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Background: Eosinophilic esophagitis (EoE) is a chronic immune mediated inflammatory disorder of the esophagus. It is still unknown why children and adults present differently, and there is little evidence about why it is more common in men than women.
Objective: Our aim was to synthesize published and unpublished esophageal bulk RNA-sequencing (RNA-seq) data to gain novel insights into the pathobiology of EoE and examine the differences in EoE transcriptome by sex and age group.
Methods: Esophageal bulk RNA-seq data from 5 published and 2 unpublished studies resulting in 137 subjects (EoE: N = 76; controls: N = 61) were analyzed. For overall analysis, combined RNA-seq data of patients with EoE were compared with those of controls and subgroup analysis was conducted in patients with EoE by age of the patient (children [<18 years] vs adults [≥18 years]) and sex (female vs male). Gene-set enrichment analysis, ingenuity pathway analysis (IPA), cell-type analysis, immunohistochemistry, and T-cell or B-cell receptor analysis were performed.
Results: Overall analysis identified dysregulation of new genes in EoE compared with controls. IPA revealed that EoE is characterized by a mixed inflammatory response compared with controls. Cell-type analysis showed that cell composition varied with age: children had more mast cells, whereas adults had more macrophages. Finally, gene-set enrichment analysis and IPA revealed pathways that were differentially regulated in adults versus children and male versus female patients with EoE.
Conclusions: Using a unique approach to analyze bulk RNA-seq data, we found that EoE is characterized by a mixed inflammatory response, and the EoE transcriptome may be influenced by age and sex. These findings enhance insights into the molecular mechanisms of EoE. (J Allergy Clin Immunol 2024;153:759-71.)

Key words: *Eosinophilic esophagitis, transcriptome, esophagus, RNA-seq*

Abbreviations used

BCR:	B-cell receptor
EoE:	Eosinophilic esophagitis
GSEA:	Gene-set enrichment analysis
IHC:	Immunohistochemistry
IPA:	Ingenuity pathway analysis
PPI:	Proton pump inhibitor
RNA-seq:	RNA sequencing
STAT:	Signal transducer and activator of transcription
TCR:	T-cell receptor

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease resulting in esophageal dysfunction.[1,2] Previous esophageal bulk RNA-sequencing (RNA-seq) studies have provided insights into the pathophysiology of EoE. However, these studies individually include a relatively small number of patients with EoE, and this small sample size has limited our ability to understand the influence of biological variables, such as age and sex, on the molecular mechanisms of EoE. As such, fundamental questions in EoE remain unanswered, such as why men develop EoE more frequently than women and why children have different symptoms than adults at presentation. Studying the esophageal transcriptome with a larger sample size may provide clues to some of these questions.

The earliest studies used microarray analysis of esophageal tissue biopsies to understand the pathogenesis of EoE.[3] More recent studies have used bulk RNA-seq,[4-8] which provides an advantage over microarray because it is an unbiased approach to defining the transcriptome.[3] One of these studies found estrogen-responsive genes to be altered in patients with EoE compared with controls and has suggested that estrogen signaling may be protective in the development of EoE.[4] Another study found that IFN-α– and IFN-γ–responsive genes are upregulated

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TABLE I. Study details*

Data set name	GEO identifier	RNA source material	RNA extraction	Library prep	Read type, length, read target no.	Platform
Choksi	GSE242373	Biopsies frozen in RNAlater (Thermofisher, Waltham, Mass) at −80°C	Trizol (Life Technologies, Carlsbad, Calif) followed by RNeasy Mini Kit with on-column DNase treatment (Qiagen, Hilden, Germany)	NEB Library preparation kit (NEBNext Poly(A) selection)	PE, 150 bp, 50 million	Illumina NovaSeq6000 (Illumina, San Diego, Calif)
Greuter et al[8]	GSE148381	FFPE biopsies	Maxwell RSC RNA FFPE Kit (Promega, Madison, Wis)	SMARTer Stranded Total RNA-Seq Kit v2–Pico Input Mammalian (Takara Bio USA, Inc, San Jose, Calif)	PE, 50 bp, NA	Illumina NovaSeq6000
Hiremath	GSE242373	Biopsies frozen in RNAlater at −80°C	Trizol (Life Technologies) followed by RNeasy Mini Kit with on-column DNase treatment (Qiagen)	NEB Library preparation kit (NEBNext Poly(A) selection)	PE, 150 bp, 50 million	Illumina NovaSeq6000
Menard-Katcher[10]	GSE197702	Biopsies in RNAlater	RNeasy Mini Kit	NEBNext Poly(A) mRNA Magnetic Isolation Module and NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	PE, 150 bp, 40-60 million	Illumina NovaSeq6000
Ruffner et al[6]	GSE156651	Biopsies frozen in RNAlater at −80°C	QIAzol lysis reagent (Qiagen, Hilden, Germany) followed by miRNeasy Kit with on-column DNase digestion	SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Takara) and NexteraXT DNA sample preparation kit (Illumina)	SE, 58 bp, 5 million	Illumina HiSeq2500
Sherrill et al[5]	GSE58640	Biopsies collected in RNAlater stored at 4°C for <4 wk	QIAzol lysis reagent followed by RNeasy Mini Kit	TruSeq mRNA Library Prep Kit (Illumina)	PE, 100 bp, NA	Illumina HiSeq2000
Wheeler et al[4]†	GSE113341	Biopsies	Qiagen kit	TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero	PE, 125 bp, 100-150 million	Illumina HiSeq2500

FFPE, Formalin-fixed paraffin-embedded; *GEO*, Gene Expression Omnibus; *NA*, not applicable; *PE*, paired-end; *SE*, single-end.
*Previously published studies have a reference in the first column.
†The Methods section in the study by Wheeler et al[4] erroneously refers to the study by Sherrill et al[5] as source for RNA-seq samples.

in children and adults with EoE.[6] Although important, these studies have not adequately answered the aforementioned fundamental questions.

In this study, we performed a synthesis of previous and new esophageal bulk RNA-seq data to examine the esophageal transcriptome of EoE and to identify differences in gene expression associated with age of onset and sex in patients with EoE. We hypothesized that in pediatric patients, gene expression would reflect more T_H2-type inflammation, whereas in adults it would be related to chronic inflammation, tissue remodeling, and fibrosis. Moreover, considering the male predisposition of EoE, we hypothesized that the immune response would include more inflammatory pathways in male patients than in female patients.

METHODS

Identification of studies

Relevant publications were identified by searching the Gene Expression Omnibus database for "Eosinophilic Esophagitis" in December 2022. Studies were included if there was esophageal bulk RNA-seq data available either from the Gene Expression Omnibus database or from the authors, as assessed by 2 authors (Y.A.C. and J.J.). In addition, 2 unpublished data sets from our institution were included. Details of these studies have been provided in the Online Repository at www.jacionline.org.

RESULTS

Seven articles describing bulk RNA-seq data of esophageal tissue biopsies from patients with EoE were identified (Table I).[4-10] The RNA-seq (meta)data from 1 study were not publicly available and were thus excluded.[7] The data from the study by Wheeler et al[4] (GSE1133341) were later reanalyzed by Miller et al,[9] and thus the data from the latter study were not included. This data set was referred to as Wheeler et al[4] in this study. In addition, we performed RNA-seq on tissue biopsies in-house from controls and patients with EoE. These 2 in-house data sets are separated by age group, that is, pediatrics (referred to as Hiremath) and adults (referred to as Choksi). The demographic data of included subjects are provided in Table II, and treatment status at the time of sample collection is provided in Table E1 (in the Online Repository available at www.jacionline.org).

