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Alimentary Tract

Gene expression-phenotype associations in adults with eosinophilic esophagitis

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ABSTRACT

Background: Gene expression patterns have not been extensively examined in the context of clinical features of eosinophilic esophagitis (EoE).
Aims: To assess whether gene expression is associated with clinically defined phenotypes in adults with EoE.
Methods: This was an analysis of prospectively collected esophageal biopsies in newly diagnosed EoE patients. We determined differential gene expression with a 94 gene panel in relation to clinical features and phenotypes. These included: endoscopic findings of esophageal rings, stricture, narrowing, linear furrows, exudates, edema, and dilation; an allergic phenotype; an inflammatory phenotype, and a fibrostenotic phenotype.
Results: In 89 EoE cases analyzed, patients with exudates on endoscopy had multiple differences in gene expression compared to patients without exudates, though patients with exudates also had higher eosinophil counts (172 vs 106 eos/hpf; p = .01). Genes associated with esophageal narrowing included CCL26 (q-value = 0.028), ALOX15 (q = 0.011), GRK5 (q = 0.029), CPA3 (q = 0.012), and TRIM2 (q = 0.0027). TRIM2 was also associated with the fibrostenotic phenotype (q = 0.0051). No genes were associated with the inflammatory or atopic phenotypes, or with dilation.
Conclusions: Multiple genes are associated with exudates, possibly related to higher eosinophil counts. However, a number of genes, including those related to both inflammation and remodelling, are associated with esophageal narrowing. In particular, TRIM2 is associated with clinical fibrotic phenotypes.

1. Introduction

Eosinophilic esophagitis is a Th2-mediated allergen/immune-mediated esophageal disorder [1,2]. Since its description in the mid-1990s, there has been rapid understanding of EoE pathogenesis. In particular, the genetic underpinnings of EoE are beginning to be elucidated [3]. Landmark work by Blanchard, Rothenberg, and co-workers described a set of genes, termed the "EoE transcriptome", that are differentially expressed in EoE patients compared to those with gastroesophageal reflux disease (GERD) and normal controls [4,5]. Recent work has identified a subset of genes that are highly discriminatory for diagnosis of EoE compared to non-EoE controls [6], and this same gene set also appears to be responsive, normalizing after successful treatment [7]. In addition, a series of genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNP) that are highly associated with EoE [8–10], and may increase EoE risk [11]

In addition to genetics, there has been recognition of different clinical phenotypes in EoE [12–15]. Perhaps the most important one categorizes patients into either an inflammatory-predominant appearance in the esophagus (endoscopic findings of edema, exudates, and linear furrows) or a fibrostenotic-predominant appearance (esophageal strictures or narrowing) [12,16]. Other phenotypes can include atopic vs non-atopic, male vs female, white vs non-white, adult vs pediatric, treatment responders vs non-responders, and connective tissue disease-associated [14,17].

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However, associations between genetics and phenotypes have not been extensively examined in EoE. Initial investigations have shown a SNP in transforming growth factor beta-1 (TGF-β1) may be associated with treatment response [18], that gene expression related to mast cells correlated with dysphagia measured by a pediatric symptom score [19], and that expression of some genes may be associated with steroid response [20].

Because there have been no studies specifically assessing the relationship between genotype and clinical phenotype, the aim of this study was to assess whether gene expression is associated with clinically defined phenotypes in adults with EoE. We hypothesized that expression of genes related to epithelial integrity and remodelling would be associated with a fibrostenotic phenotype, while expression of genes related to Th2 cytokines would be associated with an atopic phenotype.

2. Materials and methods

2.1. Study design, patients, and clinical data collection

We conducted an analysis of biopsy samples and data collected during a prospective cohort study of EoE. The parent study, details of which have been described previously [21–24], enrolled adults (≥18 years) undergoing upper endoscopy at University of North Carolina for symptoms of dysphagia, heartburn, refractory reflux, or other upper gastrointestinal symptoms. They were excluded if they had a known (prevalent) diagnosis of EoE or eosinophilic gastroenteritis, acute GI bleeding, active anticoagulation, known esophageal cancer, prior esophageal surgery, known esophageal varices, or medical instability or multiple comorbidities precluding enrollment in the clinical opinion of the endoscopist. Informed consent, including consent for future use of stored biopsy specimens, was obtained prior to the endoscopy. This study was approved by the UNC Institutional Review Board.

Only incident cases of EoE, with baseline (pre-steroid or dietary treatment) samples and data available, were included in the present study. EoE was diagnosed as per 2011 consensus guidelines [1]. Specifically, cases were had at least one symptom of esophageal dysfunction, ≥15 eos/hpf on esophageal biopsy after an 8-week proton pump inhibitor trial (20–40 mg twice daily of any of the available agents, prescribed at the discretion of the clinician), and exclusion of other causes of esophageal eosinophilia. Of note, subjects with proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) were excluded from the present analysis because we did not have sufficient pre-PPI treatment banked samples available to conduct the analysis, and it has previously been shown that gene expression profiles in this subgroup normalize after PPI administration [25], so post-PPI samples would not be appropriate to address to the study question.

Demographics, clinical, and endoscopic data were recorded prospectively using standardized case report forms. During the endoscopy, research protocol esophageal biopsies were obtained (two from the proximal, one from the middle, and two from the distal esophagus) in order to determine tissue eosinophil counts and ensure EoE diagnostic sensitivity [26,27]. We also collected research-protocol gastric and duodenal biopsies to exclude concomitant eosinophilic gastroenteritis. After masking the slides as to clinical status, peak esophageal eosinophil counts were determined based on our previously validated methodology [28,29].

2.2. Sample collection, RNA extraction, and gene expression

In addition to the biopsies that were obtained for histologic analysis, additional esophageal biopsy samples were collected, labeled with a de-identified study number, masked as to clinical status, and stored at −80 °C RNA-later (Life Technologies/Thermo-Fisher Scientific, Grand Island, NY) for future use. Samples remained frozen until study enrollment was complete and we were ready to perform the present analysis. At that time, we retrieved a single RNA-later-preserved biopsy from the mid-esophagus (10 cm above the gastroesophageal junction) for gene expression determination. The rationale for using a single mid-esophageal biopsy was based on our prior work showing that gene expression in EoE was similar throughout the esophagus [30].

Gene expression testing was performed at Miraca Life Sciences (Phoenix, AZ) as previously described [7]. After thawing and tissue homogenization, RNA was extracted using the RNeasy Mini Extraction Kit (Qiagen, Valencia, CA) per the manufacturer's instructions. The concentration was measured using spectrophotometry (NanoDrop, Wilmington, DE) with 16.5 ng/μl of RNA for a total of 500 ng considered acceptable. cDNA synthesis was carried out using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) with PCR performed on ABI 9700 (Applied Biosystems, Foster City, CA). The cDNA and TaqMan Universal Master Mix II, no UNG (Life Technologies) were loaded onto custom Taqman TLDA cards containing preloaded Taqman gene expression assays in a 384-well format. This consisted of the 94 gene panel that was previously developed for EoE and 2 housekeeping genes (GAPDH and 18S). PCR was performed on Quant Studio 7 (Life Technologies) to determine the gene expression levels measured as threshold cycles (Ct). Samples with a GAPDH value of <30 Ct were considered acceptable. A gene expression summary score was calculated using a previously established algorithm [6]. Specifically, the Ct value of the housekeeping gene was subtracted from the Ct value of each gene of interest to acquire the ΔCT, and then the absolute values of the normalized gene Ct values were summed for each gene in the gene expression panel. In general, a gene score below 333 is characteristic of EoE, with lower scores being more reflective of a diagnosis of EoE [6].

2.3. Clinical phenotype definition and statistical analysis

Clinical findings and phenotypes for analysis were defined as follows. Endoscopic findings of interest were reported by the endoscopist and included: esophageal rings, stricture, narrowing, furrows, exudates, and edema, as well as whether esophageal dilation was performed. Strictures were defined as the presence of a focal area of constriction in the esophagus. Narrowing was defined as an area where the lumen had a smaller caliber, but was more diffuse than what would be expected for a focal stricture. The fibrostenotic phenotype was defined as the presence of stricture, narrowing, or dilation, regardless of other endoscopic findings. A "severe" fibrotic phenotype was defined if there was a focal stricture or narrowing with an esophageal diameter measured as ≤10 mm (such that a standard adult upper endoscope used in our procedures unit would not pass). The inflammatory phenotype was defined as the presence of exudates, edema, or furrows, in the absence of dilation being performed, and regardless of other endoscopic findings. An inflammatory "only" phenotype was defined as having exudates, edema, or furrows without other endoscopic findings. The allergic phenotype was defined as the presence of any atopic condition.

We used summary statistics to describe the clinical features of the EoE cases. Among the EoE cases, the mean summary gene score was compared for the patients with and without each endoscopic finding or phenotype using a two-sample *t*-test. We then determined differential gene expression for each endoscopic finding or phenotype using the Mann-Whitney *U* test with a false discovery rate, calculated using the Benjamini-Hochberg procedure, of 0.1. Differential gene expression was then plotted on a heat map. Finally, we performed unsupervised consensus clustering with the R package, ConsensusClusterPlus (parameters maxK = 6, reps = 50,

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