Analysis of eosinophilic esophagitis in children with repaired congenital esophageal atresia

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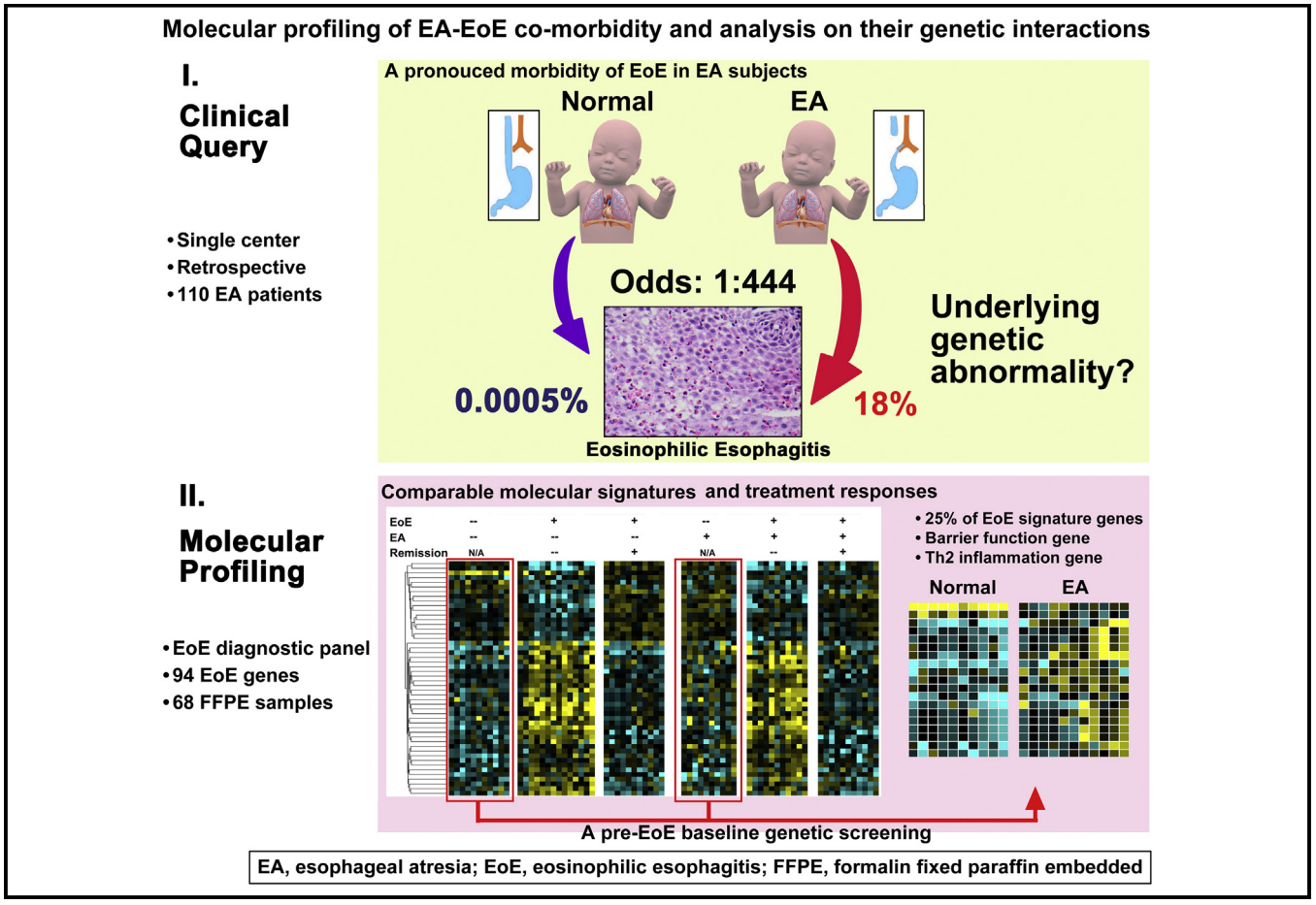
GRAPHICAL ABSTRACT



Background: A high prevalence of eosinophilic esophagitis (EoE) has been preliminarily reported in patients after repair of esophageal atresia (EA), but the basis of this association is unknown.

