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RNA sequencing confirms similarities between PPI-responsive oesophageal eosinophilia and eosinophilic oesophagitis

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Summary

Background: Although current American guidelines distinguish proton pump inhibitor-responsive oesophageal eosinophilia (PPI-REE) from eosinophilic oesophagitis (EoE), these entities are broadly similar. While two microarray studies showed that they have similar transcriptomes, more extensive RNA sequencing studies have not been done previously.

Aim: To determine whether RNA sequencing identifies genetic markers distinguishing PPI-REE from EoE.

Methods: We retrospectively examined 13 PPI-REE and 14 EoE biopsies, matched for tissue eosinophil content, and 14 normal controls. Patients and controls were not PPI-treated at the time of biopsy. We did RNA sequencing on formalin-fixed, paraffin-embedded tissue, with differential expression confirmation by quantitative polymerase chain reaction (PCR). We validated the use of formalin-fixed, paraffin-embedded vs RNAlater-preserved tissue, and compared our formalin-fixed, paraffin-embedded EoE results to a prior EoE study.

Results: By RNA sequencing, no genes were differentially expressed between the EoE and PPI-REE groups at the false discovery rate (FDR) $\leq$0.01 level. Compared to normal controls, 1996 genes were differentially expressed in the PPI-REE group and 1306 genes in the EoE group. By less stringent criteria, only MAPK8IP2 was differentially expressed between PPI-REE and EoE (FDR = 0.029, 2.2-fold less in EoE than in PPI-REE), with similar results by PCR. KCNJ2, which was differentially expressed in a prior study, was similar in the EoE and PPI-REE groups by both RNA sequencing and real-time PCR.

Conclusion: Eosinophilic oesophagitis and PPI-REE have comparable transcriptomes, confirming that they are part of the same disease continuum.

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1 | BACKGROUND

Although a close relationship between eosinophilic oesophagitis (EoE) and proton pump inhibitor-responsive oesophageal eosinophilia (PPI-REE) has long been hypothesised,[1] American guidelines for eosinophilic oesophagitis have distinguished PPI-REE from EoE.[2,3] Since then, extensive evidence has shown that, other than by proton pump inhibitor-response, these entities are mostly indistinguishable by clinical presentation,[4-9] endoscopic appearance[4-7,9] and histology.[4,5,7,9] The one exception is patients with more florid[4] or more extensive[10] oesophageal eosinophil infiltrates are modestly less likely to be PPI-responsive. A recent European task force concluded that PPI-REE is highly similar to EoE and potentially an EoE treatment variant.[11] Their main qualm was that it had not yet been shown that PPI-REE cases respond to dietary exclusion and thus antigen-induced immune responses. Shortly thereafter, 2 studies showed that proton pump inhibitor-responsive cases can also respond to food exclusion.[12,13] In addition to reducing gastric acidity (which reduces acid-related effects including altered permeability), PPIs, via Signal Transducer and Activator of Transcription 6 (STAT6), inhibit synthesis of eotaxin-3,[14,15] the dominant EoE chemokine.

Prior microarray-based gene expression studies comparing PPI-REE and EoE showed modest differences in the transcriptome, but these studies were either limited to 94 transcripts[16] or studied a limited number of subjects (6 PPI-REE and 4 EoE).[17] Wen et al[16] found that potassium voltage-gated channel subfamily J member 2 gene (KCNJ2) was a potential distinguishing factor in a study with 33 EoE and 28 Pre-PPI-REE subjects. The other study failed to identify this transcript as 1 of 35 transcripts found to be differentially regulated.[17] To our knowledge, neither study assessed MAPK8IP2 gene (C-jun-amino-terminal kinase-interacting protein 2).

Proton pump inhibitor-responsive oesophageal eosinophilia is worthy of study because it is a common finding. In prospective studies of patients presenting with oesophageal eosinophilia (≥15 eosinophils/maximal high power field [abbreviated as eos/HPF]), 36%-69% of patients have <15 eos/HPF after proton pump inhibitor therapy.[4,9,18-21] RNA sequencing (RNA-Seq) quantifies a large dynamic range of gene expression levels, improving the ability to detect differential expression. We hypothesised that although PPI-REE is closely related to EoE, there still could be transcriptome differences between them. We sought to further examine whether there are previously undetected differences between these 2 groups.

