



Published in final edited form as:

Allergy. 2023 February ; 78(2): 579–583. doi:10.1111/all.15557.

Fibrostenotic eosinophilic esophagitis phenotype is defined by a proliferative gene signature

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To the Editor,

Eosinophilic Esophagitis (EoE) is a chronic inflammatory esophageal disease in which a subset of patients undergo pathologic tissue remodeling. This fibrostenotic EoE (FS-EoE) phenotype is defined by the presence of esophageal strictures and narrowing.¹ FS-EoE risk factors include age and persistent inflammation^{1,2} and these patients can be treatment refractory.³ Cellular pathways and molecular targets of FS-EoE remain under investigation.⁴⁻⁶ Here, using unbiased whole-transcriptome RNA-sequencing of well-defined pediatric and adolescent patients, we identify novel genes and signaling pathways that distinguish FS-EoE, setting the stage for understanding approaches to early therapeutic interventions.

We performed RNA-sequencing of mucosal biopsies from 9 FS-EoE patients, 11 EoE patients with no evidence of fibrosis (non-fs EoE), and 7 non-EoE controls. Biopsies were collected from patients after informed consent as part of an IRB approved study at the Children's Hospital Colorado (Appendix S1: Methods). FS-EoE and non-fs EoE did not differ with respect to peak eos/hpf or inflammatory endoscopic features (EREFSi: exudate, edema, and furrows scores⁹). Study participants did differ with respect to the use of

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CONFLICT OF INTEREST

The authors have no competing financial and/or non-financial interests in relation to the work described.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

swallowed topical steroids, history of food impaction, and, by definition, fibrotic endoscopic features (EREFSf; esophageal rings and stricture scores), and esophageal distensibility. FS-EoE patients also had more endoscopies and appeared to be more recalcitrant to treatment (Appendix S1: Table S1 and Figure S1).

Distinct gene expression profiles were identified between controls (red) and both EoE groups [FS-EoE (blue) and non-fs EoE (green)] by way of principal components analysis (Figure 1A) and unsupervised hierarchical clustering (Figure 1B). After adjusting for unmeasured expression heterogeneity, we performed three pairwise comparisons of non-fs EoE versus controls (grey in Figure 1C), FS-EoE versus controls (white), and FS-EoE versus non-fs EoE (blue). The final dataset quantified the expression levels of 17,190 genes and detected 5866, 8097, and 1643 differentially expressed genes (DEGs), respectively, for the 3 comparisons (FDR<0.05). Although overlap exists between these comparisons, about one third of FS-EoE vs non-fs EoE DEGs are unique and distinguish the FS phenotype. (Figure 1C) Volcano plots demonstrate the relative distribution of up- and down-regulated genes (Figure 1D and Appendix S1: Figure S2). Approximately 90% of the DEGs identified between FS-EoE versus control follow a pattern in which non-fs EoE has expression intermediate between controls and FS EoE. (Figure 1E) Representative DEGs, were validated by RT-PCR analysis, including APOBEC3A, COL8A2, and TSPAN12 (Figure 1F). Genes exhibited the same directionality between the RNASeq analysis and RT-PCR results.

In addition to the primary comparisons, we assessed differential gene expression in relation to two ordinal features; patient reported dysphagia and EREFSf. There were 5507 genes significantly associated with dysphagia severity and 3846 genes significantly associated with EREFSf. The top 20 DEGs by p-value and effect size associated with EREFSf and dysphagia severity are presented (Figure 2A-D).

We utilized Enrichr overrepresentation analysis and fGSEA gene set enrichment analysis to assess gene ontogeny. Pathways enriched in the non-fs EoE vs control group were reflective of inflammatory pathways including interferon- γ response and interferon- α response, along with cell proliferation (Figure 2E). Pathways enriched in the FS-EoE vs non-fs EoE were related to cell proliferation, specifically DNA and chromosome replication including E2F targets. Cell proliferation pathways were also represented in the complementary fGSEA analysis; we measured a significant downregulation of GO:0006355 (regulation of transcription, DNA-templated) in FS-EoE compared with non-fs EoE and upregulation of GO:0007059 (chromosome segregation). Other pathways identified suggestive of extracellular matrix organization included GO:0030198 (extracellular matrix organization), GO:0030574 (collagen catabolic process), and GO:0044267 (cellular protein metabolic process) (Figure 2F).

Using an unbiased next generation sequencing, we identified 1643 genes differentially expressed in a pediatric and adolescent cohort FS-EoE compared with non-fs-EoE. Gene ontology analysis identified that cellular proliferation, extracellular matrix organization, and inflammatory processes gene sets to be significantly enriched in FS-EoE compared with

non-fs EoE. Our results can be leveraged to evaluate molecular indicators of this severe phenotype and identify potential therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

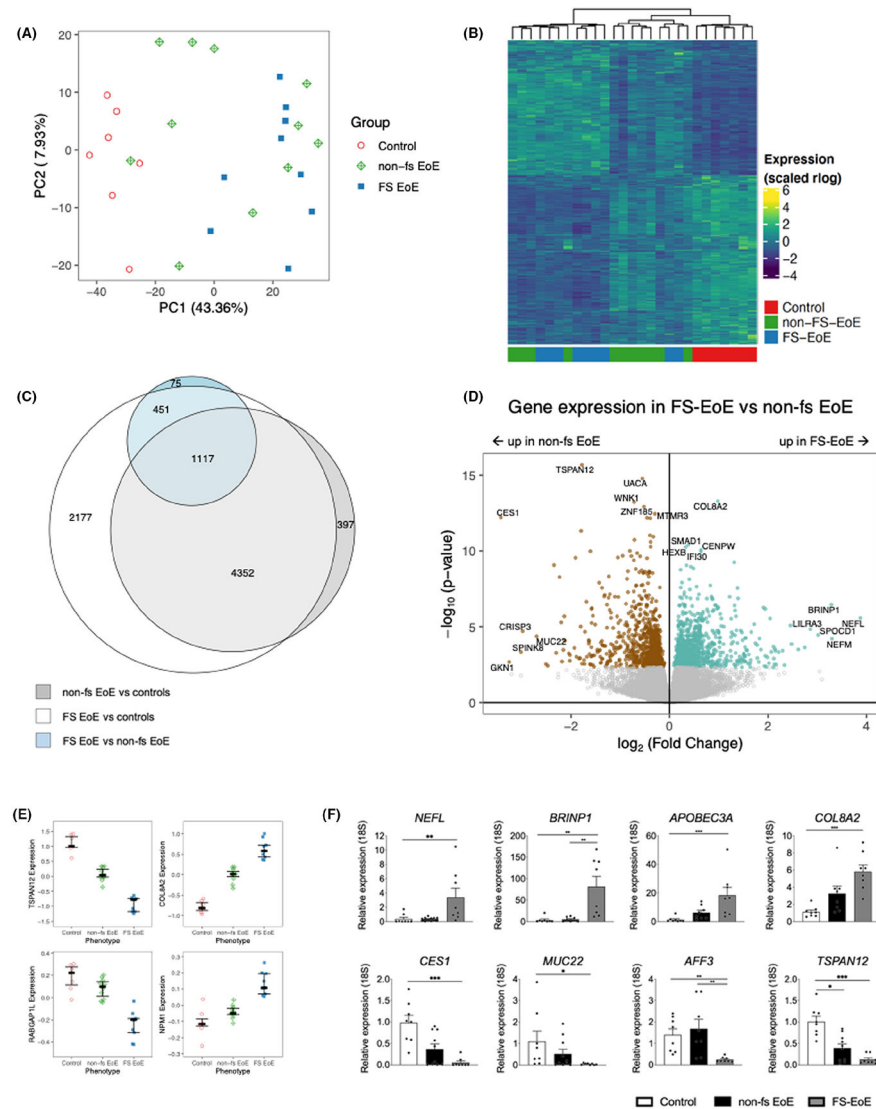
Drs. Amanda Muir and Alain Benitez are gratefully acknowledged for their input in the development of the algorithm used to calculate distensibility from raw EndoFLIP data. We thank our patients and study participants.

FUNDING INFORMATION

This publication has emanated from research supported by the NIH NIDDK under grant number K23DK109263 (CMK) and in part by a Grant from Science Foundation Ireland under Grant number FRL4863 (JCM). The opinions, findings and conclusions or recommendations expressed are those of the author(s) and do not necessarily reflect the views of the Science Foundation Ireland. Support (GTF) from LaCache Chair in Gastrointestinal Allergic and Immunological Diseases, Children's Hospital Colorado.

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**FIGURE 1.**

Differential gene expression in FS-EoE by RNA sequencing. (A) PCA plot. Each point represents one sample, colored by group. Samples closer in PC-space have more similar overall expression profiles. (B) Heatmap showing expression values and unsupervised clustering for genes identified to be significant in any pairwise between-group (FDR < 0.05, effect size of fold-change ≥ 2). Each column represents one sample. (C) The number of significant genes with differential expression (FDR < 0.05) for each pairwise comparison depicted in an Euler diagram. A total of 1643 were identified in the FS-EoE vs. non-fs EoE comparison. (D) Volcano plot depicting statistical significance, effect size, and directionality of FS-EoE vs non-fs EoE gene expression. Each point represents one gene; genes in the top 10 by either significance (p -value, y -axis) or effect size (x -axis) are labeled in text. (E) Residualized expression values for representative genes by phenotype. As seen, expression of non-fs EoE is intermediate between FS-EoE and controls in these examples regardless if statistically different between FS-EoE and non-fs EoE or not. (F) RT-PCR relative gene

