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Very Early Onset Eosinophilic Esophagitis is Common, Responds to Standard Therapy, and Demonstrates Enrichment for *CAPN14* Genetic Variants

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Abstract

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Background: Eosinophilic esophagitis (EoE) is a chronic, food antigen–mediated disease characterized by esophageal dysfunction and intraepithelial eosinophil accumulation.

Objective: We hypothesized that very early onset EoE (V-EoE) would be enriched for early life and genetic factors and have worse presentation and prognosis than later onset pediatric EoE (L-EoE).

Methods: We conducted a single-site, retrospective review comparing patients diagnosed at ≤12 months (V-EoE, n=57) and 14–18 years (L-EoE, n=70) of age. These patients underwent medical record, EoE Histology Scoring System, Endoscopic Reference Score, and EoE Diagnostic Panel assessment when sample availability permitted. Genetic association utilized two EoE genotype repositories. Data were analyzed using chi-square, t-tests, Wilcoxon rank sum, Spearman correlations, cluster analysis, and logistic regression.

Results: Amongst pediatric patients with EoE, diagnosis most commonly occurred within early life (0–24 mo, 17%). V-EoE was more likely to attain histologic remission via dietary restriction ($p<.0001$). Basal zone hyperplasia and eosinophil inflammation were greater in V-EoE ($p<.05$). Esophageal strictures more commonly occurred in L-EoE ($p=.03$). V-EoE had lower endoscopic scores ($p<.05$). Molecular expression was very similar between groups. Caesarean delivery was more common in V-EoE ($p=.03$). V-EoE demonstrated enrichment of *CAPN14* common genetic variants.

Conclusions: Early life diagnosis of EoE is a common occurrence. V-EoE responds to standard therapy without early evidence for complications, suggesting a less severe prognosis than hypothesized. Molecular pathogenesis is preserved between V-EoE and L-EoE. Caesarean delivery and *CAPN14* genetic variation likely promote earlier disease development.

Capsule Summary:

V-EoE is enriched in early life environmental factors and *CAPN14* genetic variants. V-EoE should be considered in the appropriate clinical setting due to its response to standard therapy with potential for reduction in complications.

Keywords

infancy; environment; cesarean delivery; genetics; molecular pathogenesis; EoEHSS; EREFS; EDP; *CAPN14*; *ACTG2*

Introduction

Eosinophilic esophagitis (EoE) is a chronic, food antigen–mediated disease characterized by esophageal dysfunction and intraepithelial eosinophil accumulation (≥15 eosinophils per high-power field [eos/hpf]).(1) Its etiology involves the complex interplay of genetic and environmental factors(2, 3) and is associated with strong heritability, characterized by high sibling recurrence risk,(4) and early life environmental exposures.(3, 5, 6) Genetic variants at 2p23 and 5q22 promote disease susceptibility, likely through the esophageal epithelial proteins calpain 14 (CAPN14) and thymic stromal lymphopoietin (TSLP), respectively.(7–10) EoE molecular pathophysiology is underscored by esophageal epithelial dysfunction and $CD4^+$ T cell–associated type 2 immune responses associated with *CAPN14*(8–10) and

TSLP(7, 9, 11–13) induction. Specifically, CAPN14 is an esophagus-specific intracellular cysteine protease whose increased expression is induced by IL-13 which leads to epithelial barrier dysfunction through the downregulation of desmoglein 1 (*DSG1*) (14). Subsequently, downregulation of *DSG1* induces periostin (*POSTN*) leading to increased eosinophil adhesion (15) and production of TSLP, a pro-atopy cytokine central to the type 2 immune response in EoE (7, 11–13).

Though EoE typically presents in school-aged children (6–12 yo) and young adults (3rd-4th decade of life) (16), it can also present in infancy (≤12 mo). EoE natural history studies support progression from an inflammatory condition in children to one characterized by fibrosis in adults (16, 17), and esophageal molecular expression demonstrates that EoE exists as multiple endotypes, including a fibrostenotic endotype. (18) Thus, if disease develops earlier in life, the potential risk of fibrostenosis and associated complications might increase. Therefore, we hypothesized that earlier disease presentation would represent increased severity and worse prognosis compared to those of later disease presentation due to enrichment of genetic and environmental factors. This hypothesis is based on observations in other complex immune-mediated diseases, including monogenic very early onset inflammatory bowel disease, (19) systemic lupus erythematosus, (20) and type 1 diabetes mellitus.(21) We aimed to test whether EoE presenting very early in life, represents a separate disease endotype with worse clinical features, distinct molecular properties, and enrichment for early life environmental exposures and genetic variants compared with later onset pediatric EoE.

Methods

Patient Identification and Clinical Data Collection

We performed a single-site, retrospective review approved by Cincinnati Children's Hospital Medical Center's (CCHMC) Institutional Review Board. Within the CCHMC EoE research database, we identified patients diagnosed with EoE at ≤12 months of age, herein referred to as very early onset EoE (V-EoE, n=57) and compared them to patients diagnosed with EoE between 14 and 18 years of age, herein referred to as later onset pediatric EoE (L-EoE, n=70). Diagnosis of EoE was defined as symptoms consistent with esophageal inflammation/dysfunction and ≥15 eos/hpf in distal esophageal biopsies regardless of proton pump inhibitor (PPI) use, consistent with recent diagnostic criteria.(1) Patients were excluded for inaccurate diagnosis, inaccurate age at diagnosis, and concurrent eosinophilic gastritis or eosinophilic gastroenteritis. Figure 1 summarizes cohort selection and exclusion. The following data were collected: demographics, presenting symptomatology, histologic response to standard therapy, non-adherence to therapy, early life environmental exposures, and family history of atopic disorders. Sue to limitations with the CCHMC electronic medical record (EMR) we were unable to document antibiotic and PPI exposure during infancy as components of early life environmental exposure. The EoE Histology Scoring System (EoEHSS) is a validated score of eight histologic features characteristic of EoE that distinguishes treated from untreated EoE (22) while composite (23, 24) and individual (24) scores correlate with symptoms. Due to limited sample availability in the CCHMC pathology core, 62 (29 V-EoE, 33 L-EoE) pre-treatment, diagnostic distal esophageal

biopsies were evaluated by a blinded pathologist (M.H.C.). Individual features were given a score between 0 – 3 for both stage and grade. The total grade and stage score was the ratio of the summation of the scores of the features divided by the total possible score given the number of features assessed. The EoE Endoscopic Reference Score (EREFS) is a validated and reproducible score that evaluates the severity of edema, rings, exudates, furrows, and strictures. (25) Due to limited video availability in the CCHMC EMR, EREFS were independently completed on 22 (9 V-EoE, 13 L-EoE) video recordings of pre-treatment, diagnostic esophagoduodenoscopies (EGD) by the observer (J.L.L.) and a blinded expert (V.A.M.). Scores were compared and consensus reached for each video.