2 | METHODS

To assess EoE and PPI-REE gene expression, under Institutional Review Board (University of Utah #63027) approval, we examined 14 subjects with EoE by consensus criteria, 13 subjects with PPI-REE and 14 controls. Each biopsy was done prior to any proton pump inhibitor therapy. EoE patients were defined using 2011 consensus criteria[2]—requiring oesophageal eosinophilia on biopsy (≥15 eos/HPF) whose eosinophilia remained ≥15 eos/HPF after 8 weeks of double-dose proton pump inhibitor therapy. Chart review and other biopsies were also reviewed to confirm that other EoE consensus criteria were met, such as the presence of appropriate oesophageal symptoms and lack of comorbid disease such as Crohn's disease, etc. PPI-REE patients were defined as those patients with ≥15 eos/HPF on oesophageal biopsy prior to proton pump inhibitor therapy whose eosinophilia fell to <10 eos/HPF after 8 weeks of high-dose proton pump inhibitor therapy. Controls were age- and gender-matched patients with no history of current or prior oesophageal disease whose oesophageal biopsies were histologically normal. We found 64 patients with oesophageal biopsies meeting these criteria between 2012 and 2015. Oesophageal biopsies were located on 61 patients. Biopsies were examined for depth of tissue, eosinophil counts, and gastric or other tissue contamination. Twenty samples were excluded because they either had gastric, intestinal, or glandular metaplastic tissue contamination or had extensive subepithelial tissue (extending 10 μm or more beneath the epithelium). The remaining tissues (14 EoE, 13 PPI-REE and 14 controls) were examined by RNA sequencing. Age, atopy, serum total IgE and gender were recorded.

2.1 | RNA isolation, sequencing and genome alignment

Total RNA was isolated from 10-μm-thick, formalin-fixed, paraffin-embedded tissue using the High Pure FFPET RNA isolation kit (Roche, Indianapolis, Indiana). DNA was removed from each RNA sample by the manufacturer's suggested DNase 1-digestion step. RNA quantity was determined with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Ribosomal RNA was removed from each RNA sample prior to cDNA library preparation using the Illumina TruSeq Stranded Total RNA Sample Prep Protocol. An Illumina HiSeq 2500 instrument was used to sequence the polymerase chain reaction (PCR) amplified libraries using 50-cycle single-read chemistry. Sequencing reads were aligned to the GRCh37/Hg19 human reference genome using the Novoalign application (Novocraft, Selangor, Malaysia). The mean aligned read counts for the 41 formalin-fixed, paraffin-embedded RNA sequencing data sets was 14.9 million reads.

2.2 | Differential expression and clustering

The USeq DefinedRegionDifferentialSeq (DRDS) application was used to count reads intersecting exons of each annotated gene and score them for differential expression using DESeq2 negative binomial statistics with Benjamini-Hochberg adjustments.[22] RNA sequencing data were accepted as differentially expressed if they differed 2-fold or more with an adjusted p/false discovery rate (FDR) of ≤0.01. Visualisation tracks were prepared for each sample group using the USeq application Sam2USeq and viewed using the Integrated Genome Browser (IGB). Hierarchical clustering and

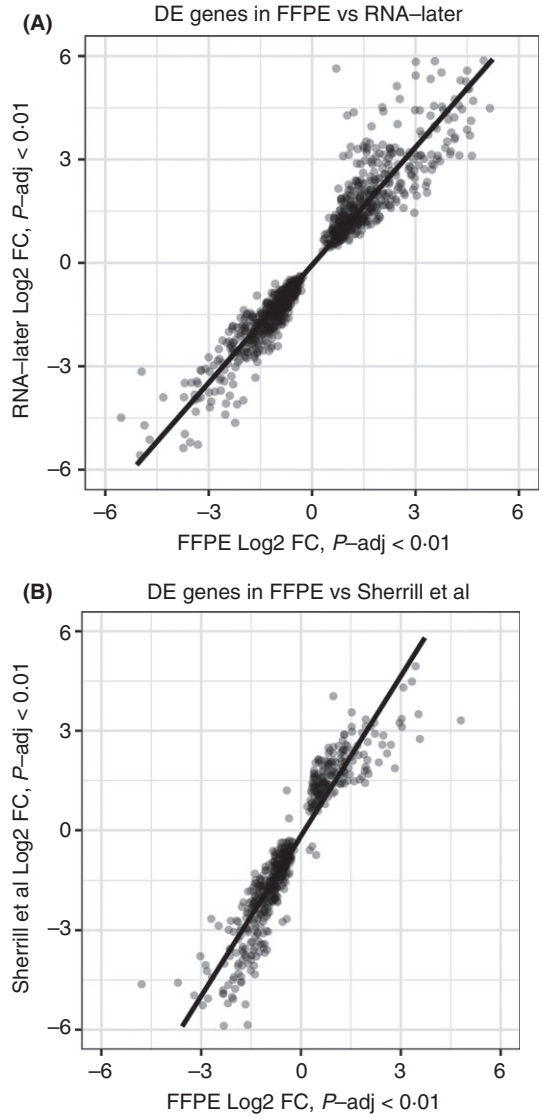


FIGURE 1 Validation comparing EoE/control expression ratios—formalin-fixed, paraffin-embedded tissues vs RNAlater, and formalin-fixed tissues vs a prior study. To validate the ratios of disease to normal RNA extracted from formalin-fixed tissues, we compared the EoE to control log 2 ratios for formalin-fixed, paraffin-embedded extracted RNA to those of well-preserved RNA. Only genes differentially expressed (DE) at the adjusted p/FDR < 0.01 level are used. In each, the formalin-fixed tissue results are on the *x* axis and well-preserved RNA results on the *y* axis. A, In a separate, preliminary data set, we compared our EoE formalin-fixed tissue results (n = 7 EoE and 6 controls) vs results from an RNAlater group (n = 6 EoE and 6 controls). There were 1529 differentially expressed genes, with excellent correlation; Pearson product moment correlation $r = .96$. B, We also compared our full formalin-fixed, paraffin-embedded tissue results (n = 14 EoE and 14 controls) to those of Sherrill et al[24] (n = 10 EoE and 6 controls). There were 624 differentially expressed genes, with very good correlation; Pearson product moment correlation $r = .92$. Both results are highly significantly correlated ($P < 2.2 \times 10^{-16}$ for each). EoE, eosinophilic oesophagitis; FDR, false discovery rate

principal component analysis were performed using Cluster 3.0 and the R application "rgl", respectively, to identify unique and common patterns of expression across each sample.

TABLE 1 Clinical characteristics of EoE and PPI-REE patients

	EoE (N = 14)	PPI-REE (N = 13)	Controls (N = 14)
Age (years, range)	38 (22-62)	48 (24-80)	43 (18-75)
% Male	71%	69%	64%
Max.tissue eos./hpf (mean, range)	39 (16-65)	35 (22-65)	0 (0-0)
Seasonal allergic conjunctivitis	62%	54%	
Asthma	25%	15%	
Allergic rhinitis	57%	54%	
Atopic dermatitis	7%	18%	
Allergic eye symptoms	57%	54%	
IgE (IU/mL serum)	399	542	

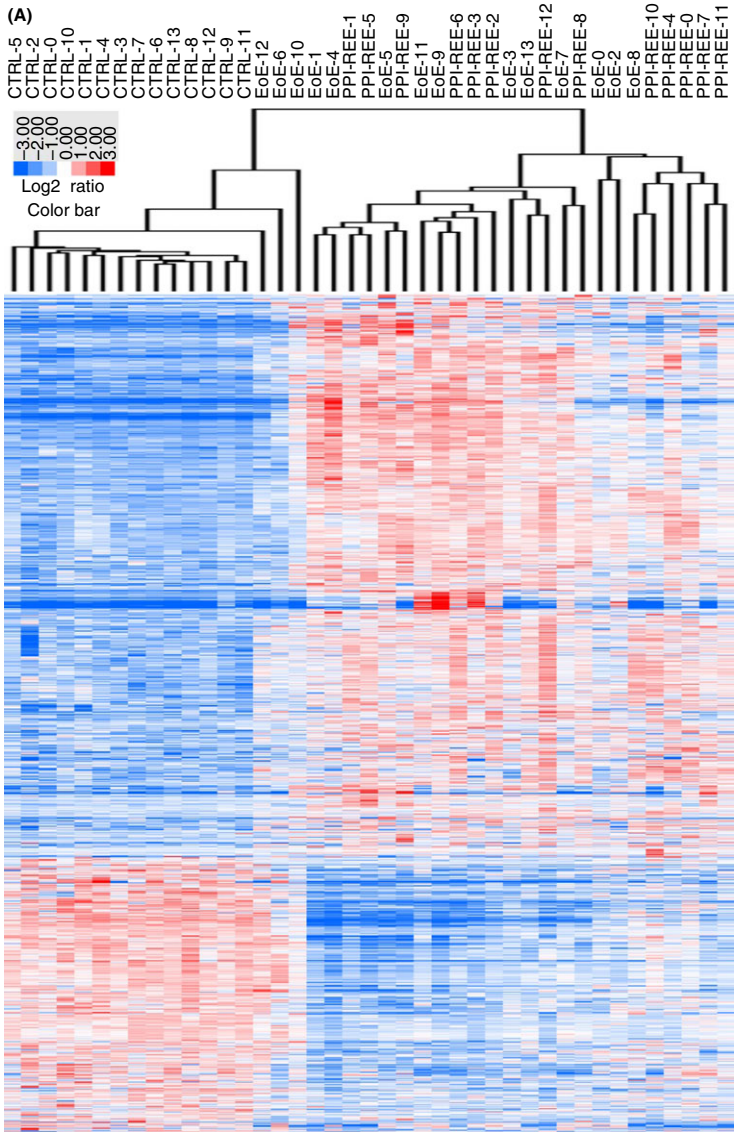
The mean age and gender of all 3 groups (EoE, PPI-REE and normal controls) did not significantly differ. Additionally, the maximal oesophageal tissue eosinophil content, IgE and clinical comorbidities did not differ significantly. Statistical comparison was done by Mann-Whitney *U* test for age, eosinophil counts and serum IgE. Proportions were compared by Fisher's exact test. $P = 0.20$ for age (EoE vs PPI-REE). For all other comparisons, $P \geq 0.37$. EoE, eosinophilic oesophagitis; PPI-REE, proton pump inhibitor-responsive oesophageal eosinophilia