Objectives: We aimed to (1) characterize the EoE transcriptome in patients with EA, (2) compare the EoE transcriptome in patients with EoE and EA with that in patients with EoE alone, and (3) identify transcripts that could predispose patients with EA to EoE.

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Disclosure of potential conflict of interest: M. E. Rothenberg is a consultant for PulmOne, Spoon Guru, Celgene, Shire, Astra Zeneca, GlaxoSmithKline, Allakos, Adare, Regeneron, and Novartis; has an equity interest in PulmOne, Spoon Guru, Celgene, and Immune Pharmaceuticals; and has received royalties from reslizumab (Teva Pharmaceuticals). M. E. Rothenberg and T. Wen are coinventors of the EDP patent owned by Cincinnati Children's Hospital. The rest of the authors declare that they have no relevant conflicts of interest.

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Methods: This single-center, population-based, retrospective study identified 4 EoE study cohorts: healthy control subjects, patients with EA and EoE (EA+EoE+), patients with EA without EoE (EA+EoE−), and patients with EoE without EA (EA−EoE+). Molecular signatures were assessed by using the EoE diagnostic panel, a 94-gene expression quantitative PCR array.

Results: In a cohort of 110 pediatric patients with surgically repaired EA, 20 (18%) patients were given a diagnosis of EoE, representing a 364-fold enrichment of EoE in patients with EA compared with the general pediatric population. EoE diagnostic panel analyses revealed a major overlap between the EA+EoE+ and EA−EoE+ cohorts. A proportion (approximately 25%) of EoE signature genes were dysregulated in patients with EA+EoE− compared with healthy control subjects, including those involved in epithelial barrier function and type 2–associated inflammatory responses. Patients with EA+EoE+ exhibit a more severe EoE clinical phenotype than those with EA−EoE+ in terms of dysphagia and dilation need.

Conclusions: Patients with EA have increased risk of EoE. Patients with EoE with EA have a similar molecular profile compared with that of patients with EoE without EA. Dysregulated baseline epithelial barrier and type 2–associated genes in EA monomorbidity might explain the higher EoE prevalence in patients with EA. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

Key words: *Eosinophils, T_H2 inflammation, EoE transcriptome, GERD*

Eosinophilic esophagitis (EoE) is an emerging chronic food-induced allergic disorder characterized by marked esophagus-specific eosinophilia associated with esophageal dysfunction (eg, dysphagia in adults)[1] diagnosed based on results of an esophageal biopsy showing 15 or more eosinophils per high-power field.[2] The reported prevalence of EoE in multiple continents consistently ranges from 1 in 2,500 to 1 in 10,000 subjects.[3-5] Since the recognition of the disease 2 decades ago, basic and translational research has elucidated a food allergen–driven, immune-mediated molecular pathogenesis.[6,7] Interestingly, an increased prevalence of EoE has been clinically observed in patients with coexisting disease or a history of connective tissue disorders,[8] aerodigestive syndromes,[9] and esophageal atresia (EA),[10-17] suggesting that the allergic components of EoE can interact with other disease processes, including inborn errors, to promote the EoE pathogenesis.

EA is a congenital digestive abnormality that requires immediate postnatal repair by means of surgery. The prevalence of EA has been estimated to be 1 in 2400 to 1 in 4500 births.[17-19] Although the cause of EA has not been associated with allergic inflammation or food allergy, an increased prevalence of EoE in the population with EA has been recently reported.[10-17] Notably, the anatomy, dysmotility, and exposure of the lumen to acid refluxate in the postsurgical esophagi of patients with EA likely differ from those of a healthy control (NL) esophagus.[13]

In this study, we examined the relationship between EoE and EA by profiling the EoE transcript signature, which is represented by an EoE diagnostic panel (EDP) of 94 esophageal mRNAs that are dysregulated in the esophagi of patients with EoE. We aimed to answer the following key questions: (1) Does the EoE transcriptome differ with EA and EoE comorbidity (EA+EoE+ vs EA−EoE+)? (2) Does the molecular signature reversal of EoE remission differ with EA comorbidity (EoE remission: EA+EoE+ vs EA−EoE+)? (3) Does a baseline gene dysregulation explain why patients with EA have greater susceptibility to EoE? Answering these questions has yielded information about the underlying pathoetiology and susceptibility mechanisms of EoE in the context of EA.

