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Gender-specific differences in the molecular signatures of adult Eosinophilic Esophagitis

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The influence of gender on the prevalence, presentation and pathogenesis of allergic diseases is being increasingly recognized [1]. Eosinophilic Esophagitis (EoE) is a chronic inflammatory disorder of the esophagus, seen in both children and adults, and is 3–4 times more common in males than females [2]. Diagnosis of EoE has relied on quantification of eosophageal eosinophils by biopsy and patient-reported symptoms but recent studies have supported the use of tissue-based molecular biomarker assessment, based on gene expression signatures [3], aiding patient diagnosis [4]. Strong correlations between gene expression profiles and symptoms were found, particularly dysphagia [4]. Early work looking at the EoE-associated transcriptional profile concluded that there were no differences between males and females [5] but this was performed in childhood EoE and recent studies have demonstrated that gender significantly influences the clinical presentation of adult EoE, particularly dysphagia [6–8]. We investigated whether the EoE-associated transcriptional profile of biopsies from adult EoE patients displays differences related to gender.

We assessed the molecular expression patterns of 40 genes associated with specific aspects of EoE pathology, including epithelial barrier, eosinophils, mast cells and cellular proliferation, in pretreatment biopsies from male and female control or EoE patients. Based on a power calculation of sample size required to detect a 30% difference between EoE patients with an alpha probability of 0.05 and power of 0.80 for the gene *MS4A2*, which encodes the β chain of the high affinity IgE receptor that we have previously described as elevated in adult EoE [9], we investigated the gene expression profiles of 22 adult EoE patients (11 male, 11 female) versus 15 control patients (4 male, 11 female). All patients were undergoing routine assessment for EoE at gastroenterology clinics at Northwestern Memorial Hospital and consented to biopsies for research under IRB approval number STU00021171. Control patients were undergoing assessment for eosophageal complaints

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but determined to not have EoE. Patients were selected randomly by the study coordinator and biopsies de-identified before being sent to the laboratory for gene expression analysis. Eosophageal biopsies were taken prior to introduction of any specific therapy but all patients were on proton pump inhibitor treatment, as per standard clinical practice, and reported dysphagia symptoms. As shown in the Supporting Information Table 1, no significant differences were found between the ages of Control versus EoE or in Male versus Female for any group. Eosinophils were significantly elevated in EoE versus control but not different between Male and Female within the EoE patients ($p=0.44$). Endoscopic features typical of EoE were also elevated in EoE but not significantly different between Male and Female EoE patients.

Real time RT-PCR (Taqman primer and probe sets, Applied Biosystems) was performed on collected biopsies that were immediately placed into RNAlater stabilizing solution (Qiagen) before gene expression assessed relative to the housekeeping gene *GAPDH*, for which all groups were within 1 cycle threshold of each other. 18/40 genes were statistically different between control and EoE patients using Nonparametric rank 2-way ANOVA analysis (full table of ranks and p values shown in Supporting Information Table 2); these included eosinophil-associated genes (*IL5*, *CCL24*, *CCL26*), mast cell-associated genes (*MS4A2*, *TPSAB1*, *HDC*, *KITLG*) and epithelial barrier genes (*SFRP1*, *KRT14*, *DSG1*, *CRISP3*). In considering gender as a variable within each group, significant differences were observed between male and female EoE patients in 8 of these 18 genes, as well as 1 (*FOS*) that had not achieved significance between the combined populations. As shown in Figure 1A, expression of *CCL24*, *CCL26* and *ALOX15*, key eosinophil-associated transcriptional signatures in EoE, as well as mast cell-associated genes *TPSAB1*, *HDC* and *KITLG* were significantly higher in Male over Female EoE patients. In contrast, barrier-related genes were significantly altered between control and EoE patients but were similar between genders, with only *SFRP1* being higher in Male than Female. In order to assess global patterns of expression, the relative expression for each individual compared to the average of the control expression level was calculated and Log2 transformed. Figure 1B shows a distinct pattern of expression in EoE Male patients that is less evident in EoE Female, particularly the high expression of mast cell and eosinophil-associated genes and the lower relative expression of some epithelial or barrier-associated signatures.

We next assessed whether the molecular signatures present in our patient cohort correlated with the pathology reported frequency of eosinophils in the biopsies. No specific gene was found to correlate with peak eosinophil counts (data not shown) and so we utilized principal component analysis (PCA), a method of orthogonal transformation on matrix-type data, to examine the variances between our entire dataset (PAST3.12 software). One patient in the EoE group was removed due to nonconformity of the matrix because of absence of expression data for more than 3 genes. Principal Component 1 (PC1) accounted for 37.7% of the overall variance within the data matrix (Supporting Information Table 3) and showed clustering of all Male EoE patients on the positive side of the axis (Figure 2A). The individual values for PC1 correlated significantly with peak eosinophils in Male EoE patients ($p<0.05$) but not in Female EoE patients (Figure 2B).

Taken together, our findings demonstrate a clear influence of gender on the molecular signatures of biomarkers for EoE. The influence of gender is most prominent within the eosinophil and mast cell-associated markers, perhaps implying a difference in inflammatory processes. Mast cells have been shown to express significant levels of estrogen, progesterone and androgen receptors [10] but the biological functions remain poorly defined. The lack of gender influences on barrier-related differences also suggests that mucosal integrity, such as has been described to be associated with the epithelial differentiation cluster (EDC) genes in EoE [11], might be a primary aspect of EoE pathogenesis but that gender-related influences then alter the inflammation-associated signature. This hypothesis might also align with the absence of gender-specific differences in the pediatric EoE patients [5], in who sex hormone effects are less likely to participate than occurs in adults. Importantly, the associations between molecular signatures and eosinophils in Male EoE patients would suggest that the pathology in these patients is driven by some synergistic influence of the signatures we examined. In contrast, the lack of association between these in females could suggest that alternative, yet to be determined, mechanisms regulate eosinophils in Female EoE. Supporting the concept that gender differences might modify disease pathogenesis, a recent study described an “EoE-like” disease within families where EoE was prevalent and that this phenotype was female dominant [12]; how this new phenotype relates to our patients remains to be determined. One limitation of our study is that we focused on self-reported gender, potentially increasing the risks of variability if there are influences of biological sex status. A second limitation relates to the potential for confounder influences that we are unable to ascertain in these patients, including atopic status, comorbid diseases, and ethnicity. We are also unable to conclude if these differences in molecular signatures between gender are specific to EoE or reflect a broader alteration in esophageal biology between men and women; gender-related differences in transcriptional signatures have been found in other esophageal diseases, including Barretts Esophagus and/or esophageal adenocarcinoma [13]. While such mechanistic research is vital to understanding differences between pediatric and adult EoE, our findings presented here offer the initial evidence that gender-related differences in the molecular biomarker signatures of EoE require consideration in understanding adult EoE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A) Gene expression levels relative to a housekeeping gene, GAPDH, was determined by real-time RT-PCR in esophageal biopsy samples from control or EoE patients. *= $p<0.05$, ***= $p<0.005$, ****= $p<0.001$ by nonparametric rank ANOVA analysis between either all controls and EoE patients or between Male and Female within each group. B) Heatmap representation of normalized and Log transformed expression for all genes tested.

Figure 2. Multivariate molecular signatures associate with eosinophils in Male but not Female EoE

A) Principal Component Analysis was utilized to determine principal components most contributing to overall variance with PC1 versus PC2 plotted. Loading influences of individual genes are represented by the linear lines. B) PC1 score values correlate with peak eosinophils in Male but not Female EoE. Linear regression for goodness of fit and P value for slope being non-zero were performed using GraphPad Prism.