2.3 | Formalin-fixed, paraffin-embedded data set validation

While RNA yields from formalin-fixed, paraffin-embedded tissues are similar to those of RNAlater-preserved tissue,[23] RNA extracted from formalin-fixed tissue is degraded to smaller fragments (typically peaking at 200-300 bases) than RNA from RNAlater-preserved tissue. Our validation study compared gene expression using formalin-fixed, paraffin-embedded tissue vs RNAlater-preserved samples. We used Illumina sequencing chemistry based on 50-base pair reads—indicating formalin-fixed tissue would be a viable alternative. We compared 7 formalin-fixed EoE oesophageal biopsies to 6 formalin-fixed controls, and 6 EoE RNAlater-preserved biopsies to 6 RNAlater-preserved normal controls. Our full study formalin-fixed, paraffin-embedded EoE and control data sets were similarly compared to those of Sherrill et al (Figure 1B)[24] which also used RNAlater-preserved mRNA.

2.4 | Real-time quantitative reverse transcription PCR validation

Subtle biological changes could be missed by DESeq2 statistics, so we also examined genes with 2-fold differences that were significant at $P < 0.05$. Thirty of our 41 RNA-Seq-analysed formalin-fixed RNA samples were used in Real-Time Quantitative Reverse Transcription PCR; 11 samples had an insufficient RNA after their use for RNA sequencing. Consequently, our real-time quantitative reverse transcription PCR sample set constituted these numbers per group: 12 EoE, 11 PPI-REE and 7 Control. For these 30 RNAs, total RNA was reverse transcribed to cDNA using the Applied Biosystems High Capacity RNA-to-cDNA Kit.



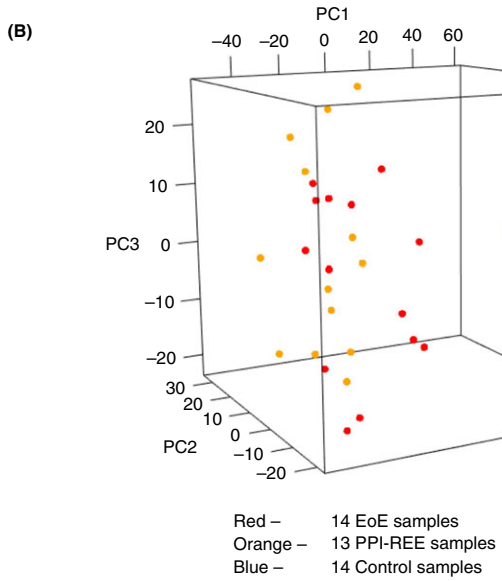


FIGURE 2 Hierarchical clustering and principal component analysis (PCA) of differentially expressed genes in EoE, PPI-REE and control oesophagus. A, Hierarchical clustering of 2247 genes differentially expressed (Fold ≥ 2, FDR < 0.01) in 1 or more patient groups. Two distinct clusters were observed including normal oesophagus (Cluster 1, blue at right) and combined EoE and PPI-REE oesophagus (Cluster 2, red plus orange, at left), suggesting a lack of appreciable difference between the 2 disease groups. B, Principle component analysis using the same 2247 genes shows 2 distinct sample groups similar to the hierarchical clustering. Principal components 1, 2 and 3 accounted for 14.8%, 5.0% and 4.6% of the variance respectively. EoE, eosinophilic oesophagitis; FDR, false discovery rate; PPI-REE, proton pump inhibitor-responsive oesophageal eosinophilia