Esophageal Molecular Analysis

RNA was extracted by the RNeasy formalin-fixed, paraffin-embedded (FFPE) kit (Qiagen, Venlo, Netherlands) from a discovery cohort of 15 pre-treatment, diagnostic distal esophageal samples (10 V-EoE, 5 L-EoE). RNA was reverse-transcribed to complementary DNA (cDNA) by the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed via the EoE Diagnostic Panel (EDP), a set of 94 informative mRNA transcripts that distinguish EoE from gastroesophageal reflux disease and normal controls, as previously described.(18, 26) Power analysis completed prior to the validation cohort demonstrated that 9 samples per group were sufficient to detect a minimum fold change of 2 with power of 0.8 and p-value of 0.05. For validation, 29 additional pre-treatment, diagnostic distal esophageal samples (15 V-EoE, 14 L-EoE) were identified. For this analysis, 9 individuals with symptoms consistent with EoE at ≤ 12 mo but who were diagnosed with EoE between 12–18 mo were included due to limited numbers of archived distal esophageal biopsy sample availability in patients diagnosed at ≤ 12 mo (Figure 1). Taqman probes (ThermoFisher Scientific, Waltham, MA, USA) (*ACTG2, F3, GCNT3,* and *GAPDH*) were used to complete real-time PCR on the Applied Biosystems 7900HT Fast Real-Time PCR system (Foster City, CA, USA) and analyzed by SDS v2.4.

Genotypic Cohort

Data from two EoE genotype repositories were compiled (1,180 patients with EoE and 11,614 controls). This cohort included 553 cases and 9,290 controls profiled on the Omni5 array (8), 627 cases genotyped on a custom genotyping array, and 2,324 external controls genotyped on the Omni2.5 array from the University of Michigan Health and Retirement Study (dbGaP accession phs000428.v2.p2). In order to obtain an adequate sample size for analysis, V-EoE (n=161) was defined as having EoE symptom onset at ≤12 mo and diagnosis by 3 yo, and L-EoE (n=140) was defined as having EoE symptom onset between 9–18 yo and diagnosis between10–20 yo.

Statistical Analysis

We applied chi square goodness-of-fit tests for categorical outcomes, t-tests for normally distributed continuous outcomes, and the Wilcoxon rank sum test for non-normally distributed continuous outcomes. Individuals with missing data on a parameter were not included in comparisons with that parameter. We recognize that multiple outcomes are evaluated, which may lead to an increased risk of false-positive associations, thus we interpreted p-values close to the 0.05 threshold for statistical significance with caution.

Esophageal Molecular Expression Analysis

For the discovery cohort, we applied the Mann-Whitney U test adjusted with the Benjamini-Hochberg method for nonparametric continuous variables. EoE scores were calculated by summing ΔCT values of the most highly dysregulated genes (ΣΔCT). (26) Cluster analysis was performed by hierarchical clustering design with Euclidean distance metric and Wald's linkage rules. Condition and gene transcripts were clustered in conjunction with expression heat map (red, up-regulation; blue, down-regulation). Principal component analysis (PCA) generated a 3D plot of the top variance contributors between groups. The resulting heat map and 3D plot by PCA were visualized using GeneSpring GX 12.6 (Agilent Technologies, Santa Clara, CA). Comparison between the transcriptomes was visualized by a volcano plot (log2 fold change as x-axis and -log10 adjusted p-value as y-axis). For the validation cohort, the minus average value for *ACTG2, F3,* and *GCNT3* expression was used to generate an expression heat map via Morpheus (https://software.broadinstitute.org/morpheus) (red, up-regulation; blue, down-regulation). We applied the Mann-Whitney U test to compare these values. A significant p-value was <0.05.

Genetic Analysis

We sought to determine whether single-nucleotide polymorphism (SNPs) in *CAPN14* and *TSLP* were differentially associated with age of diagnosis. We used PCA in Eigensoft and 1000Genomes as a referent population to identify individuals of European ancestry. We used VEP Ensembl GRCh37(27) to annotate SNPs within 5,000 base pairs of each gene. We used PLINK v1.07(28, 29) to test whether SNPs of interest exhibited association with phenotype. To evaluate baseline risk, we compared EoE, regardless of age of diagnosis, to controls. We separately compared V-EoE and L-EoE to controls to establish the odds ratio (OR) for the association based on age of diagnosis. To test for significant differences in the frequencies of the two groups, we performed genetic association comparing V-EoE to L-EoE.

Results

Patient Identification and Characteristics

Within the CCHMC EoE research database, the second year of life was the most common age at diagnosis. Indeed, 17% of patients were diagnosed with EoE within the first two years of life (n = 301 of 1813 total patients between 0–18 yo) (Figure 2). This percentage was stable over the preceding two decades (Supplementary Figure 1).

Baseline characteristics, including sex, race, and ethnicity, were similar between the groups with expected differences in V-EoE (younger age at diagnosis and longer duration of follow up) and L-EoE (longer duration of symptoms prior to diagnosis) (Table 1). When stratified by sub-group (EoEHSS, EREFS, and Molecular), baseline characteristics are similar to the groups (V-EoE, L-EoE) as a whole (Supplementary Table 1). Of note, patients with V-EoE were more likely to have first-degree relatives with a history of atopy (85% vs. 68%, p=.03) but not EoE (V-EoE 16% vs. L-EoE 8%, p=.16).

Presenting Symptoms and Environmental Factors

V-EoE was more likely to have feeding issues, weight concerns, and vomiting clinically noted at presentation. L-EoE was more likely to have dysphagia clinically noted at presentation. V-EoE was enriched for Caesarean and preterm delivery, known to be early life environmental risk factors for the development of EoE. There was no difference in exposure to breastfeeding or residence in an urban environment (Table 2).