Abbreviations used
CCHMC: Cincinnati Children's Hospital Medical Center
EA: Esophageal atresia
EDP: EoE diagnostic panel
EoE: Eosinophilic esophagitis
NL: Healthy control
PCA: Principal component analysis
SCH: Sydney Children's Hospital

METHODS

Patients

We performed a retrospective review of epidemiologic data and esophageal histopathologic biopsy slides collected at Sydney Children's Hospital (SCH) between 2000 and 2014. The patients were all between 0 and 18 years of age. On the basis of a retrospective chart review, patients were randomly selected by histopathologists and gastrointestinal physicians at SCH and divided into 4 major cohorts: NL subjects, patients with EA and EoE (EA+EoE+), patients with EA without EoE (EA+EoE−), and patients with EoE without EA (EA−EoE+). Children with no history of EA repair having more than 15 eosinophils/high-power field in their esophageal biopsy specimens were defined as the EA−EoE+ cohort, and healthy children having an endoscopy for evaluation of their gastrointestinal symptoms without detectable esophageal tissue eosinophilia or other pathologies were defined as the NL cohort. Age and sex were largely matched, although a male dominance was present in the cohorts with EoE (EA−EoE+ and EA+EoE+). The EA+EoE+ and EA−EoE+ cohorts were also analyzed before and after treatment of EoE. Patients were characterized as being atopic if they had a history of asthma, eczema, hay fever, or food allergy. Patients were characterized as having food allergy based on history, RAST or skin prick test results, or both.

Formalin-fixed, paraffin-embedded RNA extraction and reverse transcription

Formalin-fixed, paraffin-embedded blocks of esophageal biopsy specimens were shipped to Cincinnati Children's Hospital Medical Center (CCHMC) for analysis, as previously described.[20] Briefly, 6 to 8 sections at a thickness of 10 μm were immediately subjected to extraction with the miRNeasy RNA extraction kit (217004; Qiagen, Hilden, Germany), according to the manufacturer's instructions. RNA samples were stored at −80°C after extraction. An aliquot of RNA (approximately 500 ng) was reverse transcribed to cDNA by using iScript cDNA Synthesis (170-8891; Bio-Rad Laboratories, Hercules, Calif). The resulting cDNA samples were stored at −20°C before EDP analysis.

EDP experiments

Molecular signatures were analyzed by using the EDP at CCHMC and quantified by using a 94-gene mRNA expression signature array from formalin-fixed, paraffin-embedded esophageal biopsy sections, as originally described.[21] Gene expression signatures were acquired by using a low-density quantitative PCR array and analyzed with a series of algorithms, including clustering and EoE score calculation. TaqMan quantitative PCR amplification

was performed with 384-well fluidic cards. An aliquot of cDNA equivalent to 500 ng of starting RNA was adjusted to 100 μL with H_2O, mixed with 100 mL of TaqMan Universal PCR Master Mix (4440040; Applied Biosystems, Foster City, Calif), and loaded on fluidic cards. The standard amplification protocol consisted of a ramp of 50°C for 2 minutes and a hot start of 94.5°C for 10 minutes, followed by 40 cycles of 30 seconds at 97.0°C and 1 minute at 59.7°C.

Statistical methods

Patients' clinical data were analyzed by using the Fisher exact test to test for nonrandom associations between 2 categorical variables. Statistical comparisons were made with the statistical software package GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, Calif). EDP and gene expression data were analyzed by using the 2-tailed Student *t* test or 2-way ANOVA test with GraphPad Prism software. In both cases a *P* value of less than .05 was deemed statistically significant.

Principal component analysis (PCA) was performed with the embedded module in GeneSpring GX 11 (Agilent Genomics, Santa Clara, Calif), focusing on variations among conditions/samples. Volcano plots were generated by using GeneSpring GX 11 when 2 conditions were compared side by side within the scope of the 94 EDP genes. Gene ontology analysis was carried out with the CCHMC's ToppGene online gene ontology tool (https://toppgene.cchmc.org/), particularly involving the ToppFun module and the ToppGenet module, for biological function and protein-protein interaction predictions, respectively.

RESULTS

Increased EoE prevalence in patients with EA

From 2000 to 2014, 20 patients with EA were given a diagnosis of EoE from a cohort of 110 patients with EA surgically treated at SCH. The EA anatomic subtype composition is shown in Fