Published in final edited form as:

Dis Esophagus. 2012 February ; 25(2): 166–174. doi:10.1111/j.1442-2050.2011.01230.x.

Markers of tyrosine kinase activity in eosinophilic esophagitis: A pilot study of the FIP1L1-PDGFRα fusion gene, pERK 1/2, and pSTAT5

Evan S. Dellon, MD MPH[1,2], **Jacquelyn J. Bower, PhD**[1], **Temitope O. Keku, MSPH PhD**[2], **Xiaoxin Chen, MD PhD**[2,3], **C. Ryan Miller, MD PhD**[4], **John T. Woosley, MD PhD**[4], **Roy C. Orlando, MD**[1,2], and **Nicholas J. Shaheen, MD MPH**[1,2]

[1]Center for Esophageal Diseases and Swallowing, University of North Carolina School of Medicine, Chapel Hill, NC

[2]Center for Gastrointestinal Biology and Disease, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC

[3]Biomedical/Biotechnology Research Institute, North Carolina Central University, Durham, NC

[4]Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC

Abstract

Background and Aims—The pathogenesis of eosinophilic esophagitis (EoE) is incompletely understood. In certain eosinophilic diseases, activation of tyrosine kinase after fusion of the Fip1-like-1 and platelet-derived growth factor receptor-α genes (F-P fusion gene) mediates eosinophilia via downstream effectors such as extracellular-regulated kinase (ERK1/2) and signal transducers and activators of transcription (STAT5). This mechanism has not been examined in EoE. Our aim was to detect the F-P fusion gene, pERK1/2, and pSTAT5 in esophageal tissue from patients with EoE, gastroesophageal reflux disease (GERD), and normal controls.

Materials and Methods—We performed a cross-sectional pilot study comparing patients with steroid-responsive and steroid-refractory EoE, to GERD patients and normal controls. EoE cases were defined by consensus guidelines. Fluorescence in-situ hybridization (FISH) was performed to detect the F-P fusion gene and immunohistochemistry (IHC) was performed to detect pERK1/2 and pSTAT5 in esophageal biopsies.

Results—Twenty-nine subjects (median age 30 yrs (range 1–59); 16 male; 24 Caucasian) were included: 8 normal, 6 GERD, and 15 EoE (5 steroid-refractory). On FISH, 98%, 99%, and 99% of the nuclei in the normal, GERD, and EoE groups, respectively, were normal (p=0.42). On IHC, a

Corresponding Author: Evan S. Dellon MD MPH, CB#7080, Bioinformatics Building, 130 Mason Farm Rd., UNC-CH, Chapel Hill, NC 27599-7080, Phone: (919) 966-2513, Fax: (919) 843-2508, edellon@med.unc.edu.

Selected data from this paper will be presented at DDW 2011.

Author contributions (all authors approved the final draft):
Dellon: study concept and design; data acquisition; analysis; interpretation; manuscript drafting/revision
Bower: fluorescence in-situ hybridization; critical revision
Keku: immunohistochemistry; critical revision
Chen: immunohistochemistry supervision; critical revision
Miller: immunohistochemistry supervision; critical revision
Woosley: performed eosinophil counts; critical revision
Orlando: study concept and design; critical revision
Shaheen: study concept and design; supervision; interpretation; critical revision

Potential competing interests: No conflicts of interest exist for any of the authors

median of 250, 277, and 479 nuclei/mm^2 stained for pERK1/2 in the normal, GERD, and EoE groups, respectively (p=0.07); the refractory EoE patients had the highest degree pERK1/2 staining (846 nuclei/mm^2; p=0.07). No trend was seen for pSTAT5.

Conclusions—The F-P fusion gene was not detected with increased frequency in EoE. Patients with EoE had a trend towards higher levels of pERK1/2, but not STAT5, in the esophageal epithelium, with highest levels in steroid-refractory EoE patients.

Keywords

Eosinophilic esophagitis; gastroesophageal reflux disease; tyrosine kinase; ERK 1/2; STAT5

Introduction

Eosinophilic esophagitis (EoE) is an increasingly recognized clinicopathologic condition defined in recent guidelines as esophageal dysfunction and esophageal eosinophilia in the absence of other potential causes including gastroesophageal reflux disease (GERD).[1, 2] The pathogenesis of eosinophilic esophagitis (EoE) is incompletely understood, with most research to date focusing on an allergen induced Th2 response.[3–8] In particular, treatment with elimination and elemental diets in children and adults has led to resolution of symptoms and normalization of esophageal histology, thus supporting the hypothesis of EoE as an immune-mediated disease.[9–12]

However, some children and many adults with EoE have no concomitant atopic diseases,[10, 13–17] and a proportion of EoE patients do not respond to corticosteroid therapy.[18, 19] This suggests that non-immune pathogenic mechanisms may exist, and other eosinophilic diseases may provide clues. In hypereosinophilic syndrome and chronic eosinophilic leukemia, a microdeletion on chromosome 4q12 fuses the Fip1-like-1 (FLP1L1) gene to the platelet-derived growth factor receptor-α gene (PDGFRα).[20, 21] This creates the FLP1L1- PDGFRα (F-P) fusion protein, a constitutively activated tyrosine kinase which has been shown to pathologically transform hematologic stem cells via a number of signal transduction pathways, including downstream effectors such as extracellular signal-regulated kinase (ERK 1/2) and signal transducers and activators of transcription 5 (STAT5).[20, 22] This results in preferential proliferation of eosinophils and mast cells,[20, 22, 23] lineages which are both implicated in EoE.[8, 24–27] It is unknown whether this mechanism may play a role in EoE.

The aims of this pilot study were to detect the presence of the F-P fusion gene by fluorescence in-situ hybridization (FISH) and to detect markers of tyrosine kinase activity (pERK 1/2 and pSTAT5) by immunohistochemistry (IHC) in esophageal tissue from patients with EoE, and compare this to results from patients with GERD and normal controls. We hypothesized that F-P and the tyrosine kinase markers would be detected in the EoE patients but not in the other groups. We further hypothesized that the markers would be detected more prominently in steroid-refractory EoE patients compared with steroid-responsive EoE patients.

Materials and methods

Study design and patients

We performed a retrospective cross-sectional pilot study of the University of North Carolina (UNC) EoE Clinicopathologic Database utilizing subjects from 2006–2008. Details of this database have been previously reported.[17] This study was approved by the UNC Institutional Review Board.