Accounting for study heterogeneity

We first determined whether the data could be segregated by disease status. Initially, we assessed the effect of study heterogeneity, or batch effect, by performing a principal-component analysis (Fig 1, *A*). As expected, before batch correction of the data, most of the variance observed was due to which study the data originated from, suggesting that this

TABLE II. Demographic data of subjects with esophageal bulk RNA-seq from previous studies with available data and new subjects reported in this article

		Control		EoE		Mean age (y)				Eosinophils/hpf	
						Control		EoE			
Data set	N	Female, n (%)	Male, n (%)	Female, n (%)	Male, n (%)	Ped	Adult	Ped	Adult	Control	EoE
Choksi	23	9 (64)	5 (36)	3 (33)	6 (67)	NA	38	NA	36	0	42
Greuter et al[8]	17	2 (29)	5 (71)	3 (30)	7 (70)	NA	56	NA	31	0	105
Hiremath	25	7 (54)	6 (46)	2 (17)	10 (83)	14	NA	14	NA	1	64
Menard-Katcher	27	1 (4)	6 (22)	2 (7)	18 (67)	10	NA	13	19	0	70
Ruffner et al[6]	9	3 (75)	1 (25)	2 (40)	3 (60)	NA	51	NA	43	5	84
Sherrill et al[5]	16	3 (50)	3 (50)	4 (40)	6 (60)	13	NA	6	32	0	164
Wheeler et al[4]	20	5 (50)	5 (50)	5 (50)	5 (50)	11	NA	11	NA	0	52
Total	137	30 (22)	31 (23)	21 (15)	55 (40)	12	45	13	34	1	81

NA, Not applicable; *Ped*, pediatric.

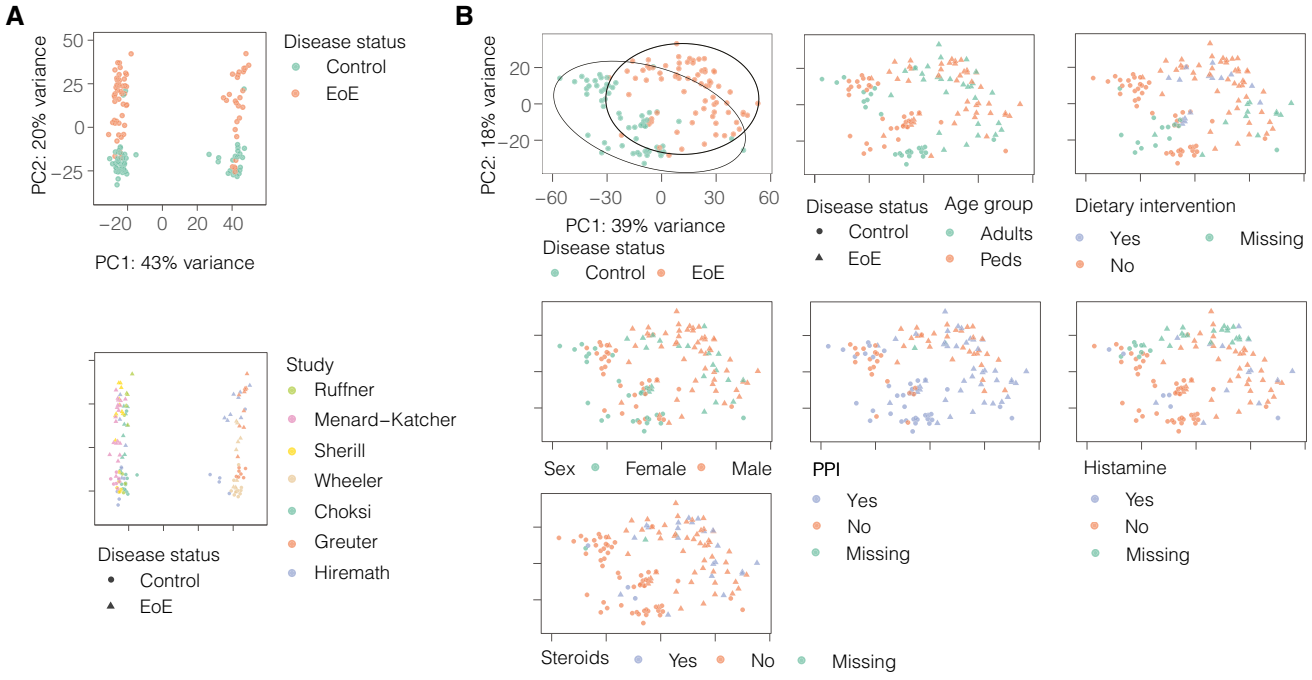

FIG 1. Synthesis of esophageal RNA-seq requires controlling for study. RNA-seq data from multiple studies (Table I) were collectively analyzed. **A** and **B,** Principal-component analyses before correcting for data set (study) (Fig 1, *A*) and after accounting for data set (Fig 1, *B*) as a variable. Only disease status exhibits segregation of patients. Age group, dietary intervention, sex, PPI use, histamine use, and steroid use do not.

approach would be unlikely to yield biological insights. Indeed, before batch correction, variance could not be explained by age, sex, or disease status (see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). Thus, we corrected for batch effect with the removeBatchEffect function in limma[11] (see the Online Repository). After correcting for variance due to study, samples segregated by disease status (EoE vs control; Fig 1, *B*), indicating that a meta-analysis was possible. We then examined which other covariates contribute to variation using principal-component analysis plots and limma and proceeded with a model including study and age group (<18 years or ≥18 years), sex (male or female), and proton pump inhibitor (PPI) use. Rather than making individual models for covariates of interest, a single model that included study and the other covariates was used for analyses in an effort to perform a conservative and rigorous assessment of the RNA-seq data.

Identification of new genes in the EoE transcriptome

First, we validated our approach of synthesizing multiple RNA-seq studies by comparing the differentially expressed genes we identified in patients with EoE versus controls with previously published results. Most of the dysregulated genes (all upregulated and downregulated genes listed in Table E2 in this article's Online Repository at www.jacionline.org) noted in our analysis have previously been shown to be associated with EoE

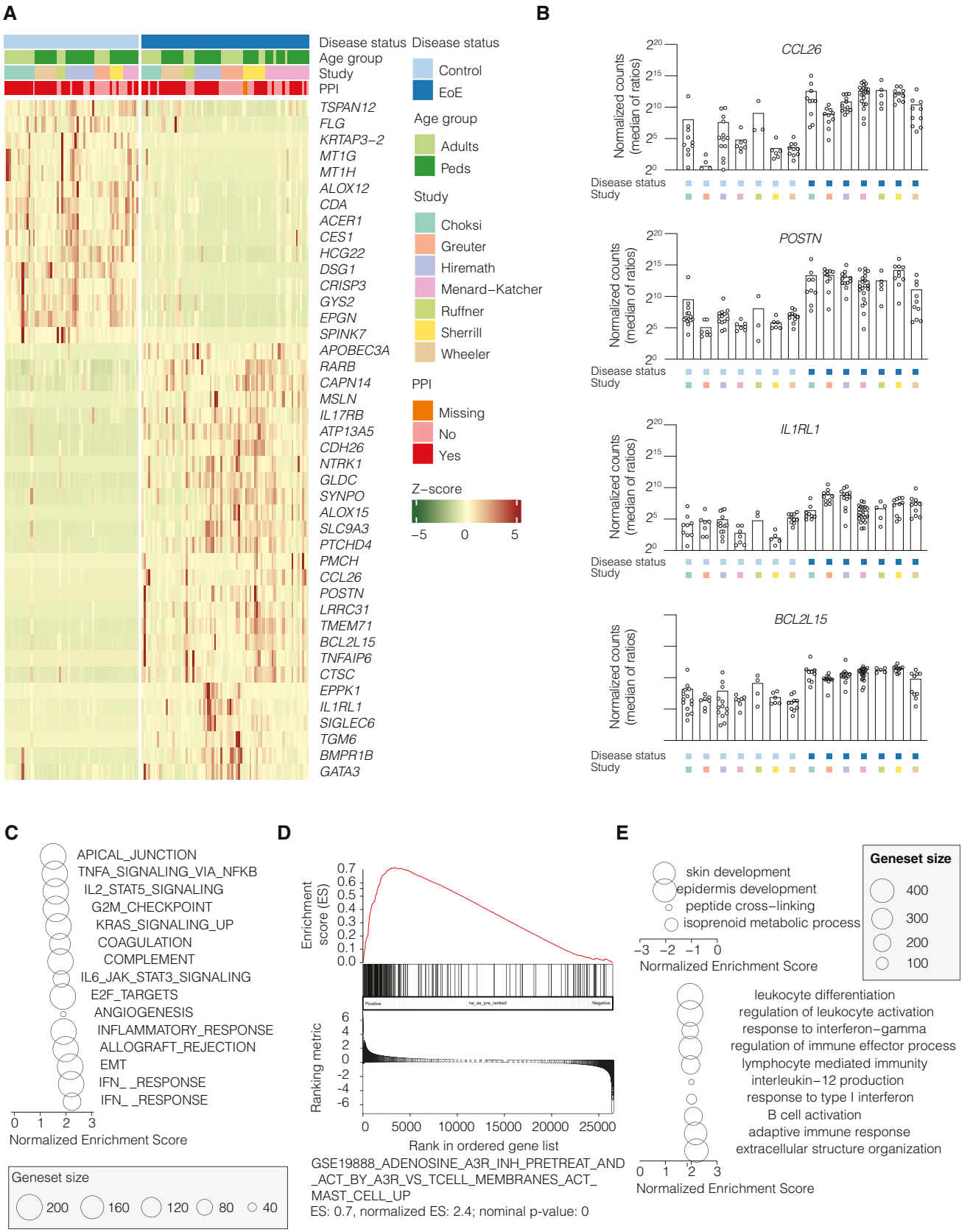

FIG 2. RNA-seq synthesis demonstrates new EoE-associated genes. **A,** Heatmap demonstrating a selection of significantly altered genes in all patients with EoE vs controls, where significance is defined as $\log_2$ fold change greater than |2| and adjusted *P* value less than .05. Many of these genes have previously been

(Fig 2, *A* and *B*). Most of these genes are listed with references in Table E3 (in the Online Repository available at www.jacionline.org), and the function of a few of these genes has already been elucidated.[12-20]

Some of the prominent genes included C-C motif chemokine ligand 26 (*CCL26*),[12] cadherin-like protein 26 (*CDH26*),[12-14] periostin (*POSTN*),[15] leucine rich repeat containing 31 (*LRRC31*),[16] sialic acid–binding Ig-like lectin 6 (*SIGLEC6*),[17] solute carrier 9, subfamily A, member 3 (*SLC9A3*),[18] neurotrophic tyrosine kinase receptor 1 (*NTRK1*),[21] and IL-33 receptor (*IL1RL1*).[20] There were other genes such as arachidonate 15-lipoxygenase (*ALOX15*),[3] ATPase 13A5 (*ATP13A5*),[19] TNF-α–induced protein 6 (*TNFAIP6*),[3] cathepsin C (*CTSC*),[22] pro–melanin- concentrating hormone (*PMCH*),[23,24] apoliprotein B (*APOBEC3A*),[25] pendrin (*SLC26A4*),[24] epiplakin 1 (*EPPK1*),[26] IL-17 receptor beta (*IL17RB*),[27] transmembrane protein 71 (*TMEM71*),[19] and retinoic acid receptor beta (*RARB*),[19] whose association with EoE has been previously described but their function has not been defined.[3,12,19,22,24-27] Notably, a subset of the upregulated genes that, to our knowledge, were not previously described to be associated with EoE were BCL2-like 15 (*BCL2L15*), patched domain containing 4 (*PTCHD4*), mesothelin (*MSLN*), transglutaminase 6 (*TGM6*), and bone morphogenetic protein receptor type 1B (*BMPR1B*). A list of other newly identified genes is provided in Table E3.

Like the upregulated genes, the function of only a few of the downregulated genes in EoE such as desmogelin-1 (*DSG1*)[28] and filaggrin (*FLG*)[29] has already been elucidated. Several other downregulated genes such as carboxylesterase 1 (*CES1*),[22] cartilage acidic protein 1 (*CRTAC1*),[22] cysteine-rich secretory protein 2 (*CRISP2*) and *CRISP3*,[3] metallothionein 1H (*MT1H*),[22] alkaline ceramidase 1 (*ACER1*),[30] *MT1G*,[22] liver glycogen synthase (*GYS2*),[22] *ALOX12*,[22] cytidine deaminase (*CDA*),[22] endothelin 3 (*EDN3*),21 secreted Ly6/uPAR-related protein 1 (*SLURP1*),[3] and keratin-associated protein 3-2 (*KRTAP3-2*)[31] have been associated with EoE, but their role in EoE has not yet been delineated. Our analysis also uncovered genes such as IL12A antisense RNA1 (*IL12AS-1*) and epithelial mitogen (*EPGN*) HLA complex group 22 (*HCG22*), which have not been associated with EoE and their functionality in EoE has not been elucidated. Altogether, the analysis provided confirmation of many of the known EoE-related genes, validating our methodology, as well as identified new genes that were previously not associated with EoE.

Gene-set enrichment analysis identifies interferon response as being dysregulated in EoE

Next, a preranked gene-set enrichment analysis (GSEA) was done to identify pathways dysregulated in EoE (Fig 2, *C*). In agreement with one of the studies,[6] the gene sets with the highest normalized enrichment score out of the Broad hallmark gene sets were related to IFN-α and IFN-γ response. Most other Broad hallmark gene sets that were upregulated were related to inflammation. Among the Broad immune signature gene sets, the one most enriched (with the highest normalized enrichment score) was related to mast cell activation (Fig 2, *D*; see Tables E4 and E5 in this article's Online Repository at www.jacionline.org; GSEA data for cellular components are provided in Table E6 in this article's Online Repository at www.jacionline.org). There were no Broad hallmark pathways that were de-enriched, but in WebGestalt, the de-enriched pathways were related to loss of differentiation (Fig 2, *E*). As such, our GSEA revealed that most of the gene sets enriched in EoE are related to inflammation, and many of the gene sets de-enriched in EoE are related to epithelial differentiation.

EoE is characterized by a mixed inflammatory response

Ingenuity pathway analysis (IPA) was then used to identify canonical pathways (see Fig E2 in this article's Online Repository at www.jacionline.org) and upstream regulators that could explain these transcriptional changes. The upstream regulators with the highest predicted activity were primarily proinflammatory cytokines (see Fig E3 a nd Table E7 in this article's Online Repository at www.jacionline.org) such as IL-4, IL-5, IL-1β, IL-13, IL-15, colony-stimulating factor 1, IFN-γ, and TNF (Table E7). The only downregulated cytokine was IL1-RN. Although some of the cytokines that were dysregulated were typically associated with T-cell–mediated inflammation characterized as T_H2 (IL-4, IL-5, and IL-13), others were not (IL-1β, IL-15, and TNF).

Next, we focused on transcription factors. As expected, GATA3 and signal transducer and activator of transcription 6 (STAT6) were identified as activated in our data set. Notably, STAT3, STAT1, and V-maf musculoaponeurotic fibroscarcoma oncogene homolog B (MAFB) were also activated (Table E7). One of the downregulated transcription factors was RUNX3 (Table E7). Altogether, these altered transcription factors suggest that a mixed inflammatory milieu is present in the esophagus of patients with EoE.

Finally, altered canonical pathways identified by IPA were examined. Several canonical pathways with low *P* values had *z* scores indicating activation of B-cell–related pathways (Fig E3, *A*). This was also true in the WebGestalt enrichment analysis (Fig 2, *E*). In IPA, the upregulated pathways included a pathway called SLE signaling in B cells and signaling via FcγRIIβ signaling in B cells. Most of the former pathway consists of genes related to immunoglobulins and includes CD22 and CD79A (Fig E3, *D*). Genes from the FcγRIIβ signaling in B cells, including *BTK*, are shown in Fig E3, *B*. Another pathway with a high *z* score was IL-15 (Fig E3, *A*), and dysregulated genes from the IL-15 pathway are shown in Fig E3, *C*.

identified and/or studied, and all genes with available references are provided in Table E8. *Red* indicates high *z* scores for upregulated genes, and *green* indicates high *z* scores for downregulated genes. Supervised clustering was done in which the disease status and the study were preset. Each vertical bar is a subject. **B**, Normalized counts of select genes were plotted to show minimal variance by study. **C**, GSEA using Broad with only significantly altered hallmark gene sets shown. An FDR of less than 0.05 was used as a threshold for statistical significance. Gene sets are listed in order of the value of the NES. **D**, Enrichment plot depicting the gene set with the highest NES in the immune signatures database from Broad GSEA. **E**, GSEA depicting gene sets with an FDR of less than 0.05 as assessed by WebGestalt on Gene Ontology biological processes. The figure panel was limited to the 10 gene sets with the lowest FDR. Gene sets are listed in order of the value of the NES. *EMT*, Epithelial mesenchymal transition; *FDR*, false-discovery rate; *NES*, normalized enrichment score.

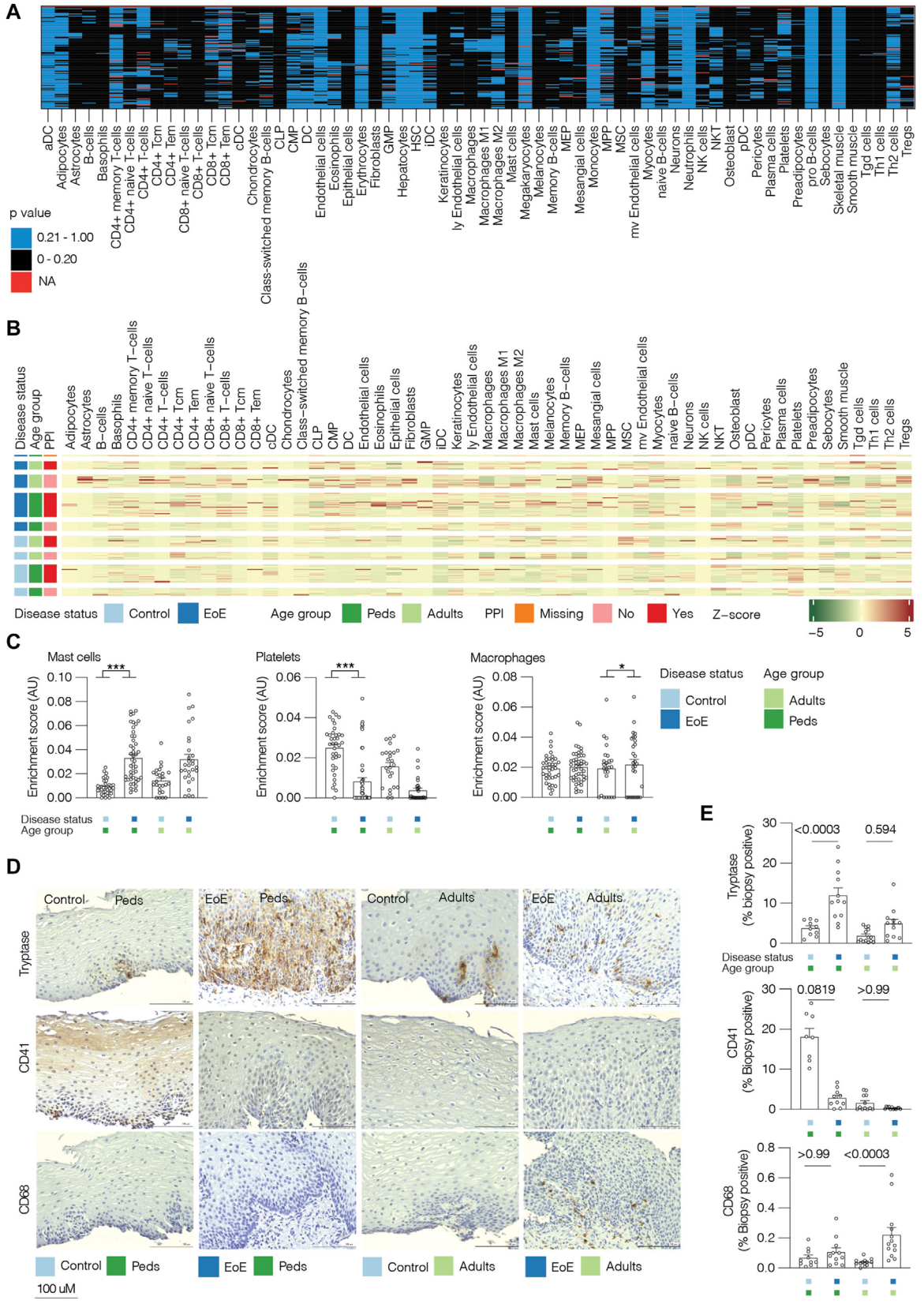

FIG 3. Cell-type analysis reveals different compositions of cell types between adult and pediatric EoE. **A**, Heatmap of *P* values of beta distribution. In this beta distribution, a *P* value of less than .20 indicates a cell type is present. Each horizontal bar represents a subject. NA indicates no *P* value could be calculated by xCell. **B**, Heatmap of all cell types that are included (ie, have *P* <.20). Cell types were removed if *P* value

In addition, canonical pathways with a negative *z* score included IL-12 signaling in macrophages, retinol biosynthesis, inhibition of matrix metalloproteinases, and erythropoietin signaling (Fig E3, *A*).

In summation, we identified upstream regulators, transcription factors, and altered canonical pathways, which suggest that inflammation in EoE is not purely T_H2 but is a mixed inflammatory milieu.

Cell-type analysis distinguishes adult EoE from pediatric EoE

To determine the cellular composition of esophageal biopsies from patients with EoE, cell-type analysis was done using a computational tool to derive cell types from RNA-seq data.[32] This analysis calculates an enrichment score that refers to the degree to which the genes in 1 or more gene sets ascribed to a particular cell type are upregulated or downregulated within a subject, which is called single-sample GSEA. The first step of this analysis consists of comparing the cell-type score to a random mixture of cell types that does not contain the particular cell type (Fig 3, *A*). This is done to reduce false-positive results. After exclusion of cell types that were predicted to be absent from esophageal biopsies (see the Online Repository; Fig 3, *A*), 55 cell types remained (Fig 3, *B*).

Disease status ($F = 22.56$; $P < .001$), study ($F = 14.76$; $P = .001$), and age group ($F = 3.74$; $P = .01$) contributed to dissimilarity in cell types. In contrast, sex did not significantly contribute to the dissimilarity in cell types ($F = 1.44$; $P = .19$). These data suggest that esophageal cell-type composition in EoE depends on disease status and study, with a minor contribution from the age group of the patient and no apparent contribution from the sex of the patient. We also examined the contribution of treatment status and found that, of the treatment parameters in this cohort, only PPI treatment status contributed to the cell-type scores ($F = 2.27$; $P = .016$). To preserve statistical power, we did not add this parameter to the final model (data not shown). As such, the final model included study ($F = 19.50$; $P = .001$) and a composite variable of disease status and age group, that is, pediatric controls, pediatric EoE, adult controls, and adult EoE ($F = 14.89$; $P = .001$).

The *post hoc* analysis demonstrated differences in cell composition between adults with EoE compared with adults without EoE (Benjamin-Hochberg–adjusted $P = .0015$) and children with EoE compared with children without EoE (Benjamin-Hochberg–adjusted $P = .001$) (Fig 3, *C*; see also Fig E4 in this article's Online Repository at www.jacionline.org).

The highlighted findings that were different in adults and children were that mast cells were enriched in pediatric patients but not in adults, platelets were de-enriched in pediatric patients but not in adults, and macrophages were enriched in adults but not in pediatric patients (Fig 3, *C*). To confirm these findings, we performed immunohistochemistry (IHC) in a separate cohort of adult and pediatric patients for tryptase (mast cells), CD68 (a macrophage marker), and CD41 (a platelet marker). Patient characteristics for this cohort are provided in Table E8 (in the Online Repository available at www.jacionline.org). We demonstrated that tryptase staining was significantly increased in pediatric patients but not in adults, CD68 was significantly increased in adults but not in pediatric patients, and CD41 showed a trend toward reduction in pediatric patients but not in adults (Fig 3, *D* and *E*).

As expected, eosinophils were increased in children and adults with EoE (Fig E4). In addition, in children and adults with EoE, the gene expression attributed to interstitial dendritic cells was increased**.** Several cell-type gene signatures seemed to be uniquely upregulated in adults with EoE, including T_H1 cells, M2 macrophages, and smooth muscle cells (Fig E4). Other cell types were enriched in children with EoE, including γδT cells. Altogether, these data suggest that esophageal cell-type composition is associated with both disease status and age.

The tissue B cells in EoE are heterogeneous

Next, we investigated the specificity of T and B cells because EoE is thought to be an antigen-mediated disease, and the B-cell activation pathway was upregulated in patients with EoE (Fig 2, *E*; see also Fig E3). To determine B-cell receptor (BCR) specificity, we derived BCR sequences from the RNA-seq data and assessed the diversity separately for children and adults. Clonotypes (IgH) were detected in 98 samples. BCR diversity was significantly higher in patients with EoE compared with controls (Fig 4, *A*), both in children and in adults. Moreover, the number of clonotypes that occupy 50% of the repertoire, a measure to assess clonality, was not different between patients with EoE and controls in either age group (Fig 4, *B*), indicating that the B-cell repertoire was not clonal. Finally, we assessed the overlap in BCR sequences between subjects to determine whether certain clonotypes were shared between subjects. The overlap analysis did not show that BCR sequences were shared between subjects (see Fig E5, *A*, in this article's Online Repository at www.jacionline.org). Overall, these data suggest that the B-cell response in patients with EoE is diverse rather than consisting of expansion of 1 or multiple B cells. We also assessed the same parameters of repertoire diversity in T cells (N = 107 samples) and did not find differences (Fig 4, *C* and *D*; see also Fig E5, *B*).

Transcriptomic analysis of subgroups with EoE

Next, we performed differential expression analysis for various covariates of interest (see metadata for all subjects in Table E9 in this article's Online Repository at www.jacionline.org), that is, age group (Fig 5; see also Fig E6 in this article's Online Repository at www.jacionline.org), sex (Fig 6; see also Fig E7 in this article's Online Repository at www.jacionline.org), and PPI

could not be calculated or if *P* value was greater than .20 in at least 12 subjects (see also the Online Repository). The values in the heatmap are the xCell enrichment scores, which indicate the gene signatures of a particular cell type present. It is only valid to compare the enrichment score between parameters within a particular cell type, not between cell types. **C**, Permutational multivariate ANOVA was used to identify cell types that were significantly different between EoE and control in adults and children (see the Online Repository for more details). The stars indicate the significance as assessed by the SIMPER analysis. *$P <$ .05; **$P <$ .01; ***$P <$ .001. The enrichment score ascribed to select cell types is shown. **D**, Representative IHC images (40×) for proteins that are typical for the cell types from Fig 3, *C*, in human samples (see Table E8). **E**, Subsequently, the amount of positive staining was quantified. The scale bar in all images is 100 μM. The adjusted *P* values were calculated using the Kruskal-Wallis test and were subsequently corrected using Bonferroni to account for assessment of multiple cell types.

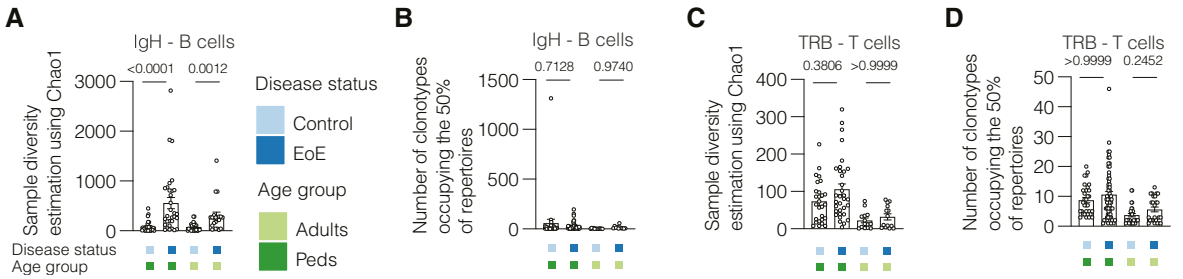

FIG 4. The B cells in EoE are heterogenous. **A-D**, MiXCR was used to derive BCR and TCR sequences from bulk RNA-seq data. Immunarch was then used to assess diversity in B cells (Fig 4, *A* and *B*) and T cells (Fig 4, *C* and *D*). The statistical test used was the Kruskal-Wallis test with the Dunn *post hoc* test.

treatment status (see Fig E8 in this article's Online Repository at www.jacionline.org). All these comparisons were done in a single model of the expression data as shown in Fig 1, *B*.

Transcriptome of EoE in children versus adults

First, we separately assessed the genes altered in EoE versus controls in children and adults (Fig 5, *A*, and Tables E10 and E11). Overall, most pathways were enriched in both adults and children, although to varying degrees. However, GSEA using Broad identified pathways that were differentially regulated in adults but not children and in children but not adults (Fig 5, *B*). Myogenesis, apical junction, and STAT3 pathways were enriched only in adults, whereas the G2M checkpoint pathway was enriched only in children. Most notably, we identified the gene set "Myogenesis," which contains many collagen genes, as upregulated in adults but not in children (Fig 5, *B*). In a heatmap, we listed the individual genes within the myogenesis pathway that were significantly altered in at least 1 comparison (Fig 5, *C*); that is, they were significantly altered in adults or in children. The fold changes are listed on the *right*. We have also graphically represented normalized counts (Fig 5, *D*) for 1 gene (*CLU*) in which the difference between controls and EoE is greater in adults than in children, 1 gene (*MEF2C*) that is upregulated in adults but downregulated in children, and 1 gene (*GADD45B*) that is downregulated to a similar degree in adults and in children.

Subsequently, a comparison was done by IPA to identify pathways (Fig E6) differentially regulated in children and adults with EoE. Fig E6, *A*, shows pathways upregulated in both children and adults with EoE, and Fig E6, *B*, shows pathways downregulated in both adults and children with EoE. Fig E6, *C*, illustrates pathways significantly dysregulated only in adults, only in children, or in opposite directions. Bold text indicates that they are significantly dysregulated in opposite directions. Notably, IL-6 and STAT3 are downregulated in children but upregulated in adults. In addition, antioxidant actions of vitamin C and eicosanoid signaling are upregulated in children but downregulated in adults. Fig E6, *D*, highlights the cytokine-related pathways that are upregulated in both children and adults, whereas Fig E6, *E*, highlights the cytokine-related pathways downregulated in both children and adults. Fig E6, *F*, shows those dysregulated in opposite directions.

Transcriptome of EoE in male versus female patients

Next, we compared the patients with EoE separately on the basis of sex. For this analysis, we pooled both children and adults and did the comparison by sex. Using the significantly upregulated and downregulated genes (Fig 6, *A*, Tables E12 and E13), we performed a similar analysis as for the 2 age groups. The pathways that were upregulated in both male and female patients are shown in Fig E7, *A*, and those that were downregulated are shown in Fig E7, *B*. In IPA, the pathways that were upregulated in only males, only females, or in opposite directions are displayed in Fig 6, *B*. Notably, the complement system and IL-12 signaling and production in macrophage pathways were downregulated in male patients but upregulated in female patients. To determine which pathways were driven by sex, we repeated this analysis but removed genes on the X and Y chromosomes (Fig 6, *C*). In this analysis, the same 2 pathways were dysregulated in the same direction, but now STAT3 was significantly upregulated in male patients and downregulated in female patients. Moreover, removing the X and Y chromosome genes resulted in the stearate biosynthesis I (Animals) pathway, which was downregulated in male patients but upregulated in female patients in the analysis with X and Y genes (Fig 6, *B*), no longer being altered (Fig 6, *C*). Normalized counts from candidate genes in this pathway are shown in Fig 6, *D*. All genes from the IL-12 signaling and production in macrophages are shown in Fig 6, *E*, and all genes from the STAT3 pathway are shown in Fig 6, *F*. Collectively, these data suggest that sex-based transcriptional differences exist in the pathogenesis of EoE.

Transcriptome of PPI-treated patients with EoE

Finally, we compared gene expression of patients with EoE who were on PPIs with that of controls, and gene expression of patients with EoE who were not on PPIs with that of controls (Fig E8, *A*, and Tables E14 and E15). Overall, the number of pathways that were differentially regulated on PPI status was small (Fig E8, *B-D*).

DISCUSSION

Many questions regarding the pathophysiology of EoE remain unanswered. Because some previous esophageal bulk RNA-seq studies comparing patients with EoE versus controls were not large enough to perform subgroup comparisons,[4-8,10] we synthesized both publicly available data and new (unpublished) esophageal bulk RNA-seq data to demonstrate the EoE transcriptome and determine whether there are differences by age group and sex. Although our results corroborated several genes that have already been reported to be involved in EoE, our analysis also identified new genes and transcription factors

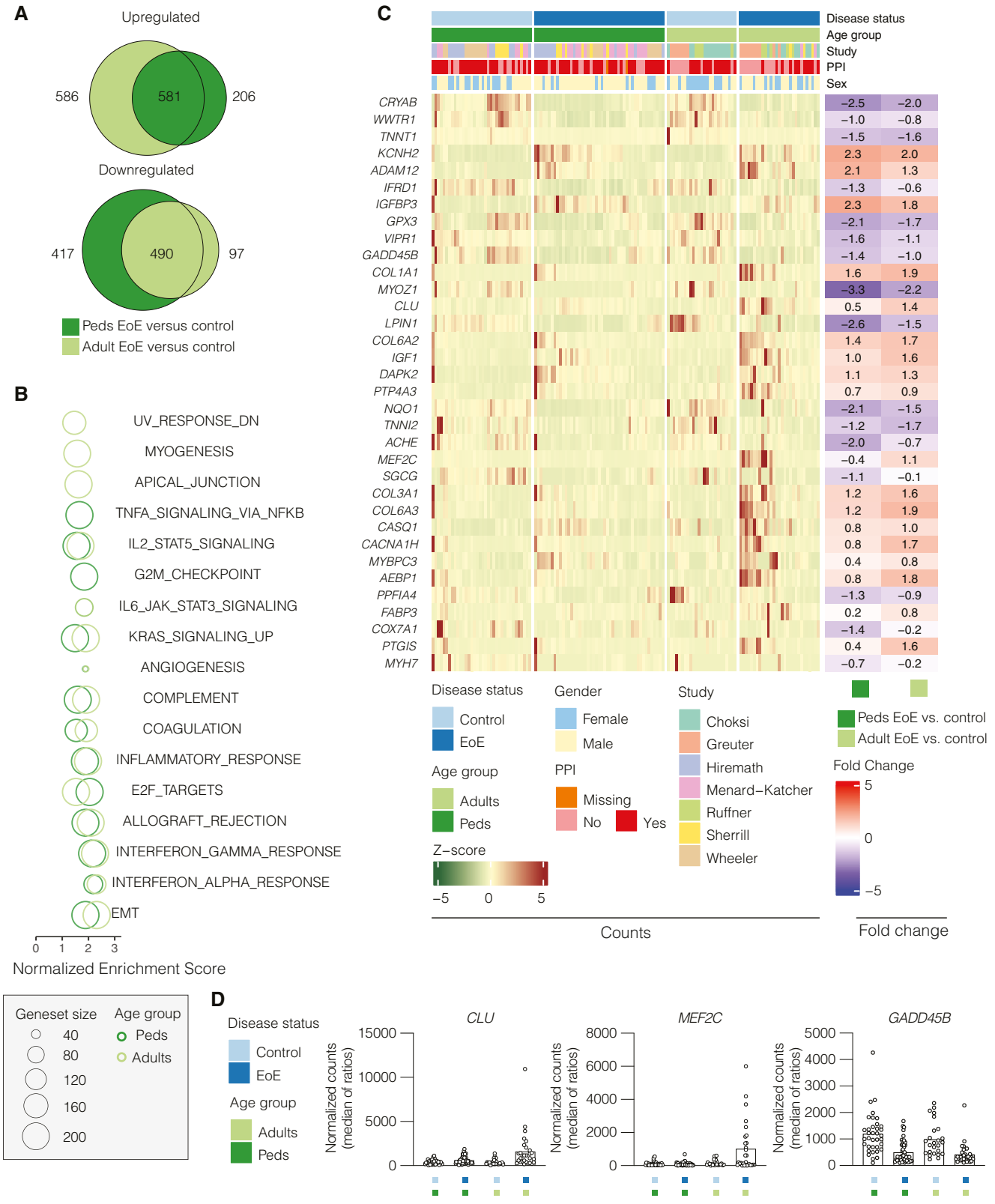

FIG 5. Myogenesis pathway is enriched in adults but not in children with EoE. **A**, Venn diagram of upregulated (*left*) and downregulated (*right*) genes comparing patients with EoE with controls in adults and pediatrics (Peds) separately. **B**, GSEA using Broad hallmark gene sets. The color of the circles indicates the comparison; that is, *dark green circles* indicate the comparison of children with EoE vs children without EoE, and *light green circles* indicate the comparison of adults with EoE vs adults without EoE. Both age group comparisons are plotted in the same graph. Only statistically significant gene sets (FDR < 0.05) are shown. **C**, Genes from the myogenesis pathway were then screened for statistical significance (adjusted $P < .05$ in at least 1 of the 2 comparisons and $\log_2$ fold change > |0.5|) and listed in this heatmap. Supervised clustering was applied. **D**, Normalized counts for select genes from the myogenesis pathway are shown. *FDR*, False-discovery rate.

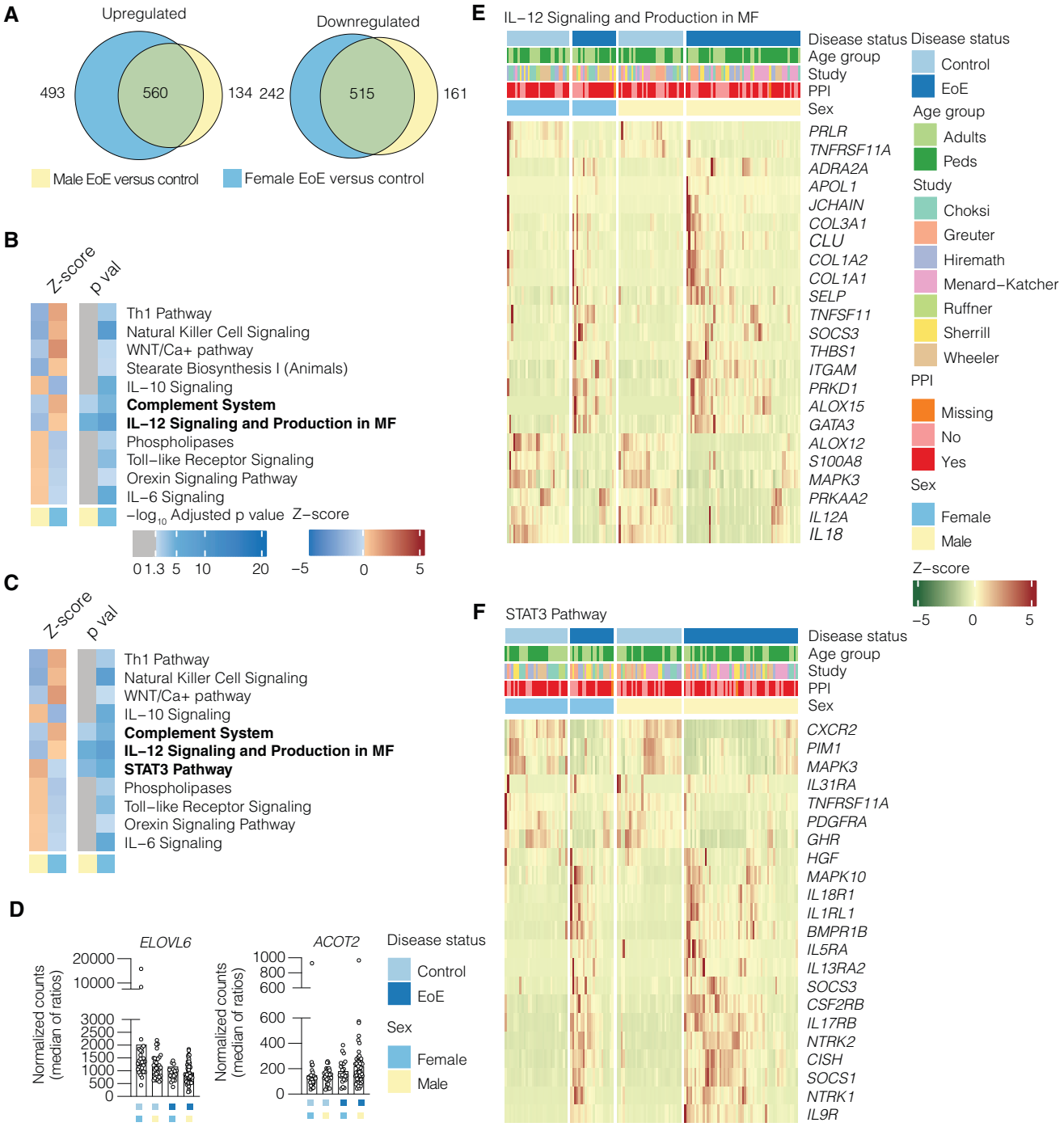

FIG 6. Sex-specific differences in EoE are related to IL-12 and STAT3 signaling. **A**, Venn diagram of upregulated and downregulated genes comparing male patients with EoE with male controls without EoE and female patients with EoE with female controls without EoE. **B**, IPA pathways that were significantly different and regulated in opposite directions. Pathways that are statistically significantly altered in both comparisons are in boldface. **C**, IPA pathways that were significantly different after excluding X and Y chromosome genes before differential gene expression analysis. **D**, Select genes from the Stearate in Biosynthesis I (Animals) pathway. **E** and **F**, Heatmap of all genes in the IL-12 signaling and production in the macrophages (MF) pathway (Fig 6, *E*) and the STAT3 pathway (Fig 6, *F*). Both Fig 6, *E*, and Fig 6, *F*, are clustered on disease status and sex.

that have not been reported to have differential expression in EoE. First, genes and pathways related to B cells were upregulated in EoE. The transcription factors STAT1 and MAFB were identified as activated in IPA on the basis of changes in the expression of their downstream targets. Analysis of upstream regulators revealed that various proinflammatory cytokines

were increased, including both T_H1 and T_H2 cytokines, as well as IL-15. In addition, several differences in gene expression, enrichment of pathways, and cell types were identified in adults compared with children. Cell-type analysis and confirmatory IHC identified mast cells as significantly increased in pediatric patients but not in adults and macrophages as significantly increased in adults but not in pediatric patients. In addition, cell-type analysis suggested that a mixed T_H1/T_H2-type inflammation was more likely in adults than in children with EoE. Finally, sex-based transcriptional differences suggested altered IL-12 signaling in macrophages (upregulated in female patients) and STAT3 (upregulated in male patients after removing genes on the X and Y chromosomes).

A central observation of our study is that there are significant differences in immune cell gene signatures besides eosinophils, basophils, and T cells,[33,34] which are yet to be studied extensively, specifically in dendritic cells, mast cells, B cells, and macrophages. The role of dendritic cell–bound IgE in EoE[35] and the limited knowledge of antigen-presenting cells in EoE in general have been reviewed elsewhere.[36] In the case of mast cells, although other studies have shown that they are present in EoE,[37] the mechanism by which mast cells contribute to EoE disease pathogenesis remains largely limited to the description of mast cell presence and their capacity to produce IL-13 in EoE.[38] One mechanistic study does show that in a murine model of EoE,[34] mast cells contribute to disease in an IL-5–independent manner.[39] In addition, we have demonstrated that mast cells are more predominant in children with EoE, and thus further study is needed because this could explain why adults and children present with different symptoms. As for B cells, in our study, we identified activation of the FcεRI signaling pathway. FcεRI, a high-affinity IgE receptor, is required for IgE-dependent mast cell degranulation and is the only receptor for IgE expressed in the esophagus.[40] We also identified dysregulation of several B-cell–related pathways containing immunoglobulin genes, including *IGHE*. Consistent with this finding, IgE^+ mast cells are present in the esophagus of patients with EoE.[41] However, arguments have been made that EoE is non–IgE-mediated because anti-IgE therapies have not been successful in the treatment of EoE.[42] Despite this, these data raise the question of whether the role of mast cells in EoE pathogenesis is through IgE-dependent mast cell degranulation or another mechanism. As for macrophages, there has been little study into their role in EoE pathogenesis, although 1 study suggests that macrophages may be a source of IL-15, which can induce epithelial cells to produce eotaxins.[43] However, to our knowledge, we were unable to find any data about macrophage infiltration in adults versus pediatric patients.

The transcription of genes associated with γδ T cells was also dysregulated in children with EoE compared with controls. To our knowledge, other studies have not detected alterations in γδ T cells, a cell type that might have been overlooked thus far, because single-cell RNA-seq shows that most of the esophageal T cells in EoE are $\alpha\beta^+$.[44] Dietary factors can modulate intestinal γδ T cells,[45] and food antigens are a known trigger for EoE. On the basis of our findings, it is possible that the dietary antigens could trigger EoE, at least in children, via regulation of γδ T cells.[46-48]

Another notable observation of our study is that EoE is characterized by a mixed inflammatory milieu (to a greater degree in adults than in children), as evidenced by cell-type analysis and the various proinflammatory cytokines found to be upstream regulators of disease by IPA. One possible explanation is that although food antigens may trigger disease, once barrier dysfunction has occurred, other types of inflammation get subsequently triggered. As such, therapies to strengthen the epithelial barrier may be instrumental in patients who are difficult to treat.

Our study has limitations. Being largely computational, experimental data would need to confirm whether the findings reported here are true. In particular, the cell-type enrichment analysis has several caveats, including that it is possible to make comparisons between subject groups but not between cell types. Moreover, the *post hoc* analysis for the permutational multivariate ANOVA to examine which factors are associated with differences in cell-type enrichment scores can be confounded by variation in enrichment values. Furthermore, cell types that are susceptible to degradation during tissue processing, such as eosinophils, might drop out of an RNA-based analysis because of degradation of the RNA. Thus, we cannot conclude that cell types are altered solely on the cell-type enrichment analysis, but we can use the data to determine what should be studied further. As such, we confirmed some of the findings with IHC.

Another limitation comes from the metadata regarding treatment status because data regarding some of the previous therapies and length of those therapies were not available. In addition, we did not have the data to determine when in the disease course the subjects were sampled. Thus, how gene expression changes as a function of treatment for EoE is an area for future studies.

Lastly, our study does not yield information about the cell-specific transcriptome. This might be addressed in the future as more studies of single-cell RNA-seq become available.[38,44,46] Despite these limitations, our study has several strengths. We curated the largest data set of bulk RNA-seq data from patients with EoE, and this allowed us to conduct meaningful subgroup analyses. We used several unique and advanced techniques to combine various studies seamlessly and accounted for batch effect. This approach is particularly relevant and may be useful in synthesizing RNA-seq data in other rare diseases such as eosinophilic gastritis or eosinophilic colitis. Finally, some of the newly identified cell types, cytokines, and metabolic pathways could serve as potential therapeutic targets to advance the management of EoE.

We applied a unique methodology for synthesizing bulk RNA-seq data from patients with EoE and controls to appraise the EoE transcriptome and perform subgroup comparisons. We found significant transcriptional differences between adults and pediatric patients and between male and female patients. Future areas of studies include substantiating the findings reported here with experimental evidence and combining data from single-cell RNA-seq data sets.

Data availability

Previously published data and metadata are detailed in Tables I and II and in Tables E1 and E9 in the Online Repository.

DISCLOSURE STATEMENT

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Key messages

- **We have identified new genes and transcription factors that are dysregulated in EoE, while confirming many of the previously reported ones, using an integrated solution for analyzing RNA-seq data in which correction for batch effect, or study heterogeneity, is possible.**
- **We have found novel transcriptional differences between adults and children with EoE, including cell types that are significantly enriched in only adult or pediatric disease.**
- **We have found pathways that are dysregulated in opposite directions in male versus female patients with EoE.**