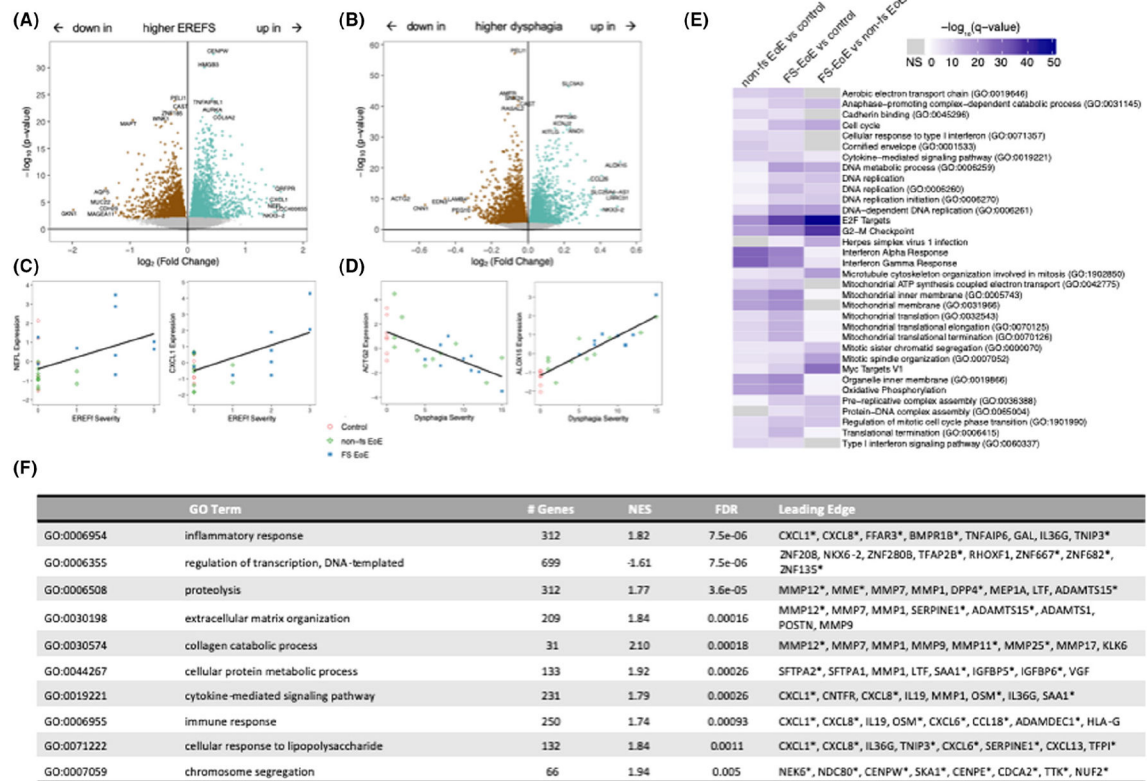
expression of representative differentially expressed genes. Control $n = 8$; non-fs EoE $n = 8$ or 10, FS-EoE $n = 8$. $* < 0.05$; $** < 0.01$; $*** < 0.001$.

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**FIGURE 2.**

(A, B) Volcano plot of genes identified to have differential expression based on ordinal variables, fibrotic endoscopic severity score and dysphagia severity. Plot depicts statistical significance, effect size, and directionality of gene expression associations. Each point represents one gene; genes in the top 10 by either p -value or by effect size labeled in text. Two genes associated with fibrotic endoscopic severity score (C) and with dysphagia severity (D) are shown with residualized expression values. Fold-changes are not directly comparable between scales and appear to be smaller for ordinal variables than categorical variables because they represent the average fold-change associated with a one-unit increase in score. (E) Heatmap of gene ontology enrichment analysis shows the top 15 most significant gene ontology (GO) terms as determined by $-\log_{10}(q\text{-value})$ for each pairwise comparison. (F) Additional pathway analysis with gene set enrichment analysis (fgSEA) for FS-EoE versus EoE comparison. Top 10 GO terms (by p -value) enriched in genes with higher effect sizes ($\log_2\text{FC}$). # Genes; e number of genes within the GO term tested within our dataset. NES; normalized enrichment score (NES; >1 if enriched in genes upregulated in FS-EoE, <1 if enriched in genes downregulated in FS-EoE). Leading edge; genes in the category that contribute most to enrichment (*significant genes within the leading edge). EREFSf = endoscopic severity score for fibrotic features (rings and stricture combined score).