Cases were a convenience sample of adults (≥ 18 years) with a new diagnosis of EoE, defined by consensus guidelines.[1] Specifically, cases were required to have: symptoms of esophageal dysfunction (for example, dysphagia, food impaction, chest pain, or heartburn); at least 15 eosinophils per high-power field (eos/hpf) on esophageal biopsy; and other causes of eosinophilia including reflux excluded. We eliminated overlapping GERD by requiring persistent esophageal eosinophilia despite acid-suppression with twice-daily proton-pump inhibitor (PPI) therapy. We also selected a subgroup of cases with steroid-refractory EoE, defined as no response in symptoms or esophageal eosinophilia after sequential 8 week courses of both topical (ie swallowed fluticasone 880 mcg twice daily) and systemic steroids (ie prednisone 40 mg daily tapered over 8 weeks).

There were two control groups also comprised of a convenience sample of patients who underwent esophagogastroduodenoscopy (EGD) during the same time frame as the cases. GERD controls were patients who underwent EGD for a primary symptom of reflux disease (for example, heartburn or regurgitation; other concomitant symptoms were allowed), had a clinical response to PPI therapy, and had other potential causes of their presentation excluded. GERD patients with high levels of esophageal eosinophilia, or patients with esophageal eosinophilia responsive to proton-pump inhibitor therapy, were not specially sought for this pilot study. Normal controls were patients who underwent EGD for a primary non-esophageal indication (for example, abdominal pain, nausea, or vomiting; other concomitant symptoms were allowed) and had a normal esophageal biopsy.

Data sources and histology review

Clinical data were extracted from the UNC electronic medical record and included: demographics (age at diagnosis, gender, race); symptoms; the presence of atopic disease (allergic rhinitis or sinusitis, documented food allergy (demonstrated by either symptomatic evidence of allergy with reintroduction of a food or by testing directed by an Allergist), or asthma); and endoscopic findings (rings, strictures, a narrow-caliber esophagus, linear furrows, crêpe-paper mucosa, white plaques or exudates, erosive esophagitis, hiatal hernia, and Schatzki's ring).

Archived pathology slides were re-reviewed by the study pathologist (JTW) to determine eosinophil counts according to our previously validated protocol.[28] In brief, slides were masked and the maximum eosinophil density (eosinophils/mm^2) was determined after examination of five microscopy fields. For purposes of comparison to previous studies, eosinophil density was then converted to eos/hpf for an assumed hpf size of 0.24 mm^2, the size of an average field as reported in the literature.[29]

Fluorescence in-situ hybridization for the FIP1L1- PDGFRα fusion gene

Archived formalin-fixed paraffin-embedded tissue blocks were masked, sectioned (5 microns thick), and randomly sorted. Tissue sections were deparaffinized with xylene. Slides were successively incubated in 0.2 N HCl, a sodium thiocyanate pre-treatment solution (Abbott Diagnostics, Abbott Park, IL), and protease (Abbott Diagnostics, Abbott Park, IL) to denature the DNA and remove proteins from the tissue sections. Slides were then sequentially dehydrated in 70%, 85%, and 100% ethanol, and air dried. A Vysis LSI 4q12 Tricolor Spectrum Orange/Spectrum Green/Spectrum Aqua rearrangement FISH probe (Abbott Molecular, Des Plaines, IL) was hybridized to the tissue sections for 48 hours using a Vysis HyBrite (Abbott Diagnostics, Abbott Park, IL) according to the probe manufacturer's instructions. Gene copy numbers were assessed from the entire esophageal biopsy sample in a blind manner using an Ikoniskope Digital Microscopy System (Ikonisys, Inc., New Haven, CT).

The tricolor probe is constructed such that the green signal is upstream of the FIP1L1 gene, the aqua signal is downstream of the PDGFRα gene, and the orange signal is located in the deleted region.[20, 21] When there is a deletion and the F-P fusion gene is created, the orange signal is no longer visualized. Therefore, a nucleus was categorized as normal if it had either one or two copies of all three color probes present (Figure 1). A nucleus was defined as diseased if there were two copies of the green signal and two copies of the blue signal, but one or zero copies of the orange signal.

Immunohistochemistry for pERK 1/2 and pSTAT5 and cell counts

Archived formalin-fixed paraffin-embedded tissue blocks were masked, sectioned (5 microns thick), and randomly sorted. Slides were deparaffinized with xylene, dehydrated with ethanol, and steam-treated for antigen retrieval (10mM citrate buffer, pH 6.0). For pERK 1/2 IHC, slides were incubated with a rabbit anti-human phosph-p44/42 ERK1/2 primary antibody (clone 20G11; 1:50 dilution; Cell Signaling Technology, Danvers, MA). For pSTAT5 IHC, slides were incubated with a rabbit anti-human phospho-STAT5 primary antibody (clone C11C5; 1:300 dilution; Cell Signaling Technology, Danvers, MA). Slides were then incubated with a peroxidase-labelled anti-rabbit secondary antibody (Envision; Dako, Carpinteria, CA), stained with a diaminobenzidine chromogen (DAB; Dako, Carpinteria, CA), and then counterstained with hematoxylin. Breast and colorectal tissue were used as controls, as per manufacturer recommendations. Positive control slides were incubated with primary antibody while only antibody diluent (Dako, Carpinteria, CA) was added to the negative control slides.

In a protocol similar to that for eosinophil counts, the masked IHC glass slides were scanned and digital slides were viewed with Aperio ImageScope (Aperio Technologies, Vista, CA). The maximum number of positively staining pERK 1/2 and pSTAT5 nuclei were quantified (nuclei/mm^2) in five microscopy fields using the Aperio Positive Pixel Count Algorithm (version 9.1, Aperio Technologies, Vista, CA). In this protocol, only positive nuclei were quantified; we did not examine cytoplasmic staining for the purposes of this study because pERK 1/2 and pSTAT5, once activated, mediate their signal transduction effects in the nucleus.

Statistical analysis

Summary statistics were used to describe the study groups. Given the small sample size and non-normal distribution of the data, non-parametric tests were used for bivariate analysis. Fisher's Exact test was used to compare categorical variables and the Kruskal-Wallis test was used for continuous variables. Correlation between markers of tyrosine kinase activation and eosinophil count was performed with Spearman's Rho. Using EoE case status as the outcome, receiver operating characteristic (ROC) curves were constructed and the area under the curve (AUC) calculated. Analyses were performed using Stata version 9 (Statacorp, College Station, TX).

Results

Patient characteristics

A total of 29 subjects (median age 30 yrs (range 1–59); 16 male; 24 Caucasian) with appropriate archived tissue were identified and included in the study; 8 were normal controls, 6 were GERD controls, and 15 were EoE cases, 5 of whom were steroid refractory (Table 1). While EoE patients had a lower median age compared to normal and GERD patients (27 vs 47 vs 41 years), this was not statistically significant. There were a higher proportion of males (80%) in the EoE group compared to the normal (13%) and GERD (50%) groups. Symptoms and endoscopic findings tended to vary by group, as expected by

the study definitions. For example, abdominal pain, nausea, or vomiting was the primary symptom/EGD indication for all of the normal patients and heartburn/reflux was the primary symptom/EGD indication for all of the GERD patients. Similarly, a normal EGD was more common in the normal group, and rings, linear furrows, and a narrow caliber esophagus tended to be more common in the EoE group. On esophageal biopsy, the maximum eosinophil count was 0, 1, and 75 eos/hpf, in the normal, GERD, and EoE groups, respectively ($p<0.001$).

FISH for the FIP1L1- PDGFRα fusion gene

Samples from all 29 subjects underwent FISH. Overall, a median of 171 nuclei per subject could be scored in the normal group, 171 in the GERD group, and 139 in the EoE group ($p=0.36$) (Table 2). There was no difference in the median proportion of normal nuclei in the normal, GERD, and EoE groups (98 vs 99 vs 99%; $p=0.42$) (Figure 1). In the subgroup of five steroid-refractory EoE patients, the F-P fusion gene was also not detected; 99% of the nuclei in this group were normal. In two patients, one in the normal group and one in the EoE group, 4% of the nuclei were classified as diseased.

IHC for pERK 1/2 and pSTAT5

Samples for 22 of the study subjects were sufficient for IHC, 7 in the normal group, 6 in the GERD group, and 9 in the EoE group, 2 of whom were steroid refractory; the other 7 patients in the study did not have adequate tissue samples remaining after the FISH analysis. Demographic characteristics and eosinophil counts were comparable to the full study population (Table 3).

There was a trend towards increased pERK 1/2 nuclear staining in the EoE group compared with the normal and control groups, but this did not reach statistical significance (Figures 2 and 3). Specifically, the median number of nuclei/mm^2 staining for pERK 1/2 was 250, 277, and 479 in the normal, GERD, and EoE groups, respectively ($p=0.07$) (Table 3). There was a similar trend ($p=0.07$) towards stronger staining when the analysis was repeated with the steroid-responsive and refractory EoE patients separated (Figure 4). Using pERK 1/2 staining alone for diagnosis of EoE vs either normal or GERD, the ROC area under the curve was 0.795. pERK 1/2 poorly correlated with eosinophil count (Spearman's rho=0.36; $p=0.10$).

No clear trend was seen with pSTAT5 staining overall. The median number of nuclei/mm^2 staining for pSTAT5 was 748, 506, and 676 in the normal, GERD, and EoE groups, respectively ($p=0.74$). While the value was higher in the refractory EoE group, this also was not statistically significant (Figure 4).

Discussion

It has been postulated that EoE is an immune-mediated disease based on data showing high rates of concomitant atopic disease and response to dietary elimination therapy, as well as pathogenic studies in both animal models and humans.[3–12] However, a proportion of patients, particularly adults with EoE, have neither associated atopic disease nor identifiable food allergies.[10, 13, 14, 16, 17] This raises a question about whether there might be a non-allergic mechanism that results in the clinical EoE phenotype. Based on the pathogenesis of other eosinophilic disorders, we aimed to detect the presence of the F-P fusion gene and markers of tyrosine kinase activity such as pERK 1/2 and pSTAT5 in patients with EoE compared to those with GERD and normal controls. In this initial pilot study, we did not detect the F-P fusion gene, but found that there was a trend towards increased pERK 1/2 but

not pSTAT5 in EoE patients. This finding is hypothesis generating, potentially suggesting activation of a tyrosine kinase in an F-P independent fashion.

ERK1/2 are members of one of the three major groups in the mitogen-activated protein kinases (MAPK) family and have myriad functions both in health and disease.[30–32] In particular, ERK1/2 play a role in translating extracellular stimuli, including growth factors and cytokines, into intracellular processes resulting in cell proliferation, differentiation, migration, inflammation, and survival.[20–23, 30–32] The role of ERK1/2 signaling has been well described in hematopoiesis, including in eosinophil maturation and development, and has also been implicated in preferential proliferation of eosinophils and mast cells in conditions such as hypereosinophilic syndrome and chronic eosinophilic leukemia.[20, 22, 23, 31] ERK1/2 have been implicated in airway eosinophilia and inflammation in patients with asthma.[33–36] They have also been shown to mediate the effects of IL-5, IL-4, IL-13, and eotaxin on eosinophils[33, 37–39] and are required for eotaxin-stimulated eosinophil degranulation.[40]

How might this trend towards increased pERK1/2 be interpreted in the context of EoE? There are several potential hypotheses. First, it is possible that pERK1/2 is increased in EoE as a consequence of the known Th2-response (stimulation via IL-4, IL-5, or IL-13) or from increased eotaxin, as suggested by the studies above. This would be in line with the presumed immune-mediated pathogenesis of EoE. Second, a recent study has shown that fibroblast growth factor, a protein that is also upregulated in EoE,[41] enhances signaling through an ERK-dependent pathway in EoE; of note is that the family of fibroblast growth factor receptors has an intracellular tyrosine kinase domain.[42] Third, upregulated ERK signaling has been shown to result in eosinophil activation following stimulation of Toll-like receptors by selected microbial products,[43] potentially suggesting a non-allergic mechanism of eosinophilia. Finally, it is also possible that the increased pERK1/2 is simply a marker of eosinophil activation, regardless of the specific cause. In our study, it does not appear the trend towards increased pERK1/2 is due to the concomitant presence of the F-P fusion gene, but the exact cause cannot be determined from our study design.

When interpreting the data from this study, there are several potential limitations to consider. First, this was a single-center retrospective pilot study with a small sample size. This is a substantial drawback as it added variability to the data (for example, esophageal biopsy location was not standardized), precluded multivariable analysis, and limited the power to detect associations, so results may be subject to type II error. However, we attempted to counter this by carefully defining the study groups and characterizing them extensively, both clinically and histologically. In particular, the EoE group was confirmed to have persistent symptoms and esophageal eosinophilia while on high dose acid suppression in accordance with consensus diagnostic guidelines, so there is likely little overlap between the GERD and the EoE groups. Moreover, we included a steroid-refractory EoE group, a sub-population of EoE patients that has yet to be studied extensively, with the hope of identifying the subgroup most likely to have a non-immune mediated esophageal eosinophilia. Second, because our main IHC measure was nuclei/mm^2, there is a possibility that the trend in the pERK 1/2 group could simply be due to increase cellular infiltration of the esophageal epithelium. However, were this to be true, there should be the same trend with pSTAT5 staining that there was with pERK 1/2, but this is not the case. Also, the eosinophil count only poorly correlates with the level of pERK 1/2 staining, which would also not be the case if the pERK 1/2 trend were only due to a general increase in cells. Third, because this study is cross-sectional, cause-and-effect cannot be determined. However, this study succeeded in generating several potential hypotheses that could explain increased pERK1/2 which would need to be addressed in subsequent work.

In addition to mechanistic questions, this study raises other issues as well. Might pERK1/2 be useful for either diagnosis or predicting treatment response in EoE? Since there was a trend towards pERK1/2 elevation in EoE as compared to GERD or normal controls, it may be a candidate tissue biomarker of the disease. Since it only poorly correlated with the eosinophil count, it might provide diagnostic utility beyond the eosinophil count alone. However, because we did not reach a threshold of statistical significance, the ROC analysis must be interpreted conservatively. Similarly, the small number of steroid refractory patients included had high levels of pERK1/2 before treatment (all examined tissue was from the time of diagnosis and prior to topical or systemic steroid therapy), suggesting an area of future study to determine whether pERK1/2 has a role in early identification of this difficult-to-treat population.

In conclusion, this pilot study found that the FIP1L1- PDGFRα fusion gene was not detected with increased frequency in EoE as compared to GERD or normal controls, even when the analysis was limited to steroid-refractory EoE patients. Therefore, the F-P fusion gene does not appear to be a pathogenic mechanism in EoE. However, the data suggest that patients with EoE may have higher levels of pERK 1/2 as a marker of tyrosine kinase activation in the esophageal epithelium. Levels are highest in the steroid-refractory EoE population. Larger studies are required to understand the role of this factor in the pathogenesis of EoE, as well as its utility for diagnosis and predicting treatment response.

Abbreviations

AUC	area under the curve
EGD	esophagogastroduodenoscopy
EoE	eosinophilic esophagitis
eos/hpf	eosinophils per high-power field
FISH	fluorescence in-situ hybridization
F-P	FIP1L1- PDGFRα fusion gene
GERD	gastroesophageal reflux disease
IHC	immunohistochemistry
mm^2	square millimeters
MAPK	mitogen-activated protein kinases
pERK 1/2	phosphorylated extracellular-regulated kinase
PPI	proton-pump inhibitor
pSTAT5	phosphorylated signal transducers and activators of transcription 5
ROC	receiver operator characteristic
UNC	University of North Carolina

Acknowledgments

We would like to acknowledge Wen Sui, PhD and Amber McCoy, BA for their assistance with technical aspects of the study.

Financial support: This work is funded, in part, by NIH award number KL2RR025746 from the National Center for Research Resources, and utilized the Histology Core of the UNC Center for Gastrointestinal Biology and Disease which is funded by NIH award number P30DK034987. The study sponsors had no role in the study design, collection, analysis, or interpretation of the data.

References

1. Furuta GT, Liacouras CA, Collins MH, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007; 133(4):1342–1363. [PubMed: 17919504]
2. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: Updated consensus recommendations for children and adults. J Allergy Clin Immunol. 2011
3. Straumann A, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. J Allergy Clin Immunol. 2001; 108(6):954–961. [PubMed: 11742273]
4. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. J Clin Invest. 2001; 107(1):83–90. [PubMed: 11134183]
5. Straumann A, Kristl J, Conus S, et al. Cytokine expression in healthy and inflamed mucosa: probing the role of eosinophils in the digestive tract. Inflamm Bowel Dis. 2005; 11(8):720–726. [PubMed: 16043986]
6. Gupta SK, Fitzgerald JF, Kondratyuk T, HogenEsch H. Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2006; 42(1):22–26. [PubMed: 16385249]
7. Blanchard C, Wang N, Stringer KF, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest. 2006; 116(2):536–547. [PubMed: 16453027]
8. Rothenberg ME. Biology and treatment of eosinophilic esophagitis. Gastroenterology. 2009; 137(4): 1238–1249. [PubMed: 19596009]
9. Markowitz JE, Spergel JM, Ruchelli E, Liacouras CA. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. Am J Gastroenterol. 2003; 98(4):777–782. [PubMed: 12738455]
10. Liacouras CA, Spergel JM, Ruchelli E, et al. Eosinophilic esophagitis: a 10-year experience in 381 children. Clin Gastroenterol Hepatol. 2005; 3(12):1198–1206. [PubMed: 16361045]
11. Kagalwalla AF, Sentongo TA, Ritz S, et al. Effect of Six-Food Elimination Diet on Clinical and Histologic Outcomes in Eosinophilic Esophagitis. Clin Gastroenterol Hepatol. 2006; 4:1097–1102. [PubMed: 16860614]
12. Gonsalves N, Yang G-Y, Doerfler B, Ritz S, Ditto AM, Hirano I. A prospective clinical trial of six food elimination diet and reintroduction of causative agents in adults with eosinophilic esophagitis. Gastroenterology. 2008; 134 Suppl 1:S104–S105. (A727).
13. Penfield JD, Lang DM, Goldblum JR, Lopez R, Falk GW. The Role of Allergy Evaluation in Adults With Eosinophilic Esophagitis. J Clin Gastroenterol. 2009
14. Roy-Ghanta S, Larosa DF, Katzka DA. Atopic Characteristics of Adult Patients With Eosinophilic Esophagitis. Clin Gastroenterol Hepatol. 2008; 6:531–535. [PubMed: 18304887]
15. Assa'ad AH, Putnam PE, Collins MH, et al. Pediatric patients with eosinophilic esophagitis: an 8-year follow-up. J Allergy Clin Immunol. 2007; 119(3):731–738. [PubMed: 17258309]
16. Spergel JM, Brown-Whitehorn TF, Beausoleil JL, et al. 14 years of eosinophilic esophagitis: clinical features and prognosis. J Pediatr Gastroenterol Nutr. 2009; 48(1):30–36. [PubMed: 19172120]
17. Dellon ES, Gibbs WB, Fritchie KJ, et al. Clinical, endoscopic, and histologic findings distinguish eosinophilic esophagitis from gastroesophageal reflux disease. Clin Gastroenterol Hepatol. 2009; 104:2695–2703.
18. Konikoff MR, Noel RJ, Blanchard C, et al. A randomized, double-blind, placebo-controlled trial of fluticasone propionate for pediatric eosinophilic esophagitis. Gastroenterology. 2006; 131(5): 1381–1391. [PubMed: 17101314]
19. Straumann A, Conus S, Degen L, et al. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. Gastroenterology. 2010; 139(5):1526–1537. 37 e1. [PubMed: 20682320]
20. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003; 348(13):1201–1214. [PubMed: 12660384]

21. Gotlib J, Cools J, Malone JM 3rd, Schrier SL, Gilliland DG, Coutre SE. The FIP1L1-PDGFRalpha fusion tyrosine kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. Blood. 2004; 103(8):2879–2891. [PubMed: 15070659]
22. Buitenhuis M, Verhagen LP, Cools J, Coffer PJ. Molecular mechanisms underlying FIP1L1-PDGFRA-mediated myeloproliferation. Cancer Res. 2007; 67(8):3759–3766. [PubMed: 17440089]
23. Robyn J, Lemery S, McCoy JP, et al. Multilineage involvement of the fusion gene in patients with FIP1L1/PDGFRA-positive hypereosinophilic syndrome. Br J Haematol. 2006; 132(3):286–292. [PubMed: 16409293]
24. Kirsch R, Bokhary R, Marcon MA, Cutz E. Activated mucosal mast cells differentiate eosinophilic (allergic) esophagitis from gastroesophageal reflux disease. J Pediatr Gastroenterol Nutr. 2007; 44(1):20–26. [PubMed: 17204948]
25. Lucendo AJ, Navarro M, Comas C, et al. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. Am J Surg Pathol. 2007; 31(4):598–606. [PubMed: 17414108]
26. Abonia JP, Blanchard C, Butz BB, et al. Involvement of mast cells in eosinophilic esophagitis. J Allergy Clin Immunol. 2010; 126:140–149. [PubMed: 20538331]
27. Dellon ES, Chen X, Miller CR, et al. Tryptase staining of mast cells may differentiate eosinophilic esophagitis from gastroesophageal reflux disease. Am J Gastroenterol. 2011; 106:264–271. [PubMed: 20978486]
28. Dellon ES, Fritchie KJ, Rubinas TC, Woosley JT, Shaheen NJ. Inter- and intraobserver reliability and validation of a new method for determination of eosinophil counts in patients with esophageal eosinophilia. Dig Dis Sci. 2010; 55(7):1940–1949. [PubMed: 19830560]
29. Dellon ES, Aderoju A, Woosley JT, Sandler RS, Shaheen NJ. Variability in diagnostic criteria for eosinophilic esophagitis: A systematic review. Am J Gastro. 2007; 102:2300–2313.
30. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta. 2010; 1802(4):396–405. [PubMed: 20079433]
31. Geest CR, Coffer PJ. MAPK signaling pathways in the regulation of hematopoiesis. J Leukoc Biol. 2009; 86(2):237–250. [PubMed: 19498045]
32. Coskun M, Olsen J, Seidelin JB, Nielsen OH. MAP kinases in inflammatory bowel disease. Clin Chim Acta. 2011; 412(7–8):513–520. [PubMed: 21185271]
33. Bates ME, Green VL, Bertics PJ. ERK1 and ERK2 activation by chemotactic factors in human eosinophils is interleukin 5-dependent and contributes to leukotriene C(4) biosynthesis. J Biol Chem. 2000; 275(15):10968–10975. [PubMed: 10753897]
34. Cui CH, Adachi T, Oyamada H, et al. The role of mitogen-activated protein kinases in eotaxin-induced cytokine production from bronchial epithelial cells. Am J Respir Cell Mol Biol. 2002; 27(3):329–335. [PubMed: 12204895]
35. Langlois A, Chouinard F, Flamand N, Ferland C, Rola-Pleszczynski M, Laviolette M. Crucial implication of protein kinase C (PKC)-delta, PKC-zeta, ERK-1/2, and p38 MAPK in migration of human asthmatic eosinophils. J Leukoc Biol. 2009; 85(4):656–663. [PubMed: 19164129]
36. Ohnishi H, Takeda K, Domenico J, et al. Mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2-dependent pathways are essential for CD8+ T cell-mediated airway hyperresponsiveness and inflammation. J Allergy Clin Immunol. 2009; 123(1):249–257. [PubMed: 19130938]
37. Adachi T, Choudhury BK, Stafford S, Sur S, Alam R. The differential role of extracellular signal-regulated kinases and p38 mitogen-activated protein kinase in eosinophil functions. J Immunol. 2000; 165(4):2198–2204. [PubMed: 10925307]
38. Moore PE, Church TL, Chism DD, Panettieri RA Jr, Shore SA. IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. Am J Physiol Lung Cell Mol Physiol. 2002; 282(4):L847–L853. [PubMed: 11880312]

39. Zhu X, Jacobs B, Boetticher E, et al. IL-5-induced integrin adhesion of human eosinophils caused by ERK1/2-mediated activation of cPLA2. J Leukoc Biol. 2002; 72(5):1046–1053. [PubMed: 12429728]

40. Kampen GT, Stafford S, Adachi T, et al. Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases. Blood. 2000; 95(6):1911–1917. [PubMed: 10706854]

41. Mulder DJ, Pacheco I, Hurlbut DJ, et al. FGF9-induced proliferative response to eosinophilic inflammation in oesophagitis. Gut. 2009; 58(2):166–173. [PubMed: 18978176]

42. Huang JJ, Joh JW, Fuentebella J, et al. Eotaxin and FGF enhance signaling through an Extracellular signal-related kinase (ERK)-dependent pathway in the pathogenesis of Eosinophilic Esophagitis. Allergy Asthma Clin Immunol. 2010; 6(1):25. [PubMed: 20815913]

43. Cheung PF, Wong CK, Ip WK, Lam CW. FAK-mediated activation of ERK for eosinophil migration: a novel mechanism for infection-induced allergic inflammation. Int Immunol. 2008; 20(3):353–363. [PubMed: 18182379]

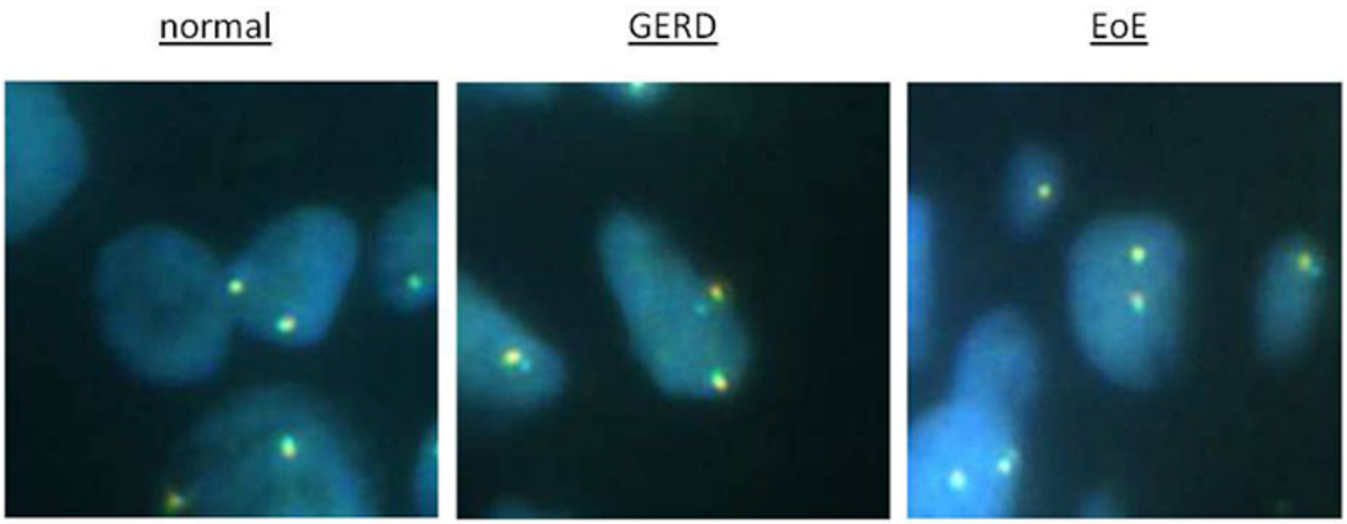

Figure 1.
Examples of FISH in normal, GERD, and EoE patients examined with composite images from fluorescence microscopy (100× magnification). The nuclei are highlighted with a DAPI stain. In each panel, the nuclei are normal and the nucleus in the middle demonstrates all three probe signals: aqua, orange, and green.

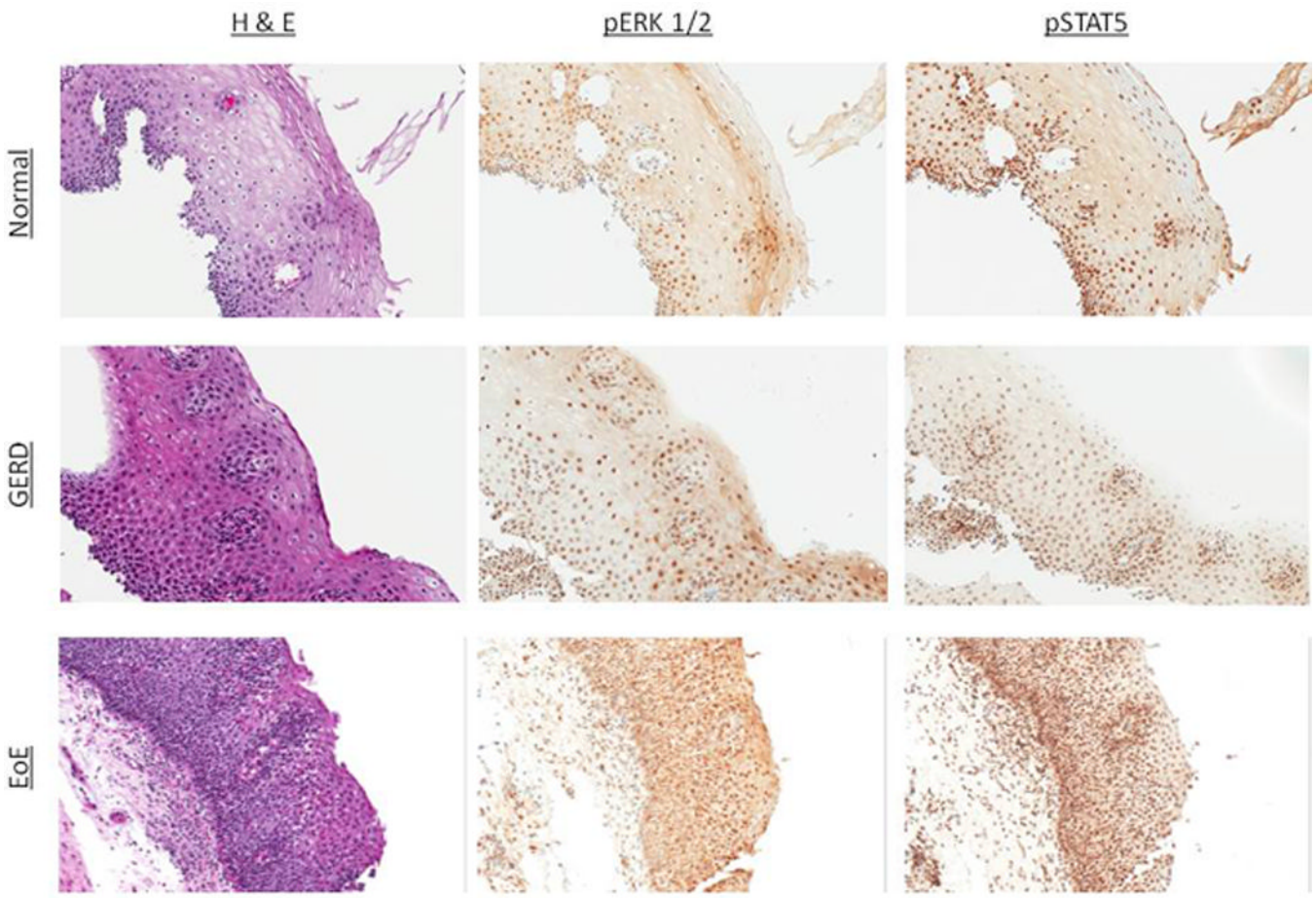

Figure 2.
Examples of H&E, pERK 1/2, and pSTAT5 staining in normal, GERD, and EoE patients. The H&E section for the normal patient is normal, the GERD patient has mild basal zone hyperplasia and a minimal non-specific inflammation, and the EoE patient has basal zone hyperplasia and a prominent esophageal eosinophilic infiltrate. There is relatively little nuclear IHC staining for pERK1/2 and pSTAT5 in the normal and GERD patients, while it is increased in the EoE patients.

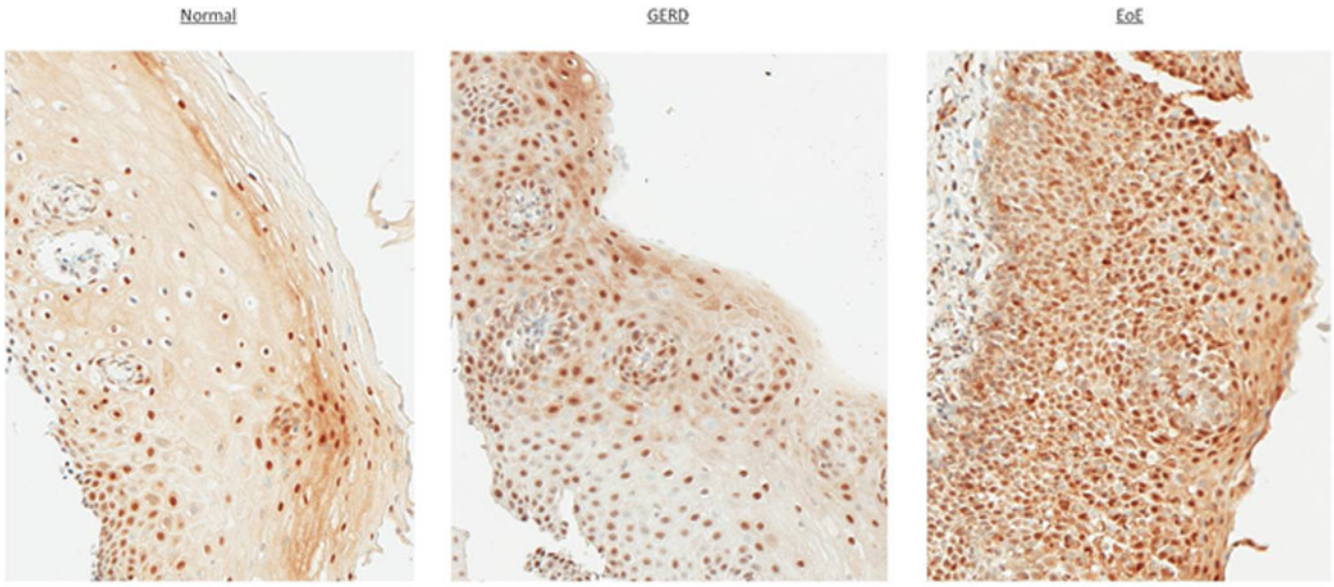

Figure 3.
Close-up view of pERK 1/2 staining in the esophageal epithelium of normal, GERD, and EoE patients. There is a small amount of nuclear staining in the normal and GERD patients, but much more prominent nuclear staining in the EoE subject. There is also minimal cytoplasmic staining compared to the nuclear staining.

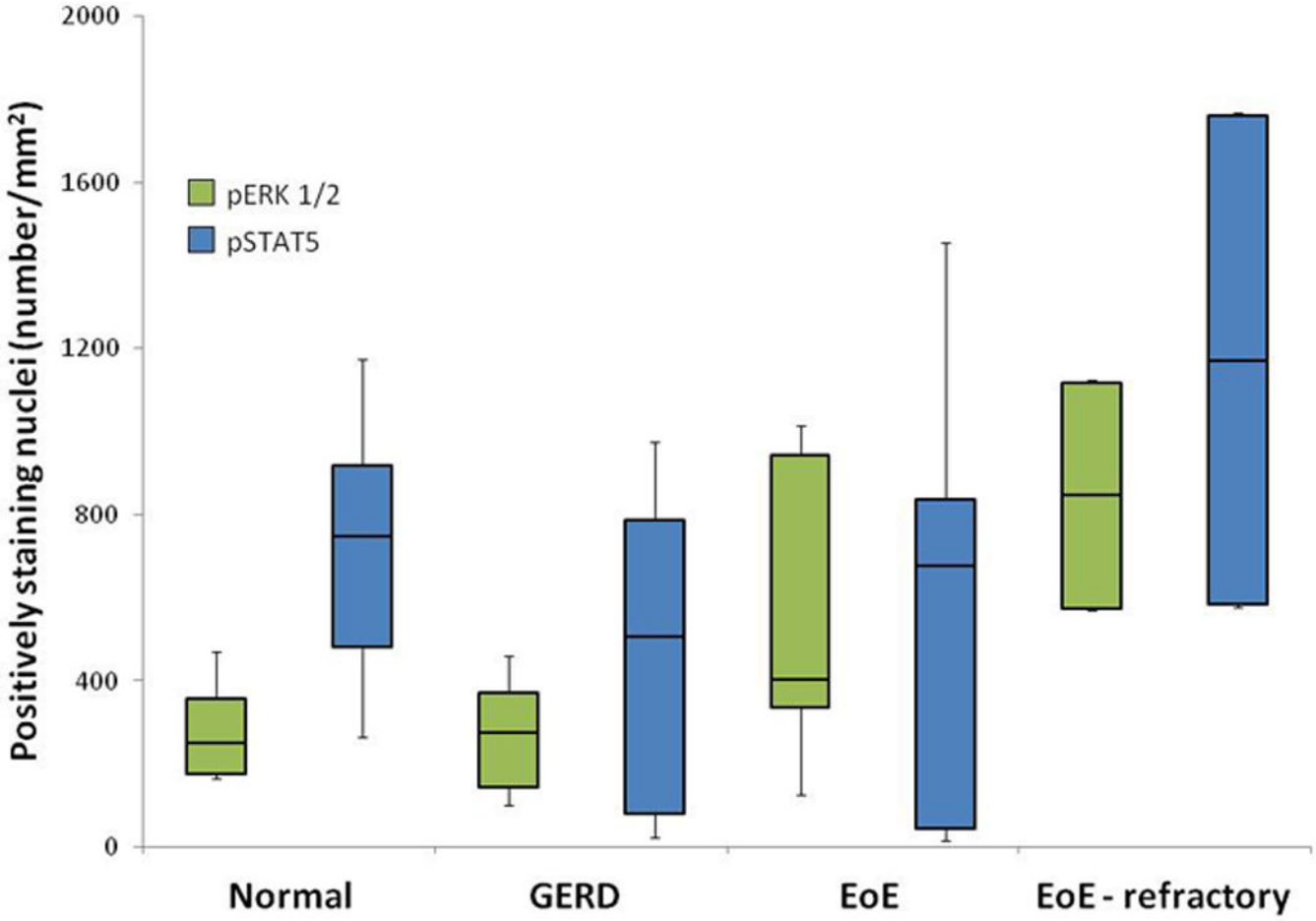

Figure 4.

A box-and-whiskers plot of for pERK 1/2 and pSTAT5 staining (nuclei/mm^2) in the normal, GERD, EoE, and refractory EoE groups. The median value for each group is noted with the horizontal line within each box, the boxes represent the range of values from the 25th percentile to the 75th percentile, and the ends of the whiskers represent the 10th and 90th percentile of values. The Kruskal-Wallis test was used to compare medians between the groups (p=0.07 for pERK1/2, p=0.74 for pSTAT5).

Table 1

Patient characteristics

	Normal (n = 8)	GERD (n = 6)	EoE (n = 15)†
Age (median; IQR)	47 (18–53)	41 (30–48)	27 (20–44)
Male (n, %)‡	1 (13)	3 (50)	12 (80)
Caucasian (n, %)	7 (88)	5 (83)	12 (80)
Primary symptoms/EGD indications (n, %)§			
Dysphagia	0	0	12 (80)
Food impaction	0	0	5 (33)
Heartburn/reflux	0	6 (100)	5 (33)
Chest pain	0	1 (17)	1 (7)
Abdominal pain	4 (50)	0	0
Nausea	2 (25)	0	0
Vomiting	2 (25)	0	0
Atopic disease (n, %)	1 (13)	0	4 (27)
Food allergy (n, %)	0	0	1 (7)
Asthma (n, %)	2 (25)	1 (17)	1 (7)
Endoscopy findings (n, %)			
Normal‡	7 (88)	1 (17)	3 (20)
Rings‡	0	2 (33)	8 (53)
Stricture	0	2 (33)	6 (40)
Narrow caliber	0	0	4 (27)
Linear furrows	0	1 (17)	5 (33)
Crêpe-paper mucosa	0	0	3 (20)
White plaques	0	1 (17)	1 (7)
Erosive esophagitis	0	3 (50)	1 (7)
Hiatal hernia	0	2 (33)	2 (13)
Schatzki's ring	1 (13)	1 (17)	2 (13)
Esophageal biopsy location (n, %)			
Distal esophagus only	2 (25)	3 (50)	4 (27)
Distal and proximal esophagus	4 (50)	2 (33)	7 (46)
Location not specified	2 (25)	1 (17)	4 (27)
Maximum eosinophil count (IQR)†,¶	0	1 (0–2)	75 (50–100)

† This group includes the 5 steroid-refractory EoE patients.

‡ $p < 0.05$ by Kruskal-Wallis for medians or by Fisher's exact for proportions.

§ Patients had could have multiple primary symptoms/EGD indications

¶ Median of the maximum number of eosinophils/high-power field, ± IQR; hpf size = $0.24mm^2$.

Table 2

Results from FISH for the FIP1L1-PDGFRα fusion gene

	Normal (n = 8)	GERD (n = 6)	EoE (n = 15)[†]	p[‡]
Median # of nuclei scored per patient (IQR)	171 (134–197)	171 (81–179)	139 (97–175)	0.36
Median % nuclei normal per patient (IQR)[§]	98 (97–99)	99 (98–100)	99 (98–100)	0.42

[†]This group includes the 5 steroid-refractory EoE patients. In this sub-group, 99% of the nuclei were normal.

[‡]Calculated with the Kruskal-Wallis test.

[§]Normal nuclei were defined as the presence of 2 copies of all colors (green, orange, blue) or 1 copy of all colors. Diseased nuclei were defined as 2 copies of green and 2 copies of blue, but only 1 or 0 copies of orange.

Table 3

Results from immunohistochemistry for pERK 1/2 and pSTAT5

	Normal (n = 7)	GERD (n = 6)	EoE (n = 9)†	p‡
Age (median; IQR)	49 (11–53)	41 (30–48)	26 (14–44)	0.29
Male (n, %)	1 (14)	3 (50)	7 (78)	0.04
Caucasian (n, %)	7 (100)	5 (83)	7 (100)	0.59
Maximum eosinophil count (IQR)§	0	1 (0–2)	75 (50–80)	< 0.001
pERK 1/2 positive nuclei (Median # nuclei/mm^2; IQR)	250 (176–357)	277 (143–373)	479 (346–943)	0.07
STAT5 positive nuclei (Median # nuclei/mm^2; IQR)	748 (482–918)	506 (79–786)	676 (45–1762)	0.75

† This group includes 2 steroid-refractory EoE patients.

‡ Kruskal-Wallis for medians; Fisher's exact for proportions.

§ Median of the maximum number of eosinophils/high-power field, ± IQR; hpf size = 0.24mm^2.