REFERENCES

1. Muir A, Falk GW. Eosinophilic esophagitis: a review. JAMA 2021;326:1310-8.
2. Furuta GT, Katzka DA. Eosinophilic esophagitis. N Engl J Med 2015;373:1640-8.
3. Matoso A, Mukkada VA, Lu S, Monahan R, Cleveland K, Noble L, et al. Expression microarray analysis identifies novel epithelial-derived protein markers in eosinophilic esophagitis. Mod Pathol 2013;26:665-76.
4. Wheeler JC, Vanoni S, Zeng C, Waggoner L, Yang Y, Wu D, et al. 17β-Estradiol protects the esophageal epithelium from IL-13–induced barrier dysfunction and remodeling. J Allergy Clin Immunol 2019;143:2131-46.
5. Sherrill JD, Kiran KC, Blanchard C, Stucke EM, Kemme KA, Collins MH, et al. Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. Genes Immun 2014;15:361-9.
6. Ruffner MA, Hu A, Dilollo J, Benocek K, Shows D, Gluck M, et al. Conserved IFN signature between adult and pediatric eosinophilic esophagitis. J Immunol 2021;206:1361-71.
7. Peterson KA, Yoshigi M, Hazel MW, Delker DA, Lin E, Krishnamurthy C, et al. RNA sequencing confirms similarities between PPI-responsive oesophageal eosinophilia and eosinophilic oesophagitis. Aliment Pharmacol Ther 2018;48:219-25.
8. Greuter T, Straumann A, Fernandez-Marrero Y, Germic N, Hosseini A, Yousefi S, et al. Characterization of eosinophilic esophagitis variants by clinical, histological, and molecular analyses: a cross-sectional multi-center study. Allergy 2022;77:2520-33.
9. Miller DE, Forney C, Rochman M, Cranert S, Habel J, Rymer J, et al. Genetic, inflammatory, and epithelial cell differentiation factors control expression of human calpain-14. G3 (Bethesda) 2019;9:729-36.
10. Menard-Katcher C, Liu C, Galbraith MD, Benson T, Burger C, Dobias D, et al. Fibrostenotic eosinophilic esophagitis phenotype is defined by a proliferative gene signature. Allergy 2023;78:579-83.
11. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
12. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest 2006;116:536-47.
13. Caldwell JM, Collins M, Blanchard C, Stucke E, Kemme K, Amin L, et al. Key function of cadherin-like 26 in eosinophilic gastrointestinal disorders. J Allergy Clin Immunol 2013;131:AB132.
14. Caldwell JM, Collins MH, Kemme KA, Sherrill JD, Wen T, Rochman M, et al. Cadherin 26 is an alpha integrin-binding epithelial receptor regulated during allergic inflammation. Mucosal Immunol 2017;10:1190-201.
15. Blanchard C, Mingler MK, McBride M, Putnam PE, Collins MH, Chang G, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. Mucosal Immunol 2008;1:289-96.
16. D'Mello RJ, Caldwell JM, Azouz NP, Wen T, Sherrill JD, Hogan SP, et al. LRRC31 is induced by IL-13 and regulates kallikrein expression and barrier function in the esophageal epithelium. Mucosal Immunol 2016;9:744-56.
17. Robida PA, Rische CH, Morgenstern NBB, Janarthanam R, Cao Y, Krier-Burris RA, et al. Functional and phenotypic characterization of Siglec-6 on human mast cells. Cells 2022;11:1138.
18. Zeng C, Vanoni S, Wu D, Caldwell JM, Wheeler JC, Arora K, et al. Solute carrier family 9, subfamily A, member 3 (SLC9A3)/sodium-hydrogen exchanger member 3 (NHE3) dysregulation and dilated intercellular spaces in patients with eosinophilic esophagitis. J Allergy Clin Immunol 2018;142:1843-55.
19. Shoda T, Morita H, Nomura I, Ishimura N, Ishihara S, Matsuda A, et al. Comparison of gene expression profiles in eosinophilic esophagitis (EoE) between Japan and Western countries. Allergol Int 2015;64:260-5.
20. Travers J, Rochman M, Caldwell JM, Besse JA, Miracle CE, Rothenberg ME. IL-33 is induced in undifferentiated, non-dividing esophageal epithelial cells in eosinophilic esophagitis. Sci Rep 2017;7:17563.
21. Rochman M, Kartashov AV, Caldwell JM, Collins MH, Stucke EM, Kc K, et al. Neurotrophic tyrosine kinase receptor 1 is a direct transcriptional and epigenetic target of IL-13 involved in allergic inflammation. Mucosal Immunol 2015;8:785-98.
22. Wen T, Stucke EM, Grotjan TM, Kemme KA, Abonia JP, Putnam PE, et al. Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. Gastroenterology 2013;145:1289-99.
23. Shoda T, Wen T, Caldwell JM, Ben-Baruch Morgenstern N, Osswald GA, Rochman M, et al. Loss of endothelial TSPAN12 promotes fibrostenotic eosinophilic esophagitis via endothelial cell–fibroblast crosstalk. Gastroenterology 2022;162:439-53.
24. Dunn JLM, Shoda T, Caldwell JM, Wen T, Aceves SS, Collins MH, et al. Esophageal type 2 cytokine expression heterogeneity in eosinophilic esophagitis in a multisite cohort. J Allergy Clin Immunol 2020;145:1629-40.e4.
25. Ruffner M, Shoda T, Muir A, Spergel J, Dellon E, Rothenberg M. Esophageal mucosal transcriptional alterations persist in eosinophilic esophagitis patients during remission. J Allergy Clin Immunol 2022;149:AB158.
26. Butz BK, Wen T, Gleich GJ, Furuta GT, Spergel J, King E, et al. Efficacy, dose reduction, and resistance to high-dose fluticasone in patients with eosinophilic esophagitis. Gastroenterology 2014;147:324-33.e5.
27. Morgan DM, Ruiter B, Smith NP, Tu AA, Monian B, Stone BE, et al. Clonally expanded, GPR15-expressing pathogenic effector T_H2 cells are associated with eosinophilic esophagitis. Sci Immunol 2021;6:eabi5586.
28. Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. Mucosal Immunol 2014;7:718-29.
29. Wu L, Tadayuki Oshima X, Li M, Tomita T, Fukui H, Watari J, et al. Filaggrin and tight junction proteins are crucial for IL-13-mediated esophageal barrier dysfunction. Am J Physiol Gastrointest Liver Physiol 2018;315:341-50.
30. Rochman M, Travers J, Miracle CE, Bedard MC, Wen T, Azouz NP, et al. Profound loss of esophageal tissue differentiation in patients with eosinophilic esophagitis. J Allergy Clin Immunol 2017;140:738-49.e3.
31. Zuo L, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C, et al. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13Rα2–inhibited pathway. J Immunol 2010;185:660-9.

32. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 2017;18:220.
33. Mishra A, Schlotman J, Wang M, Rothenberg ME. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. J Leukoc Biol 2007;81:916-24.
34. Wen T, Aronow BJ, Rochman Y, Rochman M, Kiran KC, Dexheimer PJ, et al. Single-cell RNA sequencing identifies inflammatory tissue T cells in eosinophilic esophagitis. J Clin Invest 2019;129:2014-28.
35. Mudde ACA, Lexmond WS, Blumberg RS, Nurko S, Fiebiger E. Eosinophilic esophagitis: published evidences for disease subtypes, indications for patient subpopulations, and how to translate patient observations to murine experimental models. World Allergy Organ J 2016;9:23.
36. Ryu S, Lee KH, Tizaoui K, Terrazzino S, Cargnin S, Effenberger M, et al. Pathogenesis of eosinophilic esophagitis: a comprehensive review of the genetic and molecular aspects. Int J Mol Sci 2020;21:7253.
37. Lucendo AJ, Bellón T, Lucendo B. The role of mast cells in eosinophilic esophagitis. Pediatr Allergy Immunol 2009;20:512-8.
38. Ben-Baruch Morgenstern N, Ballaban AY, Wen T, Shoda T, Caldwell JM, Kliewer K, et al. Single-cell RNA sequencing of mast cells in eosinophilic esophagitis reveals heterogeneity, local proliferation, and activation that persists in remission. J Allergy Clin Immunol 2022;149:2062-77.
39. Niranjan R, Mavi P, Rayapudi M, Dynda S, Mishra A. Pathogenic role of mast cells in experimental eosinophilic esophagitis. J Physiol Gastrointest Liver Physiol 2013;304:1087-94.
40. Yen EH, Hornick JL, Dehlink E, Dokter M, Baker A, Fiebiger E, et al. Comparative analysis of fceri expression patterns in patients with eosinophilic and reflux esophagitis. J Pediatr Gastroenterol Nutr 2010;51:584-92.
41. Vicario M, Blanchard C, Stringer KF, Collins MH, Mingler MK, Ahrens A, et al. Local B cells and IgE production in the oesophageal mucosa in eosinophilic oesophagitis. Gut 2010;59:12-20.
42. Simon D, Cianferoni A, Spergel JM, Aceves S, Holbreich M, Venter C, et al. Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. Allergy 2016;71:611-20.
43. Zhu X, Wang M, Mavi P, Rayapudi M, Pandey AK, Kaul A, et al. Interleukin-15 expression is increased in human eosinophilic esophagitis and mediates pathogenesis in mice. Gastroenterology 2010;139:182-93.e7.
44. Wen T, Douek D, Rothenberg ME. Single-cell RNA sequencing identifies inflammatory tissue T cells in eosinophilic esophagitis. J Clin Invest 2019;129:2014-28.
45. Ribot JC, Lopes N, Silva-Santos B. γ T cells in tissue physiology and surveillance. Nat Rev Immunol 2021;21:221-32.
46. Rochman M, Wen T, Kotliar M, Dexheimer PJ, Morgenstern NBB, Caldwell JM, et al. Single-cell RNA-seq of human esophageal epithelium in homeostasis and allergic inflammation. JCI Insight 2022;7:e159093.
47. Goldberg EL, Shchukina I, Asher JL, Sidorov S, Artyomov MN, Dixit VD. Ketogenesis activates metabolically protective γδ T cells in visceral adipose tissue. Nat Metab 2020;2:50-61.
48. Mehta P, Nuotio-Antar AM, Smith CW. γδ T cells promote inflammation and insulin resistance during high fat diet-induced obesity in mice. J Leukoc Biol 2015;97:121-34.