Relative mRNA levels for *KCNJ2*, *MAPK8IP2* and reference gene, zinc finger DHHC-type containing 5 (*ZDHHC5*) were determined using intron-spanning TaqMan gene expression assays and TaqMan Gene Expression Master Mix (Applied Biosystems). ZDHHC5 was chosen as the reference gene for our PCRs' relative quantitation based on its moderately high expression level and its lack of shift according to RNA sequencing reads across our sample categories. About 10 μL quantitative PCRs were performed in 384-well plates and run in triplicate in a Life Technologies 12K Flex real-time PCR instrument. Fold change was determined using the ΔΔCT method and statistical significance determined with the Mann-Whitney *U* test.

3 | RESULTS

3.1 | Clinical studies

In our main cohort, the age and gender were not statistically different among all 3 groups (EoE, PPI-REE and controls, shown in Table 1). The tissue eosinophil content and serum IgE of the EoE and PPI-REE groups were also similar, and both groups had similar proportions of other atopic diseases.

3.2 | Validation of formalin-fixed vs well-preserved RNA results

In our preliminary validation study of 6-7 subjects per group, we found very similar gene expression between formalin-fixed and RNAlater-preserved tissue, as shown by heat map (Figure S1) and in the differential expression expressed as the log 2 ratio fold change for EoE vs controls among genes significantly differentially expressed adj. P/FDR ≤0.01 (Figure 1A, Pearson product moment correlation $r = .96$). As an additional validation step, we compared the differential expression between our main study cohort (EoE vs control log 2 ratio fold change) with that of Sherrill et al,[24] the only prior EoE RNA sequencing study. This showed good correlation (Figure 1B, $r = .92$). Our formalin-fixed, paraffin-embedded results had extremely significant correlations with both our RNAlater data and with that of Sherrill et al.

3.3 | Main cohort results

Analysis of the main cohort RNA sequencing data showed 1996 genes differentially expressed between PPI-REE and controls, with 1306 genes differentially expressed between EoE and controls. However, none of the genes were differentially expressed between EoE and PPI-REE at the adj. p/FDR ≤ 0.01 level. Hierarchical clustering and principal component analysis showed no clustering that differentiated EoE from PPI-REE (Figure 2A,B).

Using the less strict criterion of adj. p/FDR < 0.05, only 1 gene transcript, *MAPK8IP2*, was statistically significant at adj. P/FDR = 0.029. *MAPK8IP2* was upregulated 2.24-fold in PPI-REE vs EoE when visualised by normalised read counts in the Integrated Genome Browser. It was also significant by real-time quantitative reverse transcription PCR (Figure 3). The RNA sequencing *MAPK8IP2* PPI-REE results were 1.69-fold those of controls (FDR 0.0049). *MAPK8IP2* EoE results were 0.76-fold those of controls (FDR 0.20).

We evaluated potassium voltage-gated channel subfamily J member 2 (*KCNJ2*) on real-time quantitative reverse transcription PCR, which had been previously identified as differentially expressed between PPI-REE and EoE in Wen et al's microarray study. *KCNJ2* mRNA expression in PPI-REE was 1.25-fold that of EoE with adj. P/FDR = 0.68. Real-time quantitative reverse transcription PCR had a similar 1.30-fold difference which was not statistically significant ($P = .37$) between the groups (Figure 3).