Initial and Successful Therapy

Standard therapy used to treat EoE consists of empiric dietary restrictions, swallowed topical corticosteroids (STC), or both. (30–32) While the treatment options presented to V-EoE and L-EoE patients are the same, dietary restriction was more likely to be the initial and successful therapy (<15 eos/hpf on both proximal and distal esophageal biopsies according to clinical reports) in V-EoE. Conversely, STC were more likely to be the initial and successful therapy in L-EoE. Patients with V-EoE were more likely to be on combination therapy (diet and STC) during remission than were patients with L-EoE (Table 2). Patients with V-EoE were more likely to attain histologic remission at the time of their last EGD. The proportion of patients who had disease refractory to standard therapy was low and similar between groups. Taken together these data demonstrate that histologic remission is attained and sustained with standard therapy in V-EoE. In addition, while a substantial proportion of patients with L-EoE did not demonstrate histologic remission at the time of their last EGD, likely due to a significantly higher rate of therapy non-adherence (Table 2), most had attained histologic remission at some point in their clinical follow up due to the low rate of refractoriness to standard therapy. Contrary to our hypothesis that V-EoE would represent an endotype with worse prognosis, these data demonstrate that V-EoE responds well to standard therapy.

Esophageal Strictures and Dilations

Preliminary longitudinal data revealed that 9% of patients with L-EoE had an esophageal stricture compared to 0% of patients with V-EoE (p=.03) during clinical follow up. Likewise, 11% of patients with L-EoE had an esophageal dilation compared to only 2% of patients with V-EoE (n=1, p=.04) (Supplementary Table 2) during clinical follow up. The median age to first esophageal stricture or dilation among patients with L-EoE was 17 yo (IQR 15–22 yo). Patients with esophageal stricture or dilation had symptoms for a longer duration [median 4.9 y (IQR 2.8–15.5 y)] than those without esophageal stricture or dilation [median 2.3 y (IQR 1.6–5.3 y), p=.03]. There was no difference in presenting symptoms between patients with and without esophageal stricture and/or dilation (Supplementary Table 3).

Histopathology

Significant differences in 3 features evaluated by the EoEHSS were found between the groups. Despite having comparable baseline peak eosinophil counts (PEC) in distal esophageal biopsies (median [IQR], V-EoE 94 [73–133] vs. L-EoE 65 [47–116], p=.07), patients with V-EoE demonstrated higher median grade scores for basal zone hyperplasia (BZH) (median [IQR], V-EoE 3 [2–3] vs. L-EoE 2 [2–3], p=.04) and eosinophil

inflammation (EI) (median [IQR], V-EoE 3 [3–3] vs. L-EoE 3 [2–3], p=.02). Of note, while the EI grade medians did not differ, the distribution of scores did with 86% of V-EoE and 61% of L-EoE being scored as 3 on this feature.

Furthermore, EoEHSS demonstrated higher median stage scores for EI in V-EoE (median [IQR], V-EoE 3 [2–3] vs. L-EoE, 2 [2–3], p=.03). V-EoE biopsies were more likely to contain muscularis mucosa (MM) and, therefore, lamina propria (LP) compared with L-EoE biopsies (96% vs. 6%, p<.0001). LP is more easily obtained and evaluated in V-EoE than L-EoE (absent in 7% of V-EoE biopsies and 45% of L-EoE biopsies, p=.0006), yet L-EoE had a higher median grade of lamina propria fibrosis (LPF) than did V-EoE (median [IQR], L-EoE 1.5 [0–3] vs. V-EoE, 0 [0–1], p=.03). The EoEHSS total grade and stage scores were similar between the groups (Table 3). These data demonstrated more histologic evidence of inflammation at presentation in V-EoE, while L-EoE had more histologic evidence of fibrotic change.

Endoscopy

V-EoE demonstrated lower overall EREFS scores (median [IQR], V-EoE 6 [2–7] vs. L-EoE 9 [6.5–9.5], p=.02) and lower EREFS scores from the distal (median [IQR], V-EoE 4 [2–5] vs. L-EoE 5 [4–5], p=.03) and proximal esophagus (median [IQR], V-EoE 2 [0–2.5] vs. L-EoE 4 [2–4], p=.02) independently. Likewise, V-EoE demonstrated lower maximum composite scores (median [IQR], V-EoE 4 [2–5] vs. L-EoE 5 [4.5–6.5], p=.008) and maximum inflammatory scores (median [IQR], V-EoE 4 [2–5] vs. L-EoE 5 [4–5.5], p=.04). Only the L-EoE cohort had patients with findings consistent with fibrostenosis (V-EoE 0% vs. L-EoE 31%, p=.03) (Table 4). These data demonstrated less evidence of macroscopic inflammation in V-EoE, while L-EoE demonstrated more evidence of macroscopic fibrotic change.

Esophageal Molecular Expression

Unsupervised cluster analysis of the EDP did not distinguish V-EoE (n=10) from L-EoE (n=5) (Figure 3A). In addition, there was no difference in the EDP EoE Score (ΣΔCT) between groups (Figure 3B). However, supervised cluster analysis (Figure 3C) and PCA (Figure 3D) demonstrated 3 differentially expressed genes (*ACTG2, F3,* and *GCNT3* with ≥2-fold change and adjusted p<.05) in V-EoE (Figure 3E). Analysis of a validation cohort (V-EoE n=15, L-EoE n=14) demonstrated increased expression of the esophageal gene transcript *ACTG2* in V-EoE (p=.0004); however, neither *F3* nor *GCNT3* were significantly different (Figure 3F and G). While the significance of *ACTG2* remains unclear and requires further study, these data demonstrated conserved esophageal molecular expression between V-EoE and L-EoE with no difference in 93 out of 94 gene transcripts evaluated by the EDP.

Genetic Analysis

We focused on disease association with the two strongest replicated genetic susceptibility loci at 2p23 and 5q22, encoding for *CAPN14* and *TSLP*, respectively. We found 20 *CAPN14* SNPs (Supplementary Table 4) and 10 *TSLP* SNPs (Supplementary Table 5) associated with EoE susceptibility. For *CAPN14*, 10 SNPs associated with V-EoE compared to controls, whereas no SNPs associated with L-EoE compared to controls. Remarkably, the

odds ratio (OR) for V-EoE, in all but 1 of the 10 identified SNPs (rs28680720), was larger than the OR for the non–age-stratified EoE group, indicating that the genetic association between 2p23 (*CAPN14*) and EoE was largely driven by patients with earlier age of disease onset and presentation. In contrast, no *TSLP* variants were significantly different between V-EoE and controls, whereas 3 were significantly different between L-EoE and controls (Figure 4). Taken together, these data support that *CAPN14* SNPs is associated with earlier age of disease onset in EoE.

Discussion

Herein, we report the first comprehensive description of V-EoE, focusing on its clinical and genetic characteristics. First, EoE commonly occurred in the first 24 months of life with 17% of patients diagnosed in that period. Second, Caesarean delivery and preterm delivery are more common in those with V-EoE than L-EoE, although preterm delivery does not reach statistical significance. Third, contrary to our hypothesis, V-EoE responded well to standard therapy and did not demonstrate evidence of more severe overall disease features, including molecular pathogenesis. Fourth, V-EoE demonstrated significant histopathologic differences from L-EoE, including more prominent BZH, more widespread EI, and reduced LPF, yet had lower endoscopic scores (less severe phenotype) suggesting that histopathologic features precede endoscopic abnormalities in EoE. Finally, V-EoE demonstrated effect modification and enrichment at *CAPN14* common genetic variants compared to non–age-stratified EoE and L-EoE, making *CAPN14* the first identified EoE genetic susceptibility variant associated with EoE age of onset. Taken together, these findings call attention to the development of EoE during infancy, particularly as a consequence of genetic and early life environmental determinants and support diagnostic evaluation for EoE in early life with successful management using standard therapy.

Three percent of patients in the CCHMC EoE research database were diagnosed in the first year of life, with an additional 14% being diagnosed during the second year of life (Figure 2). The marked increase in diagnoses between 12–24 months of age is likely due to a desire to avoid endoscopy in infants less than a year old. However, a recent study demonstrated no difference in neurodevelopment outcomes at 5 years of age for slightly less than 1 hour of general anesthesia compared to awake, regional anesthesia in infants. (33)

It is accepted that presenting symptoms of EoE vary by age with emesis, abdominal pain, and dysphagia being the most common symptoms in children. (16, 34, 35) This data supports these prior results but stratified them further, noting the distinguishing symptoms of poor appetite, oral aversions, poor weight gain, failure to thrive, and/or vomiting in V-EoE. Therefore, since the early presentation and diagnosis of EoE is relatively common and associates with identifiable and distinguishing symptomatology, clinicians should consider earlier endoscopic investigation for EoE in the appropriate clinical settings.

While clear guidelines for the histologic diagnosis of EoE exist, symptoms weakly correlate with PEC (36, 37) and inter-observer report of PEC varies (38) while other histologic features of EoE are too often overshadowed. The more comprehensive EoEHSS demonstrated higher BZH severity and EI severity and extent in V-EoE compared to L-EoE

using ordinal ranked variables. In ordinal ranked variables, statistically significant p-values could be due to differences in the median value, as in BZH in V-EoE vs L-EoE in this study, but also could be due to differences in the proportion of individuals at the specific ordinal rankings. For example, for EI grade, both the early and late EoE have a median score equaling 3. However, the interquartile range differs, from 3 to 3 in V-EoE but from 2–3 in L-EoE. These data indicate that at least 25% of the L-EoE had EI score less than or equal to 2 in contrast to less than 25% of V-EoE which had EI score less than or equal to 2. It is interesting to speculate that the observed enrichment of *CAPN14* variants in V-EoE may reflect a relatively dominant contribution of CAPN14 in eliciting epithelial hyperplasia and eosinophil infiltration but not LP related responses. (14)

V-EoE responded well to standard therapy as demonstrated by its similar, low rate of refractoriness to standard therapy as compared to L-EoE. In contrast to our initial hypothesis that earlier disease presentation would represent a more severe phenotype with worse prognosis, only L-EoE patients demonstrated histologic and endoscopic evidence of fibrostenosis at diagnosis and were more likely to have esophageal strictures and/or dilations during the course of clinical follow up. Furthermore, 96% of V-EoE biopsies contained MM compared to only 6% in L-EoE biopsies ($p<.0001$), suggesting increased esophageal wall thickness in L-EoE. While age-related differences in esophageal wall thickness (EWT) and collagen deposition could account for this difference, mean EWT in normal patients does not vary significantly by age (39, 40) and there are no pediatric studies evaluating age-related differences in esophageal collagen. Mean EWT is increased in EoE compared to normal due to mucosa/submucosa expansion (41); however, there are no studies to date evaluating EWT in EoE patients stratified by age. Thus, we cannot conclude if differences in EoE EWT are age dependent or a result of disease duration; we favor the latter due to lack of age-related EWT difference in normal patients and our current understanding of EoE's natural history. (16, 17, 42) Therefore, while longer symptom duration prior to diagnosis and increased concerns for treatment non-adherence in L-EoE likely has a role in the increased evidence of fibrostenosis, due to relatively short clinical follow-up and the retrospective nature of this study, it is difficult to state whether the lower rate of esophageal complications in V-EoE will be maintained as these patients get older. Regardless, if the process of esophageal remodeling is indeed time dependent, then earlier intervention and consistent application of effective therapies may provide the best opportunity to maintain esophageal function, likely improving patient quality of life and reducing overall healthcare costs in this patient population.

The overall esophageal molecular profile is conserved between V-EoE and L-EoE, indicating that the molecular bases for disease, including loss of barrier function, impaired epithelial differentiation, and an exaggerated type 2 immune response, are fully developed even in the early life period. Of note, *ACTG2* is increased in V-EoE compared to L-EoE. It encodes for gamma-2 actin, an enteric smooth muscle actin protein highly expressed in normal esophageal tissue(43) with demonstrated pathologic mutations in megacystis-microcolon-intestinal hypoperistalsis syndrome and intestinal pseudo-obstruction.(44) Decreased expression of *ACTG2* distinguished EoE endotype 2 (EoEe2) (characterized by increased inflammatory changes, type 2 immune responses, and refractoriness to steroids) from EoEe1 and EoEe3.(18) Yet, no studies to date have either evaluated for tissue-specific

ACTG2 expression in intestinal motility disorders or demonstrated associations between *ACTG2* variants and EoE. Thus, conclusions regarding the significance of *ACTG2* expression differences between V-EoE and L-EoE are preliminary and difficult to interpret without further study. For instance, *ACTG2* expression levels could reflect differences in EWT and disproportionate tissue sampling as V-EoE biopsies were more likely to contain MM compared to L-EoE. If this is the case, then the association between decreased *ACTG2* expression and refractoriness to steroids demonstrated in prior work could be a result of longer disease duration and/or ineffective use of standard therapy leading to fibrotic change in the esophagus with increased EWT and decreased sampling of MM. Therefore, further work is needed to qualify the significance of *ACTG2* to the molecular pathogenesis, natural history and treatment of EoE.

