FDR) of 0.05, assuming 18,000 genes whose expression can be detected and 300 genes with differential expression by RnaSeqSampleSize.²⁷ Unsupervised hierarchical clustering was done by Euclidean distance metric and Ward's linkage rule. Nonparametric correlation analysis was performed using Spearman's rank correlation coefficient. For continuous data, statistical significance was determined by the Mann-Whitney *U* test (nonparametric test, 2 groups) or Kruskal-Wallis test followed by a Dunn multiple-comparison test (nonparametric test, ≥ 3 groups). Benjamini–Hochberg correction was applied for multiple testing to control the FDR. For categorical data, the chi-square test or the Fisher's exact test was used. A significant *P* value was defined as <0.05 .

RESULTS

Subject Characteristics

Duodenal biopsies from 27 subjects (n = 8 EoD, 11 DE, 8 NL) were analyzed. Demographic and clinical characteristics of the study cohort stratified by group are detailed in Table 1.

Demographic features and clinical features (such as history of atopy, symptoms, and treatment) at the time of biopsy sampling were similar when compared among all 3 groups (EoD, DE, NL); however, subjects with EoD had significantly higher peak duodenal eosinophil counts ($P < .001$). Ages ranged from 3–59 years, with 21 pediatric (63%) and 6 adult (37%) subjects. Approximately 52% were male, and most subjects self-identified as White (93%). Peak duodenal eosinophil counts ranged from 15–129 eosinophils/HPF (EoD 60–129, DE 31–47, NL 15–27 eosinophils/HPF). There were not any significant differences in the gastrointestinal symptoms among all 3 groups (EoD, DE, NL).

Among subjects with EoD, about half of subjects had concurrent eosinophilia in the esophagus, stomach, and/or colon (50%, 50%, and 25%, respectively). As for the endoscopic findings, normal appearance (50%) was the most common finding, followed by erythema (38%), erosion (13%), and granularity (13%).

Identification of EoD transcriptome

First, we employed stringent diagnostic criteria ($>2X$ the normal number of mucosal eosinophils in duodenum [52 eosinophils/HPF in 1 HPF])¹⁵ to identify patients with EoD. We generated an RNA sequencing data set from duodenal tissue of EoD (n = 8) and NL (n = 8) subjects and

compared gene expression. We identified 382 differentially dysregulated genes in EoD versus NL biopsies (≥ 2 -fold change, FDR $P < .05$) (Fig 1, A). Unsupervised clustering analysis showed separation between EoD and NL (Fig 1, B). Of these gene signatures (e.g., EoD transcriptome), 256 transcripts were upregulated and 126 were downregulated in EoD compared to NL (see Table E2 in this article's Online Repository at www.jacionline.org). Eighteen genes were modified at least 5 fold in the EoD compared with the NL group, including eosinophil-related (*CCL26*, *CLC*), IL-13-inducible (*CCL26*, *CCL18*, *DUOX2*, *POSTN*, *ITLN1*), and duodenum-specific (*BTNL8*, *BEST4*, *MS4A18*) genes (Fig 1, C). Notably, despite clinical heterogeneity, there were no substantial molecular differences in several comparisons, such as EoD with coexisting EoE vs. EoD alone, pediatric vs. adult subjects, subjects with atopy vs. without atopy, and subjects with treated vs. untreated EoD (see Fig E1, in this article's Online Repository at www.jacionline.org).

EoD transcriptome associates with duodenal eosinophilia

We next evaluated whether the EoD transcriptome would associate with duodenal eosinophil levels. The peak duodenal eosinophil count correlated with 224 genes (59% of the EoD transcriptome), most notably with the expression of the eosinophil chemoattractant gene C-C motif chemokine ligand 26 (*CCL26*, $r = 0.80$, $P < .001$) and the eosinophil-specific gene Charcot-Leyden crystal (*CLC*, $r = 0.77$, $P < .001$) (Fig 2, A; and see Table E2 in this article's Online Repository at www.jacionline.org).

To assess the EoD transcriptome as a function of disease severity, we examined whether the magnitude of modified genes might be directly related to histologic severity. Among the groups (NL, DE, EoD), the EoD transcriptome showed a notable linear relationship according to tissue

eosinophil levels, illustrated by the heat diagram (Fig 2, B). Furthermore, principal component analysis (PCA) demonstrated separation of EoD from NL and DE (Fig 2, C). Consistently, representative genes in EGID (e.g., *CCL26*, *CLC*, *CCL11*, *CPA3*, and *POSTN*) also showed similar trends, although there was some variability among subjects (Fig 2, D). Defining EoD by at least 30 eosinophils/HPF in 3HPFs, as recently proposed,¹³ did not segregate samples as well as the >52 eosinophils/HPF, as the former criteria resulted in overlap with DE (Fig 2, B–C).

Furthermore, we developed the EoD score on the basis of the EoD transcriptome with the goal of developing quantitative values to reflect molecular changes. The score was calculated by summing the expression levels of 382 genes in the EoD transcriptome (the score increases with disease activity) (Fig 2, E). The EoD score was increased in subjects with EoD compared to subjects with DE and NL, but the score for subjects with DE did not differ from NL (Fig 2, F). We also found that the EoD score was significantly correlated with peak duodenal eosinophil counts and that there was separation of EoD from DE and NL. The highest peak eosinophil level in the DE cohort was 47 eosinophils/HPF, whereas the lowest peak eosinophil level in the EoD cohort was 60 eosinophils/HPF (Fig 2, G). Collectively, the data support that EoD emerges at a threshold between ~50-60 eosinophils/HPF. Taken together, these data suggest that the magnitude of gene changes directly correlated with tissue eosinophil levels. Furthermore, the EoD transcriptome, which distinguishes EoD from DE and NL, encompasses potential diagnostic criteria that are likely to distinguish these groups.

Functions and cell types enriched in the EoD transcriptome

To identify EoD-associated molecular pathways, we performed functional annotation enrichment analyses. The highest enrichments included extracellular matrix and IL-13/IL-4 signaling pathways (involving about 40%) and decreased transporter and metabolic pathways (involving about 70%) (Fig 3, A–B; and see Table E3 in this article’s Online Repository at www.jacionline.org). Upregulated processes of the EoD transcriptome were enriched for extracellular matrix transcripts (FDR $P < .001$), including collagen genes, and IL-13/IL-4 signaling transcripts (FDR $P < .001$), including the CC chemokine genes. Downregulated processes of the EoD transcriptome were enriched for transporter and metabolic transcripts (FDR $P < .001$), including solute carrier family and cytochrome P450 family genes. Consistent with these findings, upstream regulator analysis using IPA indicated that IL-4 and IL-13 were the most significant upstream regulators, followed by IL-6 and IL-5 (see Table E4, in this article’s Online Repository at www.jacionline.org).

We applied a computational gene expression deconvolution approach using xCell²⁴ to further evaluate the relative composition of genes related to immune cell subsets, epithelia, and other stromal cell types in EoD. Of the 64 cell types represented by gene expression, several immune cells were specifically increased in EoD, but not DE, including eosinophils, mast cells, and myeloid and granulocyte progenitor cells (Fig 3, C).

Duodenal histologic features and transcript association with disease

All subjects with EoD showed marked, though uneven, duodenal eosinophilic inflammation (Fig 4, A) even within the same biopsy, similar to the uneven esophageal and gastric eosinophilic inflammation of the other upper tract EGIDs, EoE and EoG. Assessing the relationships among

the duodenal histologic features, we generated a correlation plot with clustering arrangement (Fig 4, B). There were strong correlations for inflammatory and structural changes; the most significant was between lamina propria eosinophil sheets and reactive epithelial changes ($r = 0.79$, $P < .001$). Also, there were significant correlations within eosinophilic features, the most significant being between pericryptal circumferential eosinophil collars and lamina propria eosinophil sheets ($r = 0.72$, $P < .001$). Acute inflammatory cells that are common in diseases, such as infection, peptic duodenitis, and injury due to non-steroidal anti-inflammatory drugs, had low correlations with other histologic features.

Notably, among the histologic features, 6 features showed differences among EoD, DE, and NL; lamina propria eosinophil sheets, pericryptal circumferential eosinophil collars, eosinophilic cryptitis, eosinophils in surface epithelium, reactive epithelial changes, and eosinophils in muscularis were significantly increased in EoD compared with DE or NL (Fig 4, C).

Further dissecting the molecular basis for duodenal histopathology in EoD, we evaluated associations between the EoD transcriptome and histologic features at the gene level (Fig 4, D; and see Table E5 in this article's Online Repository at www.jacionline.org). We observed that histologic features commonly observed in EoD biopsies, such as lamina propria eosinophil sheets, eosinophilic cryptitis, and reactive epithelial changes, highly correlated with the EoD transcriptome (Fig 4, D). Genes associated with each major histologic feature ($r \geq 0.5$) showed enrichment in several molecular functions (see Table E6, in this article's Online Repository at www.jacionline.org), including phosphoenolpyruvate carboxykinase activity for genes associated with lamina propria eosinophil sheets ($P = 1.23E-05$), inorganic anion transmembrane transporter

activity for genes associated with reactive epithelial changes ($P = 1.51E-04$), extracellular matrix structural constituent for genes associated with pericryptal circumferential collars ($P = 1.36E-06$), and signaling receptor binding for genes associated with subcryptal eosinophilic inflammation ($P = 6.49E-05$). Overall, EoD had unique pathogenic gene sets and associated histologic manifestations, suggesting the potential clinical utility of these features.

EoD transcriptome distinguishes EoD from other EGIDs

To determine the relationship between EoD and other EGIDs, we compared the EoD duodenal transcriptome with the previously published EoE esophageal, EoG gastric, and EoC colonic transcriptomes (Fig 5, A; and see Table E7 in this article's Online Repository at www.jacionline.org). There were three common genes among all four EGID subtypes, *ALOX5AP*, *GAPT*, and *CLC*, which were all upregulated. Some upregulated genes in the EoD transcriptome modestly overlapped with upregulated genes in the EoE and EoG transcriptomes, whereas upregulated genes in the EoD transcriptome overlapped to a lesser degree with upregulated genes in the EoC transcriptome (Fig 5, B, *left*), suggesting that EoE and EoG share part of the inflammatory process with EoD. Downregulated genes in the EoD transcriptome had minimal overlap with downregulated genes in other EGID transcriptomes (Fig 5, B, *right*).

Given the tissue specificity in EGID, we were also interested in intersecting EGID transcriptomes with the tissue-specific genes that confer molecular identity to individual tissues, obtained from the Human Protein Atlas,²⁵ including the esophagus, stomach, duodenum, and colon. We observed the significant enrichment of duodenum-specific genes in the genes that were downregulated in the EoD transcriptome (Fig 5, C). Of note, functional enrichment analysis showed that the brush

border was most profoundly decreased in the duodenum. In addition to the significant enrichment of esophagus-specific genes in the EoE downregulated transcriptome that we previously reported,²⁸ we also observed moderate enrichment for stomach-specific genes in the EoG downregulated transcriptome and no enrichment for colon-specific genes in the EoC downregulated transcriptome (Fig 5, C). These data suggest that a profound decrease of duodenal tissue-specific genes is an integral and specific part of the pathophysiology of EoD and that loss of tissue identity by downregulation of tissue-specific genes, as evidenced in EoE, EoG, and EoD, may represent a common pathogenic process in upper EGID and partially explain the tissue specificity and frequent co-morbidity of EGID.

To further highlight the similarities and differences among EGIDs, we assessed the expression levels of several cytokines/chemokines associated with type 1-, type 2-, TH17-, natural killer (NK) cell-, regulatory T cell-, and eosinophil-related molecules using RT-qPCR of patient biopsies from NL and EGID samples. Expression of type 2 cytokines (e.g., *IL13*, *IL4*, *IL5*) was increased in subjects with EoE, EoG, and EoD, but not EoC, although there was substantial heterogeneity. In contrast, the expression of type 1-, TH17-, NK cell-, and regulatory T cell-related molecules (e.g., *IFNG*, *IL17A*, *IL15*, *IL10*) among all EGIDs was not significantly increased compared with that of the NL control subjects. Comparison of eosinophil chemoattractants showed that *CCL26* is likely to be a chemotactic factor for EoE and EoG (upper GI EGIDs), *CCL11* for EoC (lower GI EGID), and both *CCL26* and *CCL11* for EoD (Fig 5, D).

EoD transcriptome distinguishes EoD from celiac disease

Finally, to determine whether the EoD transcriptome differentiates EoD from other GI disease involving the duodenum, we extracted the previously published transcriptomes of duodenal biopsies of celiac disease (403 differentially expressed genes commonly found in ≥ 2 of 3 separate studies).²⁹⁻³¹ We manually compared all the genes in these transcriptomes (EoD vs. celiac) according to their dysregulation (upregulation or downregulation). As shown in the Venn diagram, the duodenal EoD transcriptome was distinct from the duodenal celiac disease transcriptome at the gene expression level for upregulated and downregulated genes (Fig 6, A; and see Table E8 in this article's Online Repository at www.jacionline.org). At the pathway level, the enriched epithelial and immune-associated pathways did not overlap, whereas the repressed duodenal metabolic processes and transporter activity were commonly observed in both diseases (Fig 6, B).

DISCUSSION

Herein, we provide fundamental information concerning the molecular characteristics of EoD using genome-wide duodenal expression profiling and provide insight into disease diagnosis and pathogenesis. First, we defined the EoD transcriptome, a core set of 382 genes conserved in duodenal sites across patients with EoD that robustly distinguishes patients from control subjects. Second, we observed that the EoD transcriptome associates with duodenal eosinophilia and demonstrates a generally linear relationship with duodenal eosinophil levels, with distinct clustering of EoD and DE that is greatest for peak eosinophil levels exceeding ~50-60 eosinophils/HPF. Third, we identified potential, key operational pathways in EoD pathogenesis, establishing EoD as a type 2 immune response. Furthermore, we provide evidence for the involvement of extracellular matrix, transporter activity, and metabolic pathways, which deserve further attention. Fourth, we found that the magnitude of molecular changes associates with specific histologic changes. Strong correlations with the EoD transcriptome were observed for lamina propria eosinophil sheets, providing a better understanding of histologic features of clinical biopsies. Finally, we uncover the molecular relationships of EoD to other EGIDs and celiac disease, providing a rationale for shared and unique diagnostic applications and therapeutic intervention strategies.

Using RNA sequencing, we identified 382 differentially expressed genes in EoD versus controls. The most highly upregulated genes in the EoD transcriptome were dual-oxidase 2 (*DUOX2*, 25.4 fold), C-C motif chemokine ligand 26 (*CCL26*, 18.5 fold), Charcot-Leyden crystal (*CLC*, 11.3 fold), and C-C motif chemokine ligand 18 (*CCL18*, 8.8 fold), a ligand for the TH2 cell chemokine receptor (CCR8).³² Although the highly upregulated genes in EoD included those previously found

in other EGIDs, most of the highly downregulated genes in EoD were exclusive to the duodenum. Of note, such downregulated genes [e.g., butyrophilin-like 8 (*BTNL8*, -11.6 fold), bestrophin 4 (*BEST4*, -5.5 fold), membrane spanning 4-domains A18 (*MS4A18*, -5.4 fold)] were specifically expressed in the duodenum (data available at the human protein atlas <https://www.proteinatlas.org/>),²⁵ suggesting that a profound loss of duodenal tissue identity is an integral and specific part of the pathophysiology of EoD. Importantly, along with *CLC* (eosinophil-specific gene), our study identified that both *CCL11* and *CCL26* were highly upregulated in human EoD tissue compared with human control tissue and exhibited significant positive correlation with duodenal tissue eosinophil counts. Given the role of these chemotactic factors in regulating CCR3-expressing cell responses (e.g., eosinophil),³³ their upregulation could account for eosinophil recruitment to the duodenal tissue. Interestingly, the main eosinophil-related chemotactic factor(s) for EoE and EoG was *CCL26*, EoD were *CCL26* and *CCL11*, and EoC was *CCL11*, suggesting differential chemotactic regulation by GI location for eosinophils in the GI tract.

Notably, the EoD transcriptome associates with duodenal eosinophilia, which may aid in diagnosis of EoD, particularly in individuals with intermediate eosinophil levels. This study was not intended to replace the histologic method but rather to provide mechanistic insights into EoD and a supportive platform to more precisely detect EoD. Currently, standardized diagnostic guidelines for non-EoE EGIDs are needed but do not exist.⁴ Under-recognition and substantial diagnostic delays have been reported in individuals with EoD.^{6, 34} Although multiple factors may contribute to a missed/delayed diagnosis of EoD, the differential diagnosis solely based on increased eosinophil density in duodenal mucosa might be clinically problematic because duodenal eosinophils are present during homeostasis, unlike esophageal eosinophils that are not present

during homeostasis. The threshold of ≥ 30 eosinophils/HPF (in ≥ 3 duodenal HPFs) for EoD clinical trials is supported by a recent phase 2 study.^{13, 35, 36} However, a phase 3 study using that eosinophil threshold failed to meet symptom endpoints.³⁷ Several studies reported similar levels of duodenal eosinophil levels in individuals with other GI diseases, namely celiac disease (mean 23.3 ± 9.3 eosinophils/HPF, in 1 duodenal HPF)¹⁰ and functional dyspepsia (multiple studies, mean ranges 5–42 eosinophils/HPF, in ≥ 3 duodenal HPFs).¹¹ Therefore, we stratified subjects to aid in the exploration of a threshold value for peak eosinophil count for diagnosis of EoD. The study definition of EoD in this study was applied for balanced feasibility and accuracy. We took into consideration that 30 eosinophils/HPF may be too close to normal to observe a distinct difference from EoD but was an important intermediary range and so designated 30–52 eosinophils/HPF as DE. We hypothesized that >52 eosinophils/HPF likely would yield distinct differences from normal and provide a clinically useful threshold value.^{9, 19} For clinical practice, a threshold value that is a single peak count is more practical than a criterion that requires a series of numbers in ≥ 3 HPF to be provided by the responsible pathologist. Defining EoD by other criteria was not a goal of our study; rather, we aimed to determine if a simplified peak eosinophil count (limited to 1 HPF) was sufficient for the development of a distinct molecular signature. In our study, EoD and DE were both defined by 1 HPF, and principal component analysis was able to distinguish the study cohorts, most notably the EoD group, from the others (Fig 2, C). DE separated from NL with some overlap (Fig 2, C) that was not seen with EoD. It is notable that defining EoD by at least 30 eosinophils/HPF in 3HPFs did not segregate samples as well as the >52 eosinophils/HPF, as the former criteria resulted in overlap with DE. The transcriptome score showed a roughly linear relationship with eosinophil levels, suggesting a gradation effect. Our current findings highlight a contrast and transition of the EoD transcriptome among the groups (NL, DE, EoD) according to

tissue eosinophil levels, with EoD emerging between 50-60 eosinophils/HPF. A narrowing of this threshold may be accomplished when an increased cohort size is studied but relying strictly on eosinophil levels may be an oversimplification. Taken together, our data provide a rationale for using only 1 HPF for diagnosis and substantiate the potential value of the EoD score, similar to the value of the EoE Diagnostic Panel.³⁸ These findings provide supportive evidence to begin defining specific clinicopathologic consensus diagnostic criteria in EoD. Whether individuals with intermediate duodenal eosinophil levels having 30–52 eosinophils/HPF should be considered as having non-EoD or a milder phenotype of EoD deserves further study; molecular characterization via the EoD transcriptome offers potential insight in this endeavor.

Comparing the transcriptomes of EoD, other EGIDs, and celiac disease, we establish EoD as part of the spectrum of upper EGID (joining EoE and EoG) and as distinct from EoC and celiac disease. The EoD transcriptome showed evidence of increased immune-related pathway activation and was enriched in inflammatory processes and cytokine and chemokine activity, especially with IL-4 and IL-13 being strong potential upstream regulators. Furthermore, significant increases in *IL5* and *IL13* expression were observed in EoD (Fig 5D). Comparing the EoD disease signature with previously published disease signatures of other EGIDs,¹⁶⁻¹⁸ the upper GI tract (e.g., EoE and EoG) but not the lower GI tract (EoC) showed similar upregulated pathways. Our data indicate that EoD is likely mediated by excessive type 2 immunity and imply that food antigens are driving the disease like EoE and EoG. Notably, we identified that genes associated with reduced metabolic/transporter function were uniquely enriched in the EoD transcriptome compared to those of other EGIDs. These identified shared and distinct pathways are consistent with the clinical

494 presentation of EoD because a subset of patients with EoD has anemia, hypoalbuminemia, and
495 malabsorption.^{8, 39}

496
497 Considering the observed tissue specificity of the downregulated portion of the EoD transcriptome
498 relative to other EGID transcriptomes, we compared gene expression between EoD and celiac
499 disease, a relatively common inflammatory disease involving the duodenum. Although there was
500 no substantial overlap at the individual gene expression level between EoD and celiac disease, we
501 demonstrated common repressed duodenal metabolic processes and transporter activity. Given the
502 shared clinical presentation (e.g., anemia, malnutrition, diarrhea, protein-losing enteropathy),^{8, 39}
503 the impairment of these functions suggests a link between clinical symptoms and tissue-specific
504 molecular dysfunction. In contrast, the EoD and celiac transcriptomes substantially diverged for
505 the upregulated portion of the EoD transcriptome; EoD had upregulated epithelial and immune-
506 associated pathways and an intensified signature linked to type 2 immunity and extracellular
507 matrix pathways, whereas celiac disease had upregulated pathways related to type 1 immunity.

508
509 We found that the duodenal histologic features highly correlated with the distinct molecular profile
510 of EoD (Fig 4, D; and see Table E5–6 in this article's Online Repository at www.jacionline.org).
511 For instance, reactive epithelial changes correlated with a series of downregulated duodenum-
512 specific genes, including *KCNK10*, *HACL1*, *BEST4*, and *TMEM229A*, further supporting the
513 importance of duodenal tissue identity loss in EoD (Fig 5, C). Also, multiple eosinophilic features
514 correlated with certain upregulated genes, including *CCL26* and *CLC*. Although the precise role
515 for most of these genes are still unknown, some (e.g., *UTS2*, *VSTM1*, *ITLN1*) might have potential
516 relevance: *UTS2* encodes urotensin II, which is known as a ligand for GPR14 and is reported to be

elevated in asthmatic lung and involved airway remodeling.⁴⁰ A recent study also showed elevated levels of urotensin II in the serum of patients with IBD.⁴¹ *VSTM1* is one of the genes in the eosinophilic gene expression networks associated with IgE in two distinct asthma populations.⁴² Finally, human *ITLN1* is induced during intestinal parasitemia and related type 2 immune responses, including eosinophilic airway inflammation.^{43, 44} Of the EoD histologic features, eosinophilic features were highly associated with the EoD transcriptome (Fig 4, D), with the strongest associations being lamina propria eosinophil sheets and cryptitis, warranting close attention to these altered eosinophil distributions when interpreting disease diagnosis and activity.

In addition to having increased eosinophil-related gene expression, the duodenum of individuals with EoD has increased mast cell-related gene expression (Fig 2, D and Fig 3, C). A moderate correlation between duodenal eosinophils and mast cells has been recently reported.^{35, 36} Although there have been limited prior studies assessing mast cells and their associations with non-EoE EGIDs, the duodenal mast cells involved in individuals with EoD may have unique characteristics, such as expressing high levels of *CPA3* (mast cell-specific gene over-expressed in EoD), which is consistent with mast cell phenotypes in individuals with EoE.^{45, 46}

Although this study has provided substantial progress in understanding molecular profiles in EoD, there are several limitations. First, the small sample size and inevitable heterogeneity (age, gender, comorbidity of atopy, comorbidity of other EGIDs, concurrent medications) of individuals with EoD due to the rarity of the disease limited the generalization. However, the stringent criteria that we used for EoD (>52 eosinophils/HPF, based on 2X the upper limit of normal biopsies)¹⁵ likely provided a clearer molecular understanding of this complicated condition. Second, our findings

included individuals with mixed treatments; however, because patients still exhibited signs of active disease, the key molecular pathways involved in pathogenesis were likely still active, at least partially. Third, the analyses were performed using bulk biopsies rather than single-cell separation. Additional replication, particularly in prospective and longitudinal studies and including a larger sample size from multiple centers, are warranted to examine the utility of gene signatures to diagnose, monitor, and potentially further subtype (e.g., endotype) EoD.

In conclusion, we defined the core duodenal EoD transcriptome. We reported seminal observations about the disease, including (1) establishing EoD as part of a spectrum of upper EGID (EoE, EoG) and distinct from EoC and celiac disease; (2) a linear relationship between the magnitude of EoD transcriptomic changes and the duodenal eosinophil levels even in patients designated as DE; (3) a potential diagnostic threshold of 50-60 peak eosinophils/HPF using only 1 HPF for clinical diagnosis and research purposes; (4) robust functional enrichment of type 2 immunity and extracellular matrix pathways (shared with other EGID) and reduced transporter activity and metabolic pathways (shared with celiac disease); (5) a profound loss of duodenal tissue identity, paralleling the tissue identity loss seen in upper EGID (EoE, EoG); and (6) histologic changes that correlated with distinct molecular profiles. These collective findings provide a basis for improving diagnosis and treatment.

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Table 1. Demographic and clinical characteristics of study subjects

	NL	DE	EoD	<i>P</i> value*
Subjects (n)	8	11	8	
Demographic features				
Age at biopsy [median (IQR) years]	11.6 (8.6–15.2)	12.7 (3.6–14.3)	17.3 (9.5–22.3)	.292
Range (min.–max. years)	6–59	3–54	4–38	
Gender (% Male)	3 (34%)	6 (55%)	5 (63%)	.590
Race (% White)	8 (100%)	9 (82%)	8 (100%)	.534
Duodenal eosinophil counts				
Peak [median (IQR) eosinophils/HPF]	20.5 (16.8–25.5)	35.0 (32.0–39.0)	95.0 (71.5–114.0)	<.001
Range (min.–max. eosinophils/HPF)	15–27	31–47	60–129	
Active EGID at biopsy				
EoE	0 (0%)	6 (55%)	4 (50%)	.035
(≥15 eosinophils/HPF, 1 esophageal HPF)				
EoG	0 (0%)	4 (36%)	4 (50%)	.074
(≥30 eosinophils/HPF, 5 gastric HPF)				
EoD	0 (0%)	0 (0%)	8 (100%)	<.001
(>52 eosinophils/HPF, 1 duodenal HPF)				
EoC	0 (0%)	0 (0%)	2 (25%)	.077
(≥65 eosinophils/HPF, 1 colonic HPF)				
History of atopy				
Asthma	2 (25%)	4 (36%)	4 (50%)	.584
Allergic rhinitis	4 (50%)	7 (64%)	4 (50%)	.782
Eczema	2 (25%)	7 (64%)	1 (13%)	.052
Food allergy	0 (0%)	2 (18%)	0 (0%)	.208
Treatment at biopsy				
Ongoing diet therapy	1 (13%)	6 (55%)	4 (50%)	.150
Proton pump inhibitor	5 (63%)	6 (55%)	4 (50%)	.878
Topical steroids	0 (0%)	0 (0%)	2 (25%)	.077
Systemic steroids	0 (0%)	0 (0%)	1 (13%)	.291
Symptoms at biopsy				
Abdominal pain	5 (63%)	5 (46%)	2 (25%)	.319
Nausea	1 (13%)	0 (0%)	1 (13%)	.476
Vomiting	4 (50%)	4 (36%)	4 (50%)	.782
Regurgitation	2 (25%)	2 (18%)	1 (13%)	.812
Weight loss	1 (13%)	1 (9%)	1 (13%)	.962
Eating problems	0 (0%)	3 (27%)	1 (13%)	.249
Diarrhea	1 (13%)	0 (0%)	2 (25%)	.228
Food impaction	0 (0%)	1 (9%)	0 (0%)	.470

**P* values are based on comparison among all 3 groups using the chi-square test or the Kruskal-Wallis test. EoD, eosinophilic duodenitis; NL, normal; DE, duodenal eosinophilia; EoE, eosinophilic esophagitis; EoG, eosinophilic gastritis; EoC, eosinophilic colitis; EGID, eosinophilic gastrointestinal disease; HPF, high-power microscopic field (0.27 mm² circular field); max., maximum; min., minimum.

FIGURE LEGENDS

FIG 1. A conserved pattern of gene expression from duodenal tissue of subjects with EoD. **A**, Clustering analysis based on 382 differentially expressed genes (EoD transcriptome). Heat map (*red*, upregulated; *blue*, downregulated) of expression profiles of differentially dysregulated genes between NL and subjects with EoD (FDR $P < .05$, ≥ 1.5 -fold change). Each column represents an individual subject (NL $n = 8$, EoD $n = 8$). **B**, Duodenal transcriptome data on NL (*yellow*) and subjects with EoD (*red*) reduced to 3-dimensional presentation by multidimensional scaling analysis for visual presentation of the expression distance between samples. A-B, Arrows and bold circles represent samples that have ≥ 30 eosinophils/HPF in at least 3 HPFs. **C**, The number of genes that exhibit the indicated minimum fold difference in subjects with EoD versus NL subjects is graphed (*left*). Genes with 5-fold or greater change ($n = 18$) are listed (*right*). EoD, eosinophilic duodenitis; NL, normal control; FDR, false-discovery rate; HPF, high-power microscopic field.

FIG 2. EoD transcriptome associates with duodenal eosinophilia. **A**, Peak duodenal eosinophil count and duodenal *CCL26* and *CLC* correlation. **B**, Individual EoD transcriptome expression and peak duodenal eosinophil count (subjects numbered 1-27 by increasing eosinophilia, *upper panel*); dashed lines represent thresholds (*black*; 30 eosinophils/HPF, *red*; 52 eosinophils/HPF). Heat map (*red*, upregulated; *blue*, downregulated) of 382 differentially expressed genes (EoD transcriptome, *lower panel*); columns represent individual subjects (NL $n = 8$, DE $n = 11$, EoD $n = 8$) matched vertically to *upper panel*. **C**, NL (*yellow*), DE (*blue*), and EoD (*red*) duodenal transcriptome data as 3-dimensional presentation. B-C, Arrows and bold circles represent samples that have ≥ 30 eosinophils/HPF in at least 3 HPFs. **D**, Conserved duodenal cytokine gene expression pattern of

EoD vs. NL and/or DE. **E**, EoD score generation schematic summary distinguishing EoD vs. NL and quantifying EoD disease severity. **F**, Peak duodenal eosinophil count (left) and EoD score (right). **G**, Peak duodenal eosinophil count and EoD score correlation. **A-D, F-G**, Circles represent individual subjects. **D, F**, mean $\pm$ SEM. * $P < .05$, ** $P < .01$, *** $P < .001$, and **** $P < .0001$, using Kruskal-Wallis test followed by Dunn multiple-comparison test. EoD, eosinophilic duodenitis; DE, duodenal eosinophilia; NL, normal control; HPF, high-power microscopic field; SEM, standard error of the mean; TPM, transcripts per million kilobases.

FIG 3. Functions and cell types enriched in the EoD transcriptome. **A-B**, Functional annotation enrichment analyses of 256 upregulated (*left*) and 126 downregulated (*right*) genes of the EoD transcriptome using CluGO overview charts (A) and showing the 7 most significant terms in biological process by ToppGene (full list; Table E3, in this article's Online Repository at www.jacionline.org) (B). **C**, Specific increase of gene expression—estimated proportion of cell types in EoD. Data presented as mean $\pm$ SEM. Each circle represents an individual subject or control. * $P < .05$ and ** $P < .01$, using Kruskal-Wallis test followed by Dunn multiple-comparison test. GMP, granulocyte-monocyte progenitor; MEP, megakaryocyte-erythrocyte progenitor; CMP, common myeloid progenitor, EoD, eosinophilic duodenitis; DE, duodenal eosinophilia; NL, normal control; FDR, false-discovery rate; SEM, standard error of the mean.

FIG 4. Duodenal histologic features and associations with duodenal transcripts. **A**, Representative EoD hematoxylin and eosin-stained duodenal biopsy specimens (20X). **B**, Histologic feature clustering in duodenal biopsies with features arranged to ensure that members of the same cluster are adjacent in the correlation plot and in the same order as in the cluster

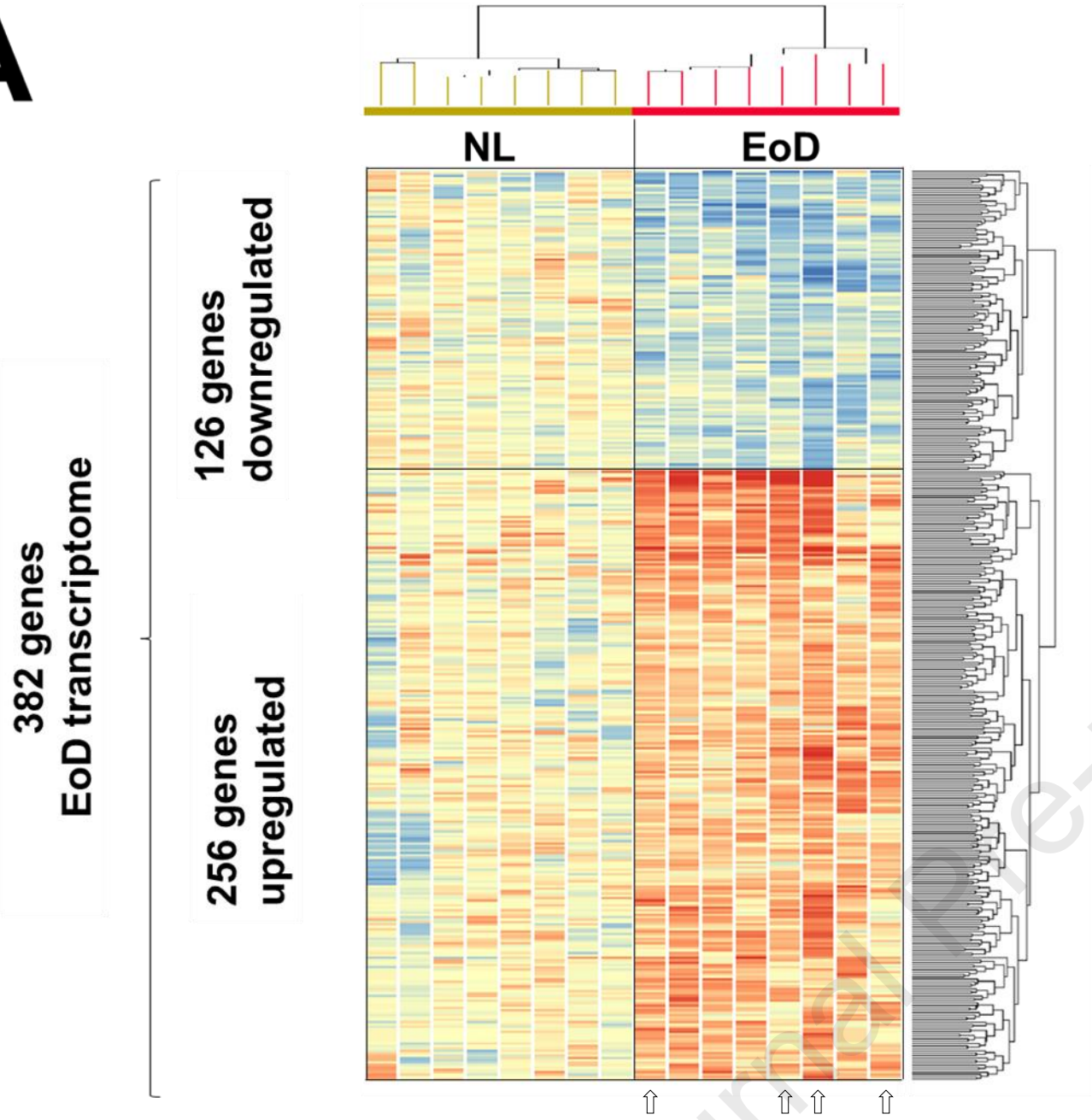
members. Color map shows correlations among histologic features; darker red shades indicate stronger positive correlations. **C**, Comparison of histologic features among NL, DE, and EoD. Data are mean $\pm$ SEM. Each circle represents an individual subject or control. $*P < .05$, $**P < .01$, and $***P < .001$, using Kruskal-Wallis test followed by Dunn multiple-comparison test. **D**, Hierarchic relationships between duodenal histologic features from EoD transcriptome gene expression profile correlations. Spearman r -based heat diagram for gene-level correlations (*upper left*). Darker shades indicate stronger correlations, red for positive and blue for negative. Associations between the EoD score and individual histologic features (*upper right*). Red coloration indicates statistical significance with a $P < .05$ with a Spearman r correlation. Top 10 genes significantly associated with each histologic feature in EoD (*lower*). EoD, eosinophilic duodenitis; DE, duodenal eosinophilia; NL, normal control; FDR, false-discovery rate; SEM, standard error of the mean.

FIG 5. Comparing the EoD transcriptome with other EGID transcriptomes. **A**, Venn diagram analysis of shared genes for upregulated (*left*) and downregulated (*right*) expression among EGID transcriptomes (EoD, EoE, EoG, EoC). **B**, The 50 most upregulated (*left*) and downregulated (*right*) transcripts in the EoD transcriptome relative to NL are given with the corresponding relative fold change in expression in tissue from subjects with EoE, EoG, and EoC relative to NL. **C**, Enrichment analysis of tissue-specific genes in the tissue-matched EGID transcriptome (i.e., esophagus-specific genes in EoE transcriptome). Each pie chart indicates the proportion of tissue-specific genes in the upregulated and downregulated portions of each tissue-matched transcriptome. P values using the Fisher's exact test. **D**, Comparisons of gene expression by RT-qPCR in active EGIDs [esophagus (EoE $n = 147$, NL $n = 48$), stomach (EoG $n = 13$, NL $n = 33$), duodenum (EoD

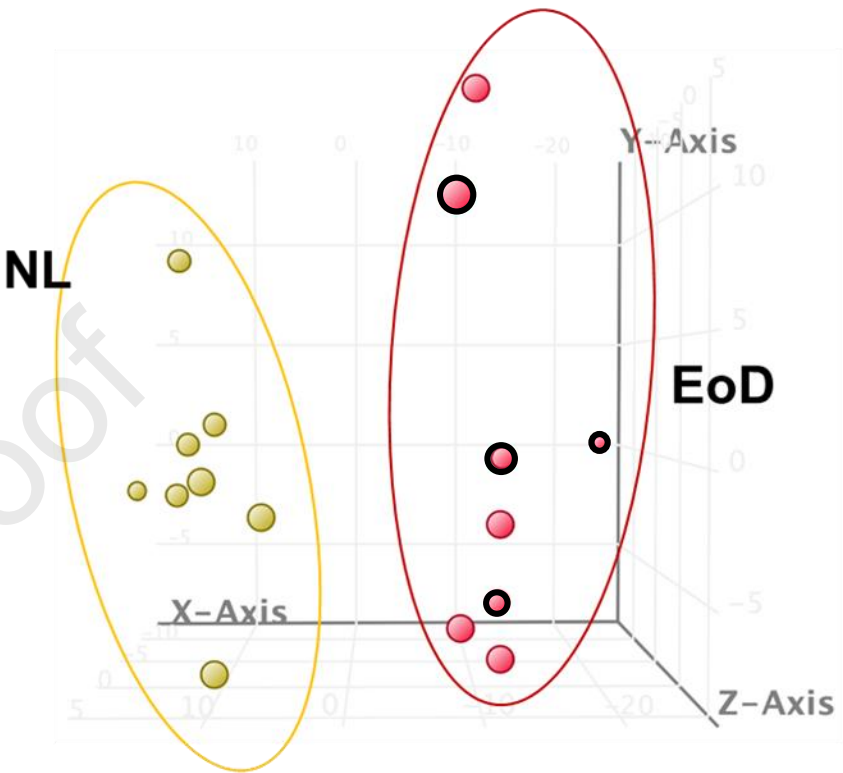
n = 8, NL n = 8), colon (EoC n = 12, NL n = 16)] relative to NL. Data are mean $\pm$ SEM. * $P < .05$ using the Mann–Whitney U test. EoD, eosinophilic duodenitis; NL, normal control; EoE, eosinophilic esophagitis; EoG, eosinophilic gastritis; EoC, eosinophilic colitis; EGIDs, eosinophilic gastrointestinal diseases; SEM, standard error of the mean.

FIG 6. Comparing the EoD transcriptome with celiac disease gene expression. **A**, Venn diagram analysis of shared genes for upregulated (*left*) and downregulated (*right*) expression between EoD and celiac disease [EoD from this study; celiac disease from 403 genes differentially expressed in at least 2 of 3 recent RNAseq transcriptomic studies comparing active celiac disease and controls (PMID: 31700112, 30097691, and 30998704)]. **B**, Comparison of top 20 gene ontology terms related to downregulated expression between EoD and celiac disease. Red bar indicates common terms between EoD and celiac disease. EoD, eosinophilic duodenitis; FDR, false-discovery rate.

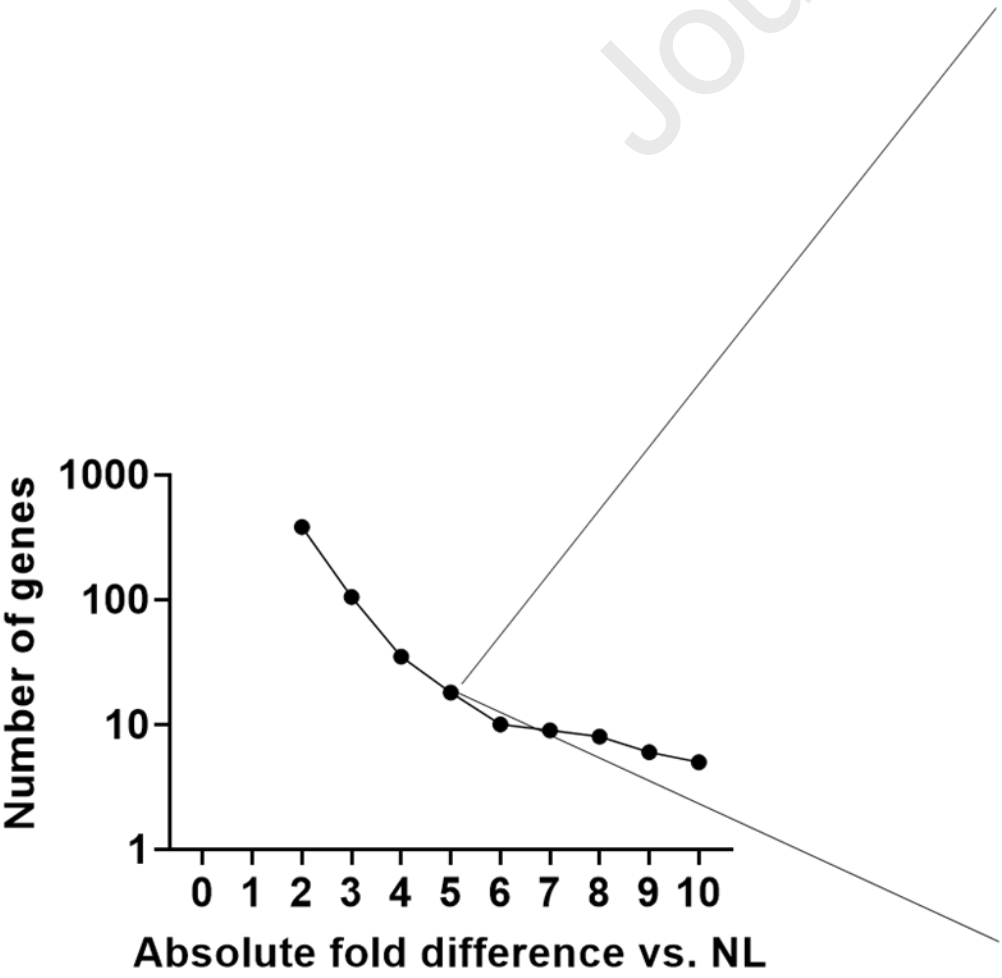
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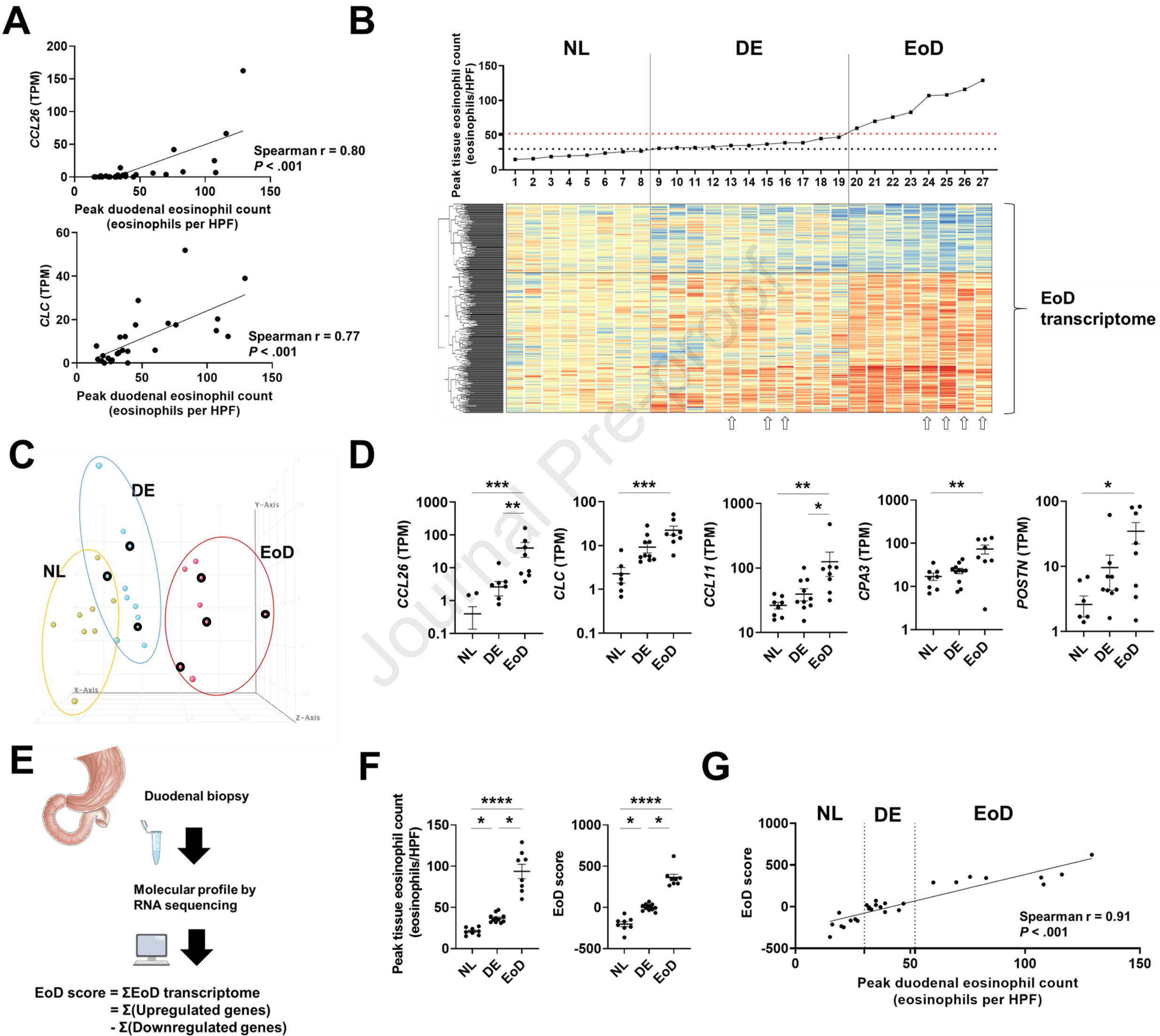
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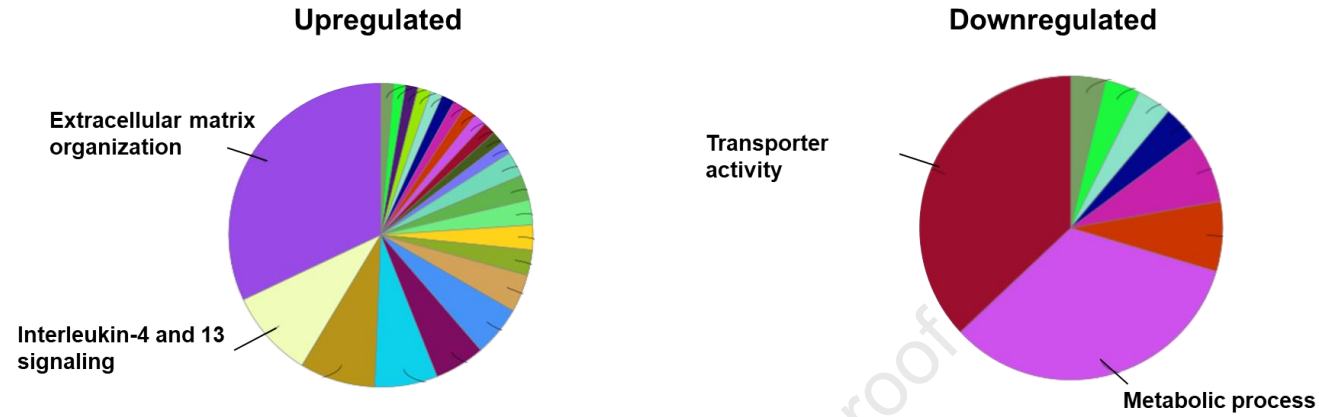
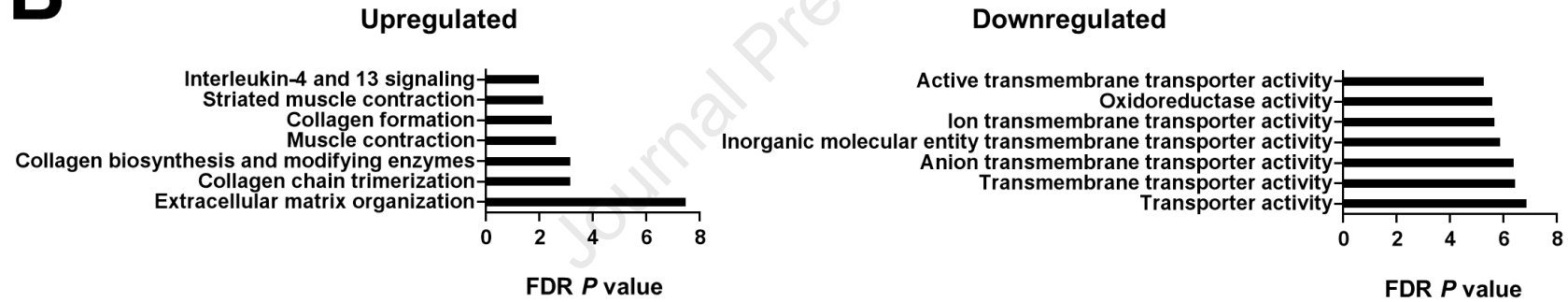
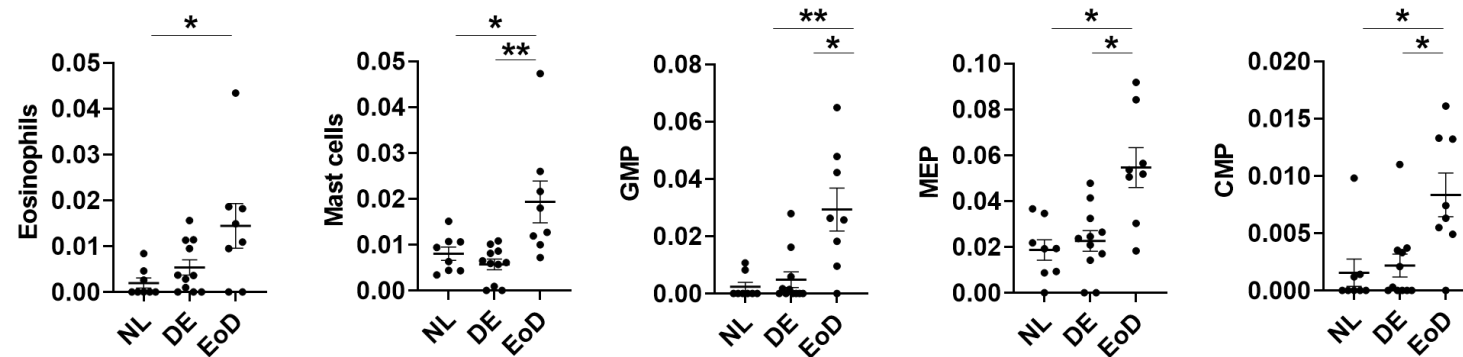


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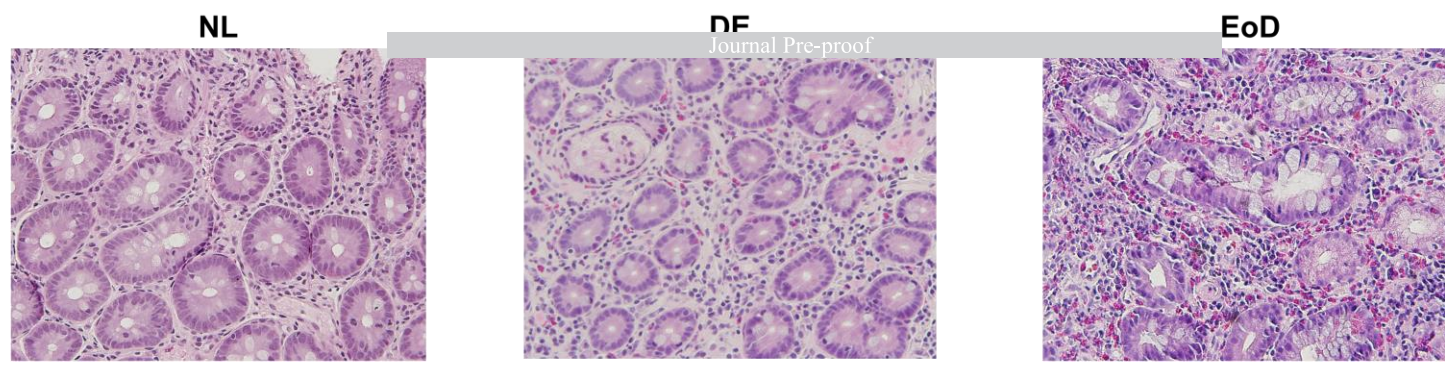


GeneSymbol	Description	Fold change	FDR P value
<i>DUOX2</i>	dual oxidase 2	25.4	0.0169
<i>CCL26</i>	C-C motif chemokine ligand 26	18.5	0.0012
<i>CLC</i>	Charcot-Leyden crystal galectin	11.3	0.0003
<i>CCL18</i>	C-C motif chemokine ligand 18	10.6	0.0272
<i>DUOXA2</i>	dual oxidase maturation factor 2	9.8	0.0251
<i>ANKRD30B</i>	ankyrin repeat domain 30B	8.9	0.0017
<i>POSTN</i>	periostin	8.2	0.0190
<i>ITLN1</i>	intelectin 1	7.4	0.0103
<i>TNXB</i>	tenascin XB	6.0	0.0046
<i>ADGRG3</i>	adhesion G protein-coupled receptor G3	5.9	0.0001
<i>LCN2</i>	lipocalin 2	5.7	0.0251
<i>CTSG</i>	cathepsin G	5.5	0.0190
<i>F13A1</i>	coagulation factor XIII A chain	5.3	0.0218
<i>SLC1A7</i>	solute carrier family 1 member 7	-5.2	0.0086
<i>MS4A18</i>	membrane spanning 4-domains A18	-5.3	0.0214
<i>PLA2G4C</i>	phospholipase A2 group IVC	-5.4	0.0032
<i>BEST4</i>	bestrophin 4	-5.5	0.0008
<i>BTNL8</i>	butyrophilin like 8	-11.6	0.0324

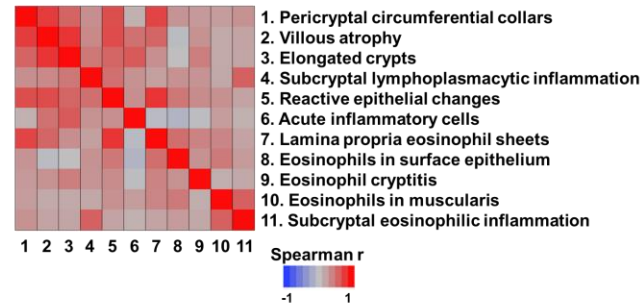


A**B****C**

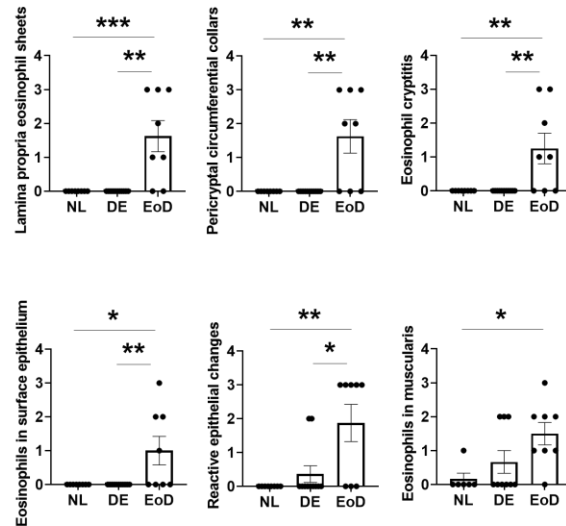
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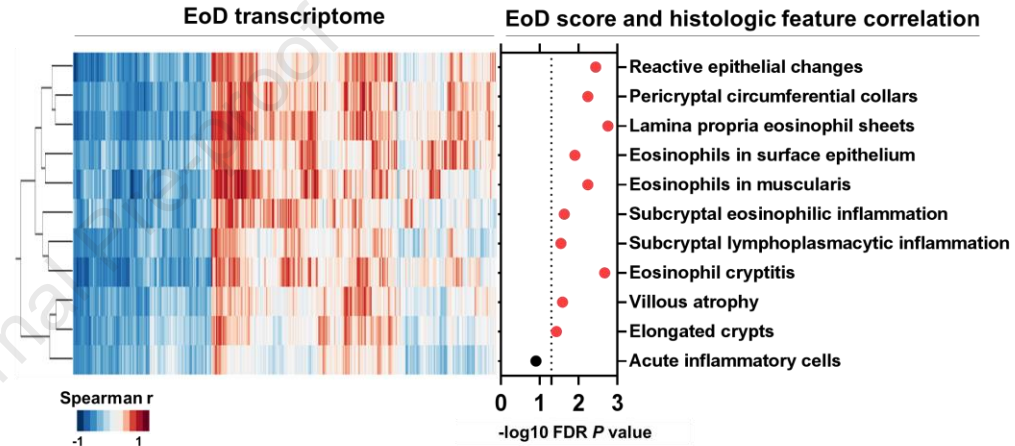
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C

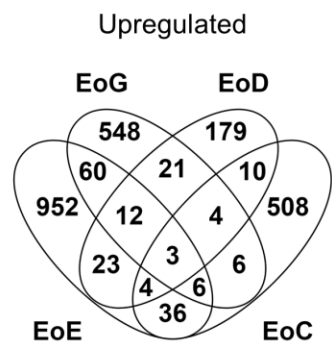


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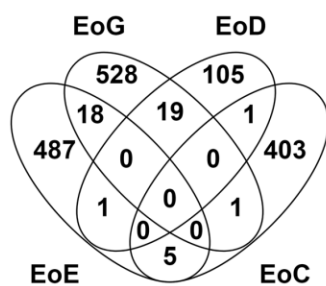


Reactive epithelial changes	Pericryptal circumferential collars	Lamina propria eosinophil sheets	Eosinophils in surface epithelium	Eosinophils in muscularis mucosa/submucosa	Subcryptal eosinophilic inflammation
<i>KCNK10</i>	<i>VSTM1</i>	<i>UTS2</i>	<i>CLEC1A</i>	<i>ITLN1</i>	<i>ANKRD30B</i>
<i>HACL1</i>	<i>NME8</i>	<i>ANKRD30B</i>	<i>BMX</i>	<i>C1QTNF1</i>	<i>JAM2</i>
<i>TNC</i>	<i>AC011511.4</i>	<i>VSTM1</i>	<i>SLC6A16</i>	<i>MS4A18</i>	<i>TNS4</i>
<i>BEST4</i>	<i>DDIAS</i>	<i>CCL26</i>	<i>GPR42</i>	<i>C11orf53</i>	<i>HPCAL1</i>
<i>WDR72</i>	<i>TNC</i>	<i>ITLN1</i>	<i>NBPF10</i>	<i>C15orf48</i>	<i>CCL26</i>
<i>TMEM229A</i>	<i>FAM189A1</i>	<i>TNC</i>	<i>DDIAS</i>	<i>THRB</i>	<i>PDPN</i>
<i>ITLN1</i>	<i>ADGRG3</i>	<i>CPA3</i>	<i>WDR72</i>	<i>DUOX2</i>	<i>POLR3G</i>
<i>SCN3B</i>	<i>WARS2</i>	<i>ADGRG3</i>	<i>FCGR2B</i>	<i>ANKRD30B</i>	<i>LIMS2</i>
<i>TMEM132E</i>	<i>CLC</i>	<i>ACTC1</i>	<i>LGR6</i>	<i>PMP22</i>	<i>RBP5</i>
<i>CPA3</i>	<i>ELN</i>	<i>MUC12</i>	<i>GAPT</i>	<i>TMEM52</i>	<i>VSTM1</i>
Subcryptal lymphoplasmacytic inflammation	Eosinophil cryptitis	Villous atrophy	Elongated crypts	Acute inflammatory cells	
<i>UTS2</i>	<i>TMEM229A</i>	<i>NME8</i>	<i>TREML2</i>	<i>GJB3</i>	
<i>GJB3</i>	<i>UTS2</i>	<i>FAM189A1</i>	<i>DUOX2</i>	<i>TREML2</i>	
<i>TREML2</i>	<i>CCL26</i>	<i>GPR183</i>	<i>ZSCAN2</i>	<i>DUOX2</i>	
<i>COL28A1</i>	<i>TREML2</i>	<i>CCR10</i>	<i>UNC93A</i>	<i>UNC93A</i>	
<i>CTSG</i>	<i>EFNA1</i>	<i>CYP2J2</i>	<i>HACL1</i>	<i>TYMS</i>	
<i>AGT</i>	<i>PCK1</i>	<i>KCNK10</i>	<i>DUOX2</i>	<i>SCN3B</i>	
<i>CD36</i>	<i>CYP3A4</i>	<i>PCK1</i>	<i>LGR6</i>	<i>FPR3</i>	
<i>CROT</i>	<i>MS4A8</i>	<i>ZSCAN2</i>	<i>DCPS</i>	<i>CYP2J2</i>	
<i>GANAB</i>	<i>HTR4</i>	<i>TNC</i>	<i>FN1</i>	<i>HACL1</i>	
<i>HACL1</i>	<i>SLC2A2</i>	<i>ADAMTSL1</i>	<i>TMEM229A</i>	<i>MS4A8</i>	

A



Downregulated

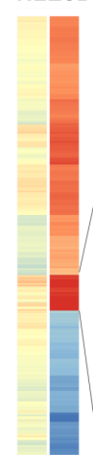


B

Upregulated

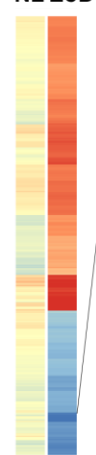
Downregulated

NLEoD



Name	EoD	EoE	EoG	EoC
DUOX2	25.4		15.7	
CCL26	18.5	387.1	3590.9	
CLC	11.3	8.5	37.9	10.2
CCL18	10.6		23.0	
DUOX2	9.8		31.9	
ANKRD30B	8.9			
POSTN	8.2	178.1		
ITLN1	7.4		243.5	
TNXB	6.0			
ADGRG3	5.9		26.8	
LCN2	5.7		4.2	
CTSG	5.5	14.3		
F13A1	5.3	3.8		4.7
C1orf186	5.0			
CTNNA1	4.8			
CISH	4.8	5.6	2.7	
C15orf48	4.8		2.1	2.2
BMX	4.7		12.4	
MXRA7	4.7			
C1QTNF1	4.4	7.4	7.2	
NBPFF10	4.1			
KCTD14	4.1			3.4
POLR3G	4.1			
LGR6	4.1			
HPGD5	4.1	4.6	3.9	
VSTM1	4.0		31.0	
CCL11	3.9		2.2	2.1
KCNQ3	3.9			
SLC39A10	3.8			
TGFB11	3.8			
LYSMD2	3.8			
WDR72	3.8		47.1	
RVR2	3.8			
HTRA3	3.8			
DNAL1	3.7			
CPA3	3.7	59.8	2.6	
THY1	3.7			
ALOX15	3.7	234.4	48.3	
TNS4	3.6	3.5	4.6	
ADCY1	3.6			
FGR	3.6	2.2	2.5	
CCL15-CCL14	3.6			
PDK1L	3.5			
CLDN5	3.5	2.6		
PTGIS	3.5			
ASNS	3.4			
CCL21	3.4			
TNC	3.4		2.8	
IL27RA	3.4	2.7		
TNNC2	3.3			

NL EoD

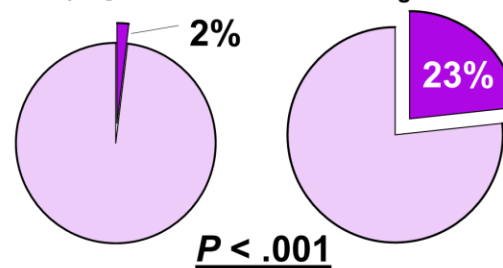


Name	EoD	EoE	EoG	EoC
BTNL8	-11.6			
BEST4	-5.5			
PLA2G4C	-5.4			
MS4A18	-5.3			
SLC1A7	-5.2		-2.8	
TIRAP	-4.4			
FAM184A	-4.2			
SLC23A1	-4.2			
CCZ1B	-4.1			
NELL2	-4.0		-3.0	
GUCA2A	-4.0			
GBP3	-3.9			
SLC2A12	-3.8		-3.1	
CYP3A4	-3.8		-5.8	
KLRK1	-3.7		-2.2	
ENPEP	-3.7			
CYP2J2	-3.6			
THRB	-3.6			
PRKG2	-3.6			
HTR4	-3.5			
SLC27A2	-3.5			
SLC5A11	-3.5			
DAB1	-3.4			
PCK1	-3.3			
GSDME	-3.3			
CYP2C9	-3.3			
SUSD2	-3.3			
DIRA3	-3.3		-3.7	
LYPD8	-3.2			
SCN3B	-3.2			
STX1A	-3.2			
TSPOAP1	-3.1		-2.2	
DRYD	-3.1			
GDPD1	-3.1		-2.0	
TMEM132E	-3.0			
SLC26A3	-2.9			
TUSC3	-2.9		-2.4	
LAMA1	-2.9			
PLCXD2	-2.9			
HIVEP3	-2.8			
RSAD2	-2.8			
C5	-2.8			
PAQR3	-2.8			
DAGLB	-2.8			
TUBE1	-2.7			
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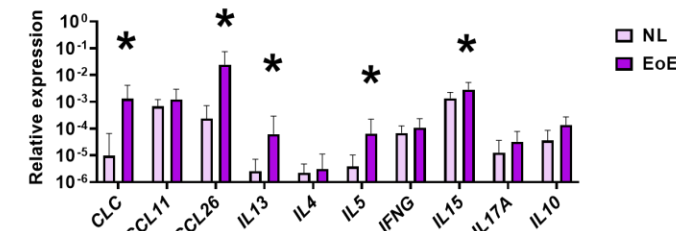
Journal Pre-proof

Upregulated

Downregulated



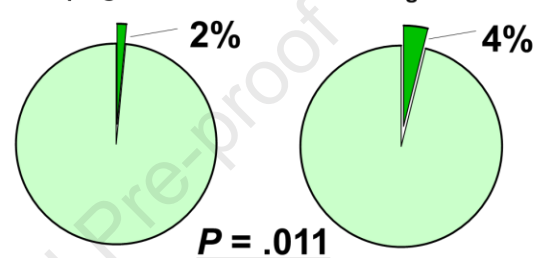
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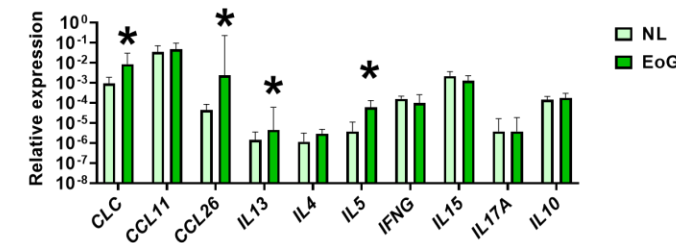
Stomach

Upregulated

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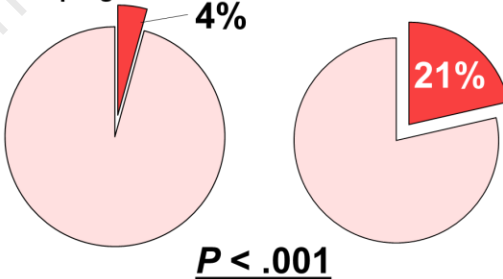
Stomach



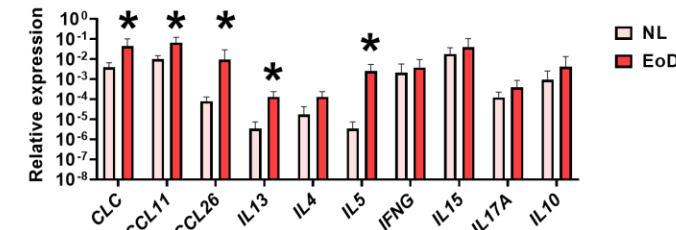
Duodenum

Upregulated

Downregulated



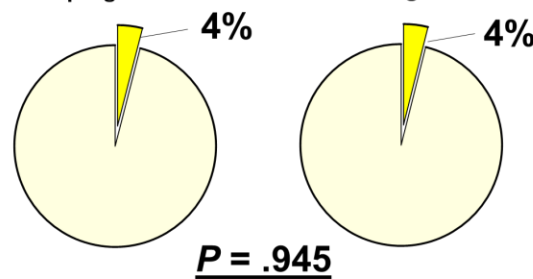
Duodenum



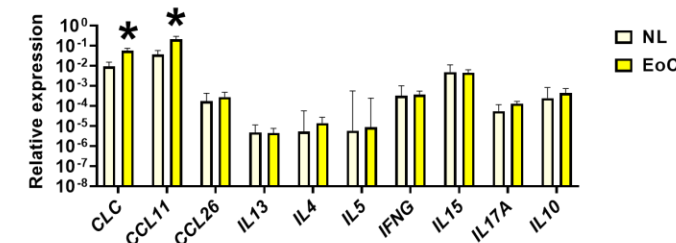
Colon

Upregulated

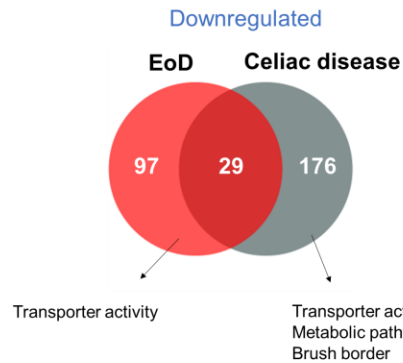
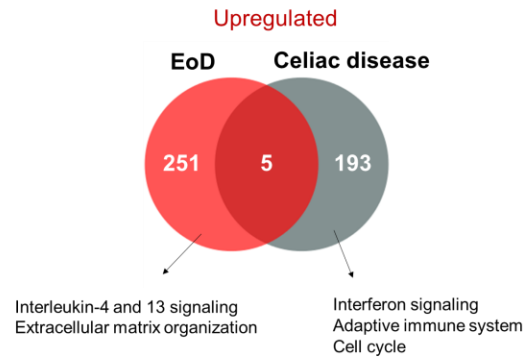
Downregulated



Colon

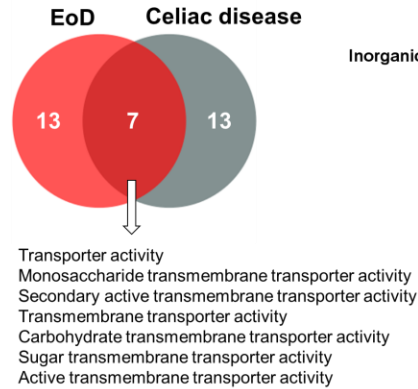


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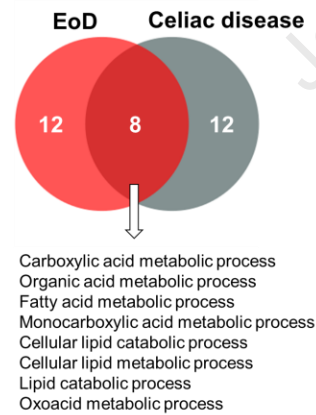


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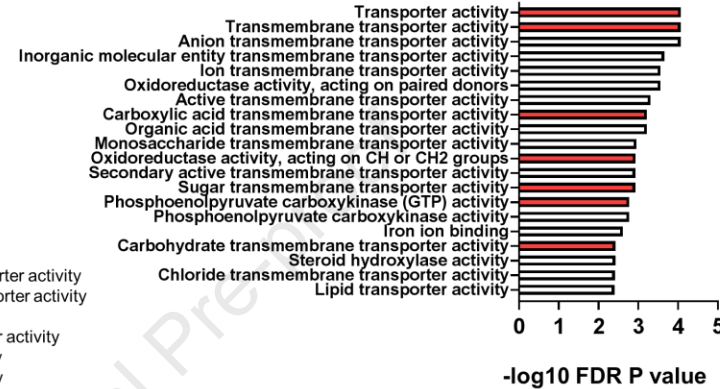
Molecular function



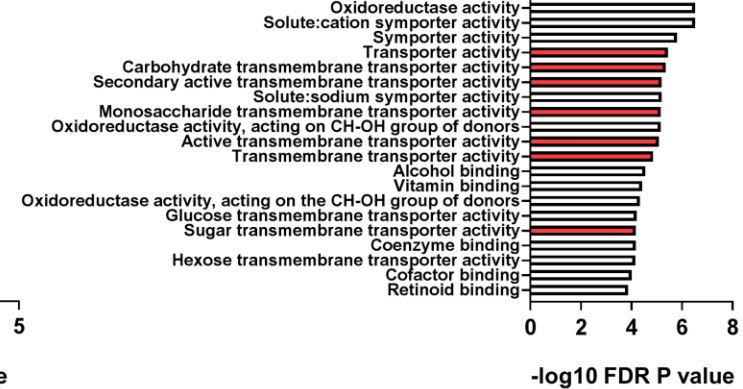
Biological process



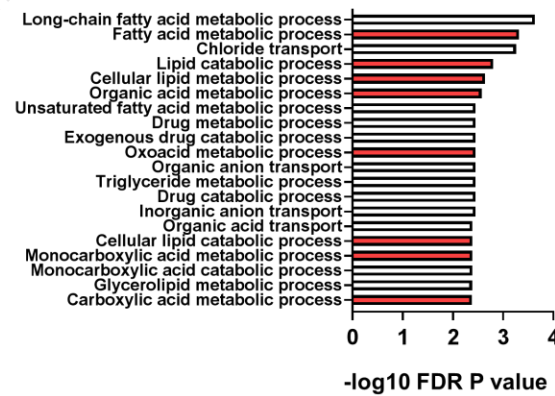
EoD



Celiac disease



EoD



Celiac disease