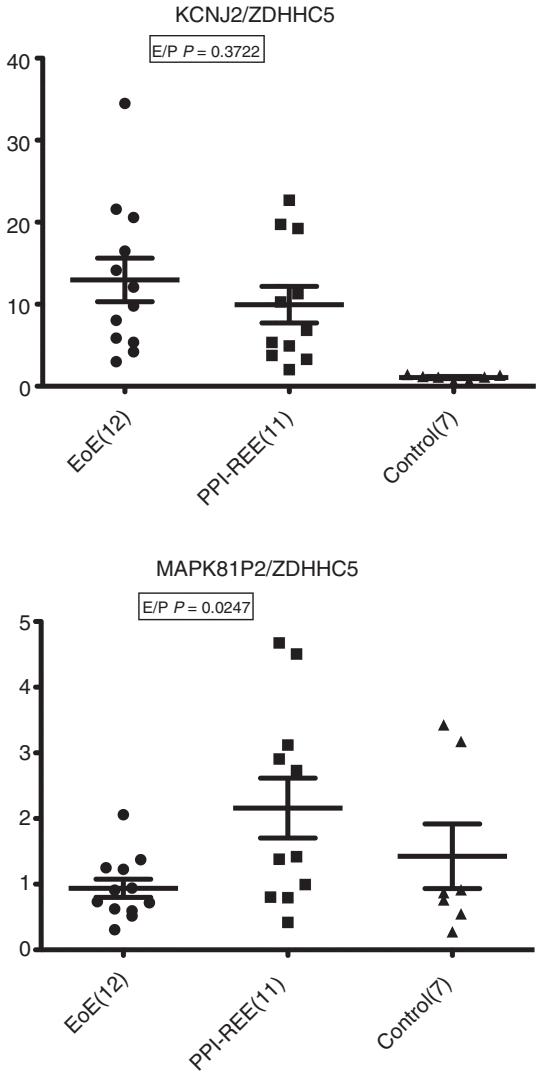


FIGURE 3 Real-time quantitative reverse transcription PCR results for KCNJ2 and MAPK8IP2. For KCNJ2, at top, the EoE cases were 1.30-fold those for PPI-REE, $P = 0.37$. By RNA sequencing they were 1.36-fold different, FDR = 0.68. For MAPK8IP2, at bottom, the EoE cases were 0.43-fold those for PPI-REE, $P = 0.025$. By RNA sequencing they were 0.45-fold different, FDR = 0.029. Although the PPI-REE cases were significantly higher, the EoE and PPI-REE ranges broadly overlapped. EoE, eosinophilic oesophagitis; FDR, false discovery rate; PCR, polymerase chain reaction; PPI-REE, proton pump inhibitor-responsive oesophageal eosinophilia

4 | DISCUSSION

In short, our results show that the EoE and PPI-REE transcriptomes are nearly identical, with no significant differences at the FDR ≤0.01 significance level. Only a single gene, MAPK8IP2, was different at the FDR ≤0.05 significance level, a finding that was confirmed by real-time quantitative reverse transcription PCR. To our knowledge, this is the first published RNA sequencing study comparing PPI-REE with EoE.

As previously mentioned, our findings fit well with the many studies showing that PPI-REE and EoE are highly related and, by many measures, indistinguishable.[4-9] Prior microarray-based studies also found relatively modest differences.[16,17]

Strengths of this study are that it is the first RNA sequencing comparison of moderately large numbers of EoE and PPI-REE samples and that the eosinophil contents were matched in both groups. By using formalin-fixed tissue, we could confirm that the tissue was representative and avoid using cases with contaminating gastric or other spurious tissue, or deep subepithelial tissue, problems that were present in 33% of our cases. In a recently published study, 43% of biopsies contain deep tissue.[25] Recently described static jaw biopsy forceps allow a far higher rate of subepithelial sampling.[26] We only studied subjects not taking proton pump inhibitors at the time of biopsy. Additionally, we compared groups of subjects that did not differ statistically from one another in regard to their oesophageal tissue eosinophil content, atopy, age and gender. Limitations include the moderate number of subjects studied, that the deep tissue, lymph nodes and other potentially relevant sites were not examined, and the use of formalin-fixed tissue with inherent RNA degradation. While our use of formalin-fixed tissue means that occasional genes will be poorly detected, our validation studies show excellent correlations with our RNAlater tissue as well as with Sherrill et al's EoE RNA sequencing data.[24] While our results are less similar to those of Sherrill et al than our own RNAlater cases, there are many possible reasons, including their different subject cohort and a variety of methodologic differences. However, our study has an average of 14.9 million reads per subject, while, among Sherrill et al's EoE group, most subjects had about 5 million reads per subject.[24] Numerous short human gene reads, rather than read lengths, appears to be a greater factor determining accurate RNA quantitation in RNA sequencing studies.[27] Finally, the RNA sequencing and real-time quantitative reverse transcription PCR results are quite similar for the 2 genes studied—KCNJ2 and MAPK8IP2. Earlier studies comparing formalin-fixed tissue vs well-preserved RNA for RNA sequencing have also supported the validity of formalin-fixed data.[23]

None of the genes were significantly different between EoE and PPI-REE at the FDR ≤0.01 significance level. Only MAPK8IP2 had a relatively weakly significant difference (FDR = 0.029) between the 2 groups. MAPK8IP2 was 2.2-fold greater in PPI-REE than in EoE, with very similar findings by real-time quantitative reverse transcription PCR analysis. Given large number of genes examined, it remains quite possible that this finding is spurious; additional validation would be needed to corroborate this finding. Furthermore, in about half of the PPI-REE subjects, the MAPK8IP2 contents are similar to those of the EoE subjects. This broad overlap suggests that, even if confirmed as statistically significant, this finding might be of little practical value. To our knowledge, this gene was not examined in the prior, microarray-based studies.[16,17] MAPK8IP2 has been identified in a panel of genes predicting clinical reactivity to food sensitivity.[28] In a murine model, it was upregulated on induction of Tregs.[29]

We found no significant difference between KCNJ2 gene in PPI-REE and EoE, which was previously identified as differentially expressed in Wen et al[16] but not Shoda et al.[17] Our care to avoid proton pump inhibitor-treated subjects and gastric or deep tissue contamination could have contributed to the difference in our findings. As mentioned above, when so many genes are analysed, it is difficult to exclude rare possible false positive findings.

These results emphasise the similarities between EoE and PPI-REE. While it remains possible that more fundamental changes will be found with further study, we theorise that PPI-REE is a subtype of EoE.

In summary, our RNA sequencing results show that EoE and PPI-REE have nearly identical transcriptomes. This corroborates abundant prior evidence that EoE and PPI-REE are closely related and appear to differ mainly in the degree of responsiveness to proton pump inhibitors. Additionally, this study helps demonstrate that formalin-fixed tissue-derived RNA can be used for RNA sequencing with results strongly correlating with those from well-preserved mRNA.

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AUTHORSHIP

Guarantor of the article: Kathryn Peterson.

Author contributions: Kathryn Peterson: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; study supervision. Don Delker, Mark Hazel: acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; administrative, technical, or material support; study supervision. Masaaki Yoshigi: study concept and design; obtained funding. Nicholas Consiglio: analysis and interpretation of data; drafting of the manuscript. Chaya Chrishnamurthy: acquisition of data; Jacob Robson: critical revision of the manuscript for important intellectual content. Frederic Clayton: acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; study supervision. Transcript Profiling: Our plan is that all data will be released/deposited to GEO once publication is accepted. Writing Assistance: Jordan Johnson B.S. Support via Utah Division of Gastroenterology.

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REFERENCES

1. Spechler SJ, Genta RM, Souza RF. Thoughts on the complex relationship between gastroesophageal reflux disease and eosinophilic esophagitis. *Am J Gastroenterol*. 2007;102:1301-1306.
2. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol*. 2011;128:3-20.
3. Dellon ES, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA; American College of Gastroenterology. ACG clinical guideline: evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol*. 2013;108:679-692; quiz 693.
4. Molina-Infante J, Ferrando-Lamana L, Ripoll C, et al. Esophageal eosinophilic infiltration responds to proton pump inhibition in most adults. *Clin Gastroenterol Hepatol*. 2011;9:110-117.
5. Warners MJ, van Rhijn BD, Curvers WL, Smout AJ, Bredenoord AJ. PPI-responsive esophageal eosinophilia cannot be distinguished from eosinophilic esophagitis by endoscopic signs. *Eur J Gastroenterol Hepatol*. 2015;27:506-511.
6. Iwakura N1, Fujiwara Y, Tanaka F, et al. Basophil infiltration in eosinophilic oesophagitis and proton pump inhibitor-responsive oesophageal eosinophilia. *Aliment Pharmacol Ther*. 2015;41:776-784.
7. Moawad FJ, Schoepfer AM, Safroneeva E, et al. Eosinophilic oesophagitis and proton pump inhibitor-responsive oesophageal eosinophilia have similar clinical, endoscopic and histological findings. *Aliment Pharmacol Ther*. 2014;39:603-608.
8. Molina-Infante J, Rivas MD, Hernandez-Alonso M, et al. Proton pump inhibitor-responsive oesophageal eosinophilia correlates with downregulation of eotaxin-3 and Th2 cytokines overexpression. *Aliment Pharmacol Ther*. 2014;40:955-965.
9. Dellon ES, Speck O, Woodward K, et al. Clinical and endoscopic characteristics do not reliably differentiate PPI-responsive esophageal eosinophilia and eosinophilic esophagitis in patients undergoing upper endoscopy: a prospective cohort study. *Am J Gastroenterol*. 2013;108:1854-1860.
10. Sayej WN, Patel R, Baker RD, Tron E, Baker SS. Treatment with high-dose proton pump inhibitors helps distinguish eosinophilic esophagitis from noneosinophilic esophagitis. *J Pediatr Gastroenterol Nutr*. 2009;49:393-399.
11. Molina-Infante J, Bredenoord AJ, Cheng E, et al. Proton pump inhibitor-responsive oesophageal eosinophilia: an entity challenging current diagnostic criteria for eosinophilic oesophagitis. *Gut* 2016;65:524-531.
12. Sodikoff J, Hirano I. Proton pump inhibitor-responsive esophageal eosinophilia does not preclude food-responsive eosinophilic esophagitis. *J Allergy Clin Immunol*. 2016;137:631-633.
13. Lucendo AJ, Arias Á, González-Cervera J, Olalla JM, Molina-Infante J. Dual response to dietary/topical steroid and proton pump inhibitor therapy in adult patients with eosinophilic esophagitis. *J Allergy Clin Immunol*. 2016;137:931-934. e2.
14. Cheng E, Zhang X, Huo X, et al. Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. *Gut*. 2013;62:824-832.
15. Zhang X, Cheng E, Huo X, et al. Omeprazole blocks STAT6 binding to the eotaxin-3 promoter in eosinophilic esophagitis cells. *PLoS ONE*. 2012;7:e50037.
16. Wen T, Dellon ES, Moawad FJ, Furuta GT, Aceves SS, Rothenberg ME. Transcriptome analysis of proton pump inhibitor-responsive esophageal eosinophilia reveals proton pump inhibitor-reversible allergic inflammation. *J Allergy Clin Immunol*. 2015;135:187-197.
17. Shoda T, Matsuda A, Nomura I, et al. Eosinophilic esophagitis versus proton pump inhibitor-responsive esophageal eosinophilia: Transcriptome analysis. *J Allergy Clin Immunol*. 2017;139:2010-2013. e4.
18. Gutiérrez-Junquera C, Fernández-Fernández S, Cilleruelo ML, et al. High prevalence of response to proton-pump inhibitor treatment in children with esophageal eosinophilia. *J Pediatr Gastroenterol Nutr*. 2016;62:704-710.
19. Peterson KA, Thomas KL, Hilden K, Emerson LL, Wills JC, Fang JC. Comparison of esomeprazole to aerosolized, swallowed fluticasone for eosinophilic esophagitis. *Dig Dis Sci*. 2010;55:1313-1319.
20. Vazquez-Elizondo G, Ngamruengphong S, Khrisna M, Devault KR, Talley NJ, Achem SR. The outcome of patients with oesophageal eosinophilic infiltration after an eight-week trial of a proton pump inhibitor. *Aliment Pharmacol Ther*. 2013;38:1312-1319.
21. van Rhijn BD, Weijenborg PW, Verheij J, et al. Proton pump inhibitors partially restore mucosal integrity in patients with proton pump inhibitor-responsive esophageal eosinophilia but not eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014;12:1815-1823. e2.
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57:289-300.
23. Zhao W, He X, Hoadley KA, Parker JS, Hayes DN, Perou CM. Comparison of RNA-Seq by poly (A) capture, ribosomal RNA depletion, and DNA microarray for expression profiling. *BMC Genom*. 2014;15:419.
24. Sherrill JD, Kc K, Blanchard C, et al. Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. *Genes Immun* 2014;15:361-369.
25. Wang J, Park JY, Huang R, Souza RF, Spechler SJ, Cheng E. Obtaining adequate lamina propria for subepithelial fibrosis evaluation in pediatric eosinophilic esophagitis. *Gastrointest Endosc* 2018;87:1207-1214.
26. Bussmann C, Schoepfer AM, Safroneeva E, et al. Comparison of different biopsy forceps models for tissue sampling in eosinophilic esophagitis. *Endoscopy*. 2016;48:1069-1075.
27. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*. 2011;12:323.
28. Martino D, Dang T, Sexton-Oates A, et al. Blood DNA methylation biomarkers predict clinical reactivity in food-sensitized infants. *J Allergy Clin Immunol*. 2015;135:1319-1328. e1-12.
29. Haghikia A, Jörg S, Duscha A, et al. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. *Immunity*. 2015;43:817-829.

SUPPORTING INFORMATION

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

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