The increased incidence of Caesarean delivery in V-EoE suggests the importance of early life environmental exposures in EoE (5, 6). This finding suggests both a strong environmental component to the development of V-EoE and potential etiology for the observed esophageal dysbiosis in EoE (45, 46) via early microbial alteration in the developing esophagus. Due to limitations of our single-center retrospective data, future studies should focus on whether more early life environmental exposures associate with earlier disease presentation and study their impact on the esophageal microbiome.

The data demonstrated an increased association between V-EoE and *CAPN14* genetic variants and argue that the association between *CAPN14* and EoE is largely driven by those patients with earlier disease presentation and diagnosis. Prior studies on *CAPN14* genetic risk did not stratify by patient age (8, 9); whereas, the data herein establish that *CAPN14* variants increased the likelihood of earlier disease onset and presentation. However, these data should be interpreted with caution until replicated. Breastfeeding has been observed to be protective for the development of EoE in the context of *CAPN14* genetic variants, (47) which is consistent with our findings that *CAPN14* variants associate with earlier disease onset. Combined with the relatively strong influence of Caesarean delivery, in which patients are not exposed to vaginal microflora, these collective data suggest a possible two hit disease mechanism involving gene-environment interaction particularly important to the development of EoE in early life.

Due to the retrospective nature of this study, some of our data, including early life environmental factors (exposure to antibiotics and PPI during the first year of life), initial pre-treatment distal esophageal biopsies for EoEHSS and esophageal molecular expression profiles, and diagnostic EGD videos, are limited by their availability in the CCHMC pathology repository or EMR. In addition, a validated symptom instrument was not used, and non-verbal patients were compared to verbal patients, limiting the specificity of presenting symptomatology. As such, the conclusions drawn from this data are exploratory endpoints needing further study. As a large, tertiary, pediatric EoE referral center, the presence of V-EoE may reflect a referral bias in part, but nearly 40% of V-EoE patients lived within the Cincinnati Metropolitan Area. Additionally, the conclusion that V-EoE has a lower risk of fibrostenosis is limited by the relatively short duration of longitudinal data.

In conclusion, very early onset EoE is a common presentation of EoE that shows no evidence of increased severity or worse prognosis (as assessed by response to standard therapy, endoscopic findings, and complications), and is likely due to enrichment of Caesarean delivery and *CAPN14* SNPs. Therefore, these data highlight the value of early recognition, diagnosis, and treatment of EoE in infancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ACTG2	gamma-2 actin
BZH	basal zone hyperplasia
CAPN14	calpain 14
CCHMC	Cincinnati Children’s Hospital Medical Center
cDNA	complementary DNA
DSG1	desmoglein 1
EDP	Eosinophilic Esophagitis Diagnostic Panel
EGD	esophagogastroduodenoscopy
EI	eosinophilic inflammation
EoE	eosinophilic esophagitis
EoEe1	eosinophilic esophagitis endotype 1
EoEe2	eosinophilic esophagitis endotype 2
EoEe3	eosinophilic esophagitis endotype 3
eos/hpf	eosinophils per high-power field
EoEHSS	Eosinophilic Esophagitis Histology Scoring System
EREFS	Endoscopic Reference Score

EWT	esophageal wall thickness
FFPE	formalin-fixed, paraffin-embedded
IQR	interquartile range
L-EoE	later onset eosinophilic esophagitis
LPF	lamina propria fibrosis
MM	muscularis mucosa
OR	odds ratio
PCA	principal component analysis
PEC	peak eosinophil count
POSTN	periostin
PPI	proton pump inhibitor
SNPs	single-nucleotide polymorphisms
STC	swallowed topical corticosteroids
TSLP	thymic stromal lymphopoietin
V-EoE	very early onset eosinophilic esophagitis

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Key Messages:

- V-EoE demonstrates a similar molecular pathogenesis to later onset disease.
- V-EoE responds to standard therapy.
- V-EoE demonstrates enrichment for Caesarean section and *CAPN14* genetic variants.
- *CAPN14* is the first identified EoE genetic susceptibility variant that affects EoE age of onset.

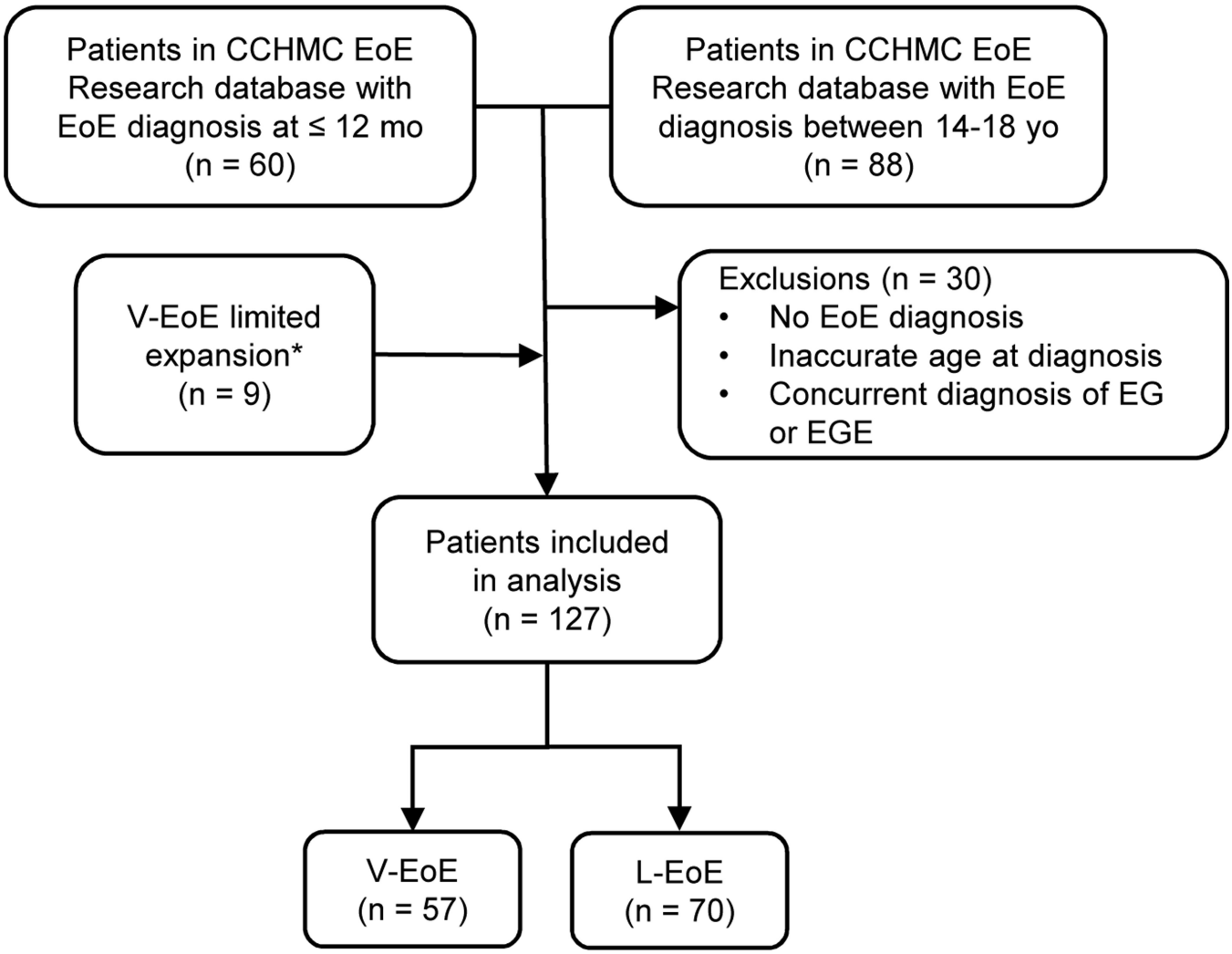

Figure 1. Patient selection and exclusion.

Flow diagram of patient selection and exclusion. V-EoE limited expansion (n=9) represents patients with symptoms consistent with EoE prior to 12 mo but diagnosed with EoE between 12–18 mo needed for esophageal molecular analysis validation due to inadequate sample availability in patients diagnosed ≤12 mo. These patients were included in all analyses when data was available. EG, eosinophilic gastritis. EGE, eosinophilic gastroenteritis; EoE, eosinophilic esophagitis; L-EoE, late onset eosinophilic esophagitis; V-EoE, very early onset eosinophilic esophagitis.

*Symptoms present at ≤ 12 mo; diagnosis at 12–18 mo.

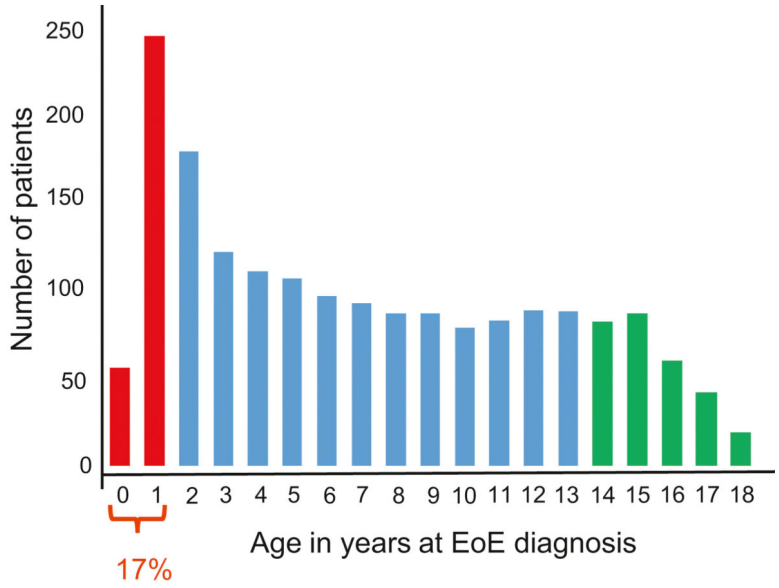

Figure 2. EoE prevalence by age of diagnosis.

Bar graph depicting the prevalence of EoE by age of diagnosis in years within the CCHMC EoE Research Database. EoE, eosinophilic esophagitis. Red and green bars are from where the V-EoE and L-EoE groups, respectively, are derived.

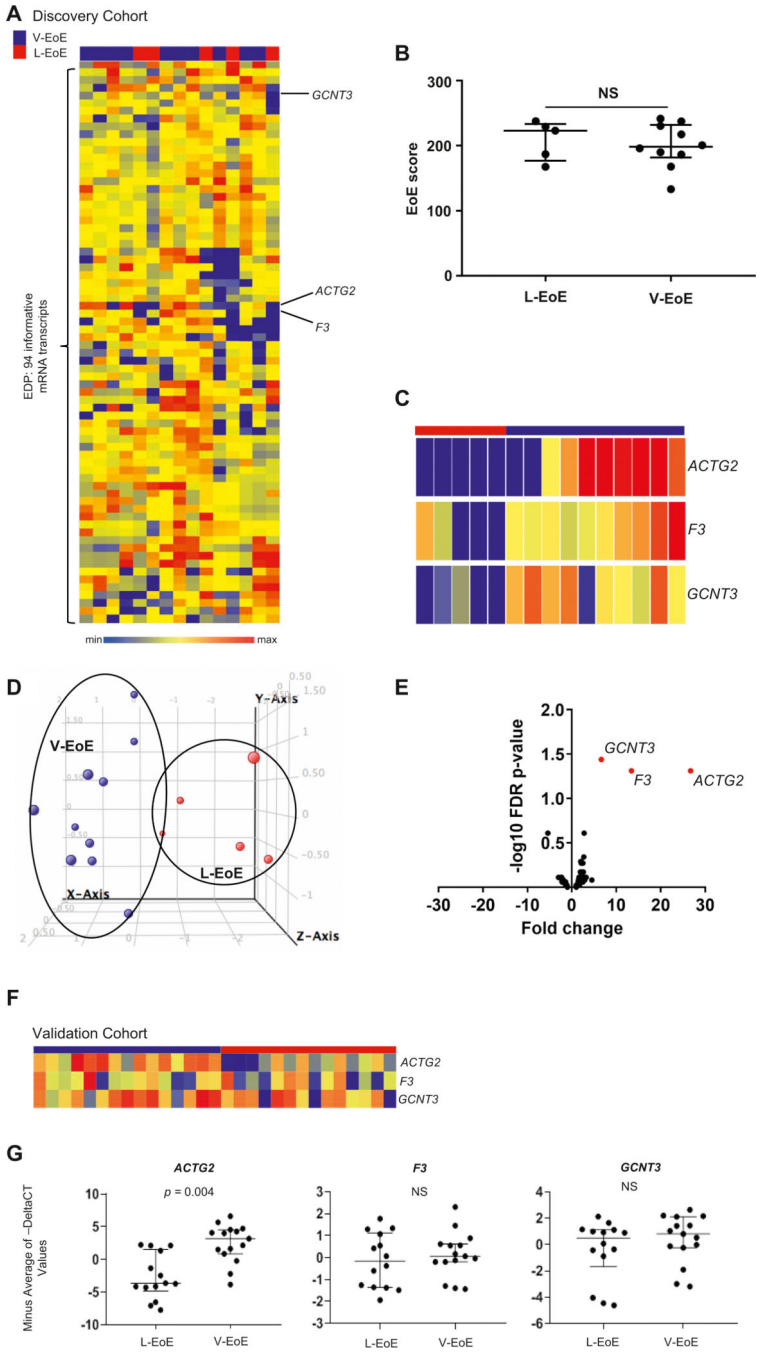

Figure 3. Esophageal molecular expression of the discovery cohort.

A) Heat diagram of unsupervised cluster analysis of EDP for the discovery cohort. B) Graph of EDP EoE Score, summation of delta CT values of the most highly dysregulated genes within the EDP, between V-EoE and L-EoE. C) Heat map of supervised cluster analysis of the discovery cohort EDP. D) Principal component analysis based on three genes identified through supervised cluster analysis. The x-axis is PCA 1. The y-axis is PCA 2. The z-axis is PCA 3. E) Volcano plot of fold change and FDR p-value for the three genes identified through supervised cluster analysis. F) Heat diagram of gene transcripts of interest identified

in discovery cohort for the validation cohort. G) Graphs of gene transcript expression (minus average of negative deltaCT values) between V-EoE and L-EoE. For panels B and G, data are shown as mean ± SEM with each circle representing an individual patient. In C and F, the horizontal bar above the graph designates control patients (red) and patients with EoE (blue). EDP, EoE Diagnostic Panel; EoE, eosinophilic esophagitis; L-EoE, late onset eosinophilic esophagitis; V-EoE, very early onset eosinophilic esophagitis. NS, not significant.

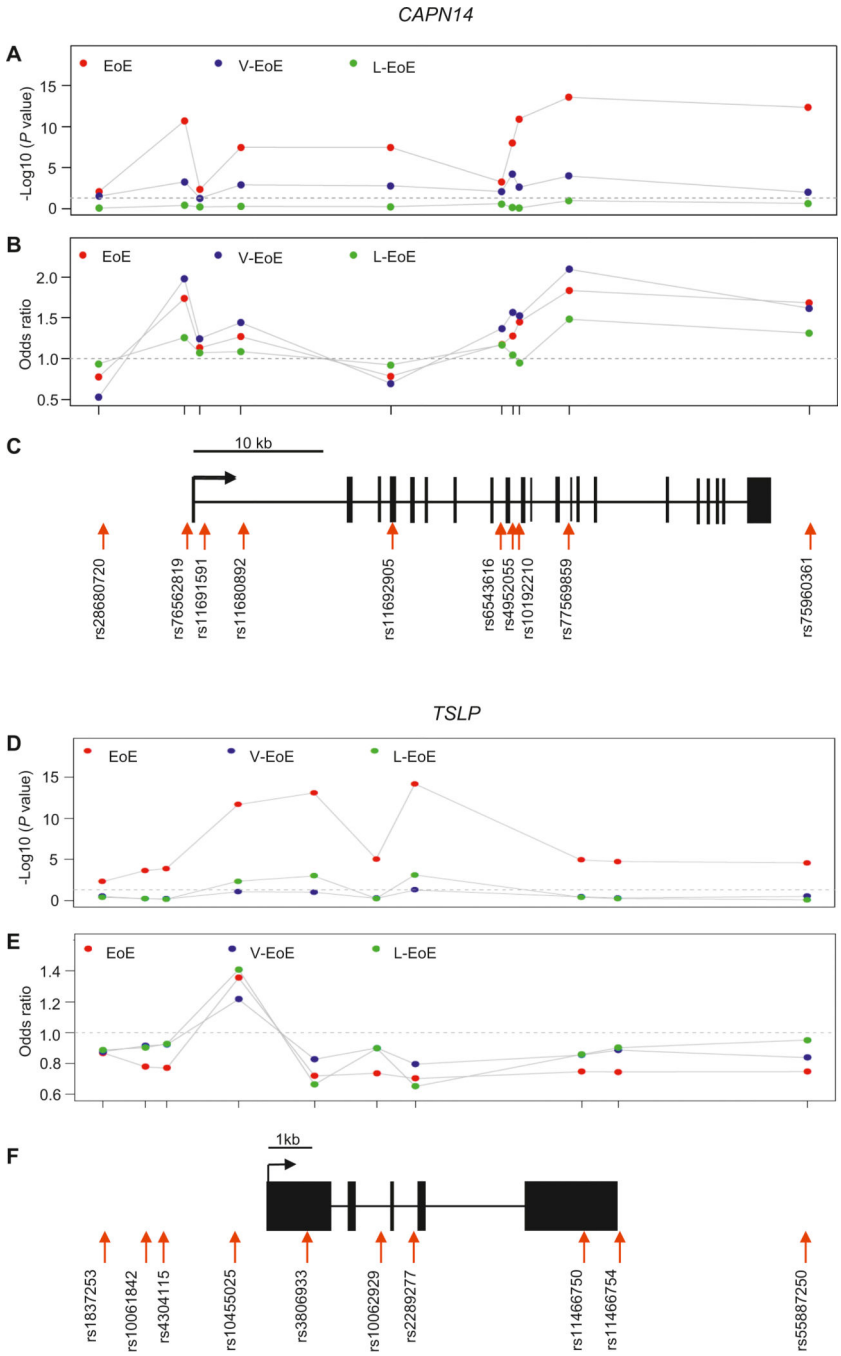

Figure 4.

The A and B panels demonstrate the negative log p-values and odds ratios, respectively, for 9 *CAPN14* SNPs associated with EoE versus controls (red), V-EoE versus controls (blue), and L-EoE versus controls (green). The gray dotted lines in A and B represent p-value = 0.05. The C panel demonstrates where the *CAPN14* SNPs of interest occur in relation to *CAPN14* with black bars representing exons. The D and E panels demonstrate the negative log p-values and odds ratios, respectively, for all 10 *TSLP* SNPs found with association to EoE versus controls (red), V-EoE versus controls (blue), and L-EoE versus controls (green).

The gray dotted lines in D and F represent p-value = 0.05. The F panel demonstrates where the 10 *TSLP* SNPs of interest occur in relation to *TSLP* with black bars representing exons.

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Table 1.

Baseline patient characteristics

	V-EoE (n = 57)	L-EoE (n = 70)	*p*
Sex (Male, n [%])	41 (72%)	45 (64%)	.36
Age at EoE diagnosis (mean ± SD, y)	0.93 ± 0.26	15.7 ± 1.0	<.0001
Race (White, n [%])	52 (91%)	68 (97%)	.14
Ethnicity* (Hispanic, n [%])	1 (2%)	0 (0%)	.47
Duration of follow up (mean ± SD, y)	5.6 ± 3.3	4.3 ± 1.8	.008
Duration of symptoms prior to EoE diagnosis (median [IQR], y)	0.74 (0.61, 0.86)	2.5 (1.7, 5.9)	<.0001

*Data missing for this parameter for 5 patients with L-EoE.

Table 2.

Clinical EoE history

	n	V-EoE	n	L-EoE	*p*
Presenting symptoms					
Dysphagia *	49	16 (33%)	54	39 (72%)	**<.0001**
Pain **	55	33 (60%)	70	50 (71%)	.18
Feeding issues	48	15 (31%)	48	5 (10%)	**.02**
Weight concerns ǂ	53	40 (75%)	54	11 (20%)	**<.0001**
Nausea/vomiting	48	38 (79%)	53	30 (57%)	**.02**
Initial treatment	57		68		**<.0001** ¶
Dietary restriction		34 (60%)		10 (15%)	
Swallowed topical corticosteroids		12 (21%)		46 (68%)	
Both		7 (12%)		5 (7%)	
Other		4 (7%)		7 (10%)	
Successful treatment	55		57		**<.0001** ¶
Dietary restriction		27 (49%)		10 (18%)	
Swallowed topical corticosteroids		7 (13%)		33 (58%)	
Both		19 (35%)		7 (12%)	
Other		2 (4%)		7 (12%)	
Clinical outcomes					
Histologic remission at last endoscopy	56	42 (75%)	69	26 (38%)	**<.0001**
Refractory to therapy	56	3 (5%)	65	4 (6%)	1.0
Concern for therapy non-adherence	57	3 (5%)	70	35 (50%)	**<.0001**
Environmental factors					
Preterm (≤36 wga)	56	9 (16%)	61	3 (5%)	.07
Caesarean section delivery	46	23 (50%)	35	9 (26%)	**.03**
Any breastfeeding	38	29 (76%)	31	20 (65%)	.28
Urban #	50	41 (82%)	69	62 (90%)	.22

Weeks gestational age (wga)

* Includes choking/gagging with feeding

** Includes irritability, heartburn, chest and abdominal pain

Oral aversions and poor appetite

ǂ Poor weight gain and failure to thrive with growth chart verification

¶ P-values denote the global difference between V-EoE and L-EoE across four treatment categories.

Residence in an urban setting, as defined by the 2010 US Census, at time of diagnosis.

Table 3.

Histologic characteristics

	n	V-EoE	n	L-EoE	*p*
Peak eosinophil count	29	94 (73–133)	33	65 (47–116)	.07
EoEHSS grade					
Basal zone hyperplasia	29	3 (2–3)	33	2 (2–3)	**.04**
Eosinophilic inflammation	29	3 (3–3)	33	3 (2–3)	**.02**
Eosinophilic abscesses	29	0 (0–1)	33	0 (0–1)	.51
Eosinophil surface layering	29	2 (0–3)	33	2 (0–2)	.22
Dilated intercellular spaces	29	2 (2–2)	33	2 (2–2)	.18
Surface alteration	29	1 (0–2)	33	2 (0–2)	.91
Apoptotic epithelial cells	29	0 (0–0)	33	0 (0–0)	.10
Lamina propria fibrosis	27	0 (0–1)	18	1.5 (0–3)	**.03**
Total grade	29	0.46 (0.42–0.58)	33	0.48 (0.33–0.60)	.93
EoEHSS stage					
Basal zone hyperplasia	29	3 (3–3)	33	3 (2.5–3)	.34
Eosinophilic inflammation	29	3 (2–3)	33	2 (2–3)	**.03**
Eosinophilic abscesses	29	0 (0–1)	33	0 (0–1)	.49
Eosinophil surface layering	29	1 (0–1)	33	1 (0–1)	.13
Dilated intercellular spaces	29	3 (2–3)	33	3 (3–3)	.57
Surface alteration	29	1 (0–2)	33	1 (0–2)	.52
Apoptotic epithelial cells	29	0 (0–0)	33	0 (0–0)	.10
Lamina propria fibrosis	27	0 (0–3)	18	3 (0–3)	.10
Total stage	29	0.50 (0.41–0.54)	33	0.42 (0.33–0.54)	.20
Muscularis mucosa present	28	27 (96%)	32	2 (6%)	**<.0001**

Data reported as median and interquartile ranges. The EoEHSS scale ranges from 0 to 3 with 3 being the maximum and indicating greater severity. Total scores are the ratio of the summation of the scores of the features over the total possible score based on the number of features assessed.

Table 4.

Endoscopic characteristics as assessed by EREFS

	n	V-EoE	n	L-EoE	*p*
Overall sum	9	6 (2–7)	13	9 (6.5–9.5)	**.02**
Distal sum	9	4 (2–5)	13	5 (4–5)	**.03**
Proximal sum	9	2 (0–2.5)	13	4 (2–4)	**.02**
Maximum composite*	9	4 (2–5)	13	5 (4.5–6.5)	**.008**
Maximum inflammatory	9	4 (2–5)	13	5 (4–5.5)	**.04**
Presence of fibrostenosis¶	9	0%	13	31%	**.03**
Peak eosinophil count	9	101 (73–159)	13	62 (43–144)	.37

Data, with exception of Presence of fibrostenosis (%), are reported as medians and interquartile ranges.

The possible data range is 0–10 with the exception of Overall sum, which is 0–20; maximum inflammatory, which is 0–6; and peak eosinophil count, which has an unlimited range.

*Summation of the maximum score between proximal and distal esophagus scores for each feature.

Summation of the maximum score between proximal and distal esophagus scores for edema, exudates, and furrows.

¶Presence of esophageal rings or strictures in either proximal or distal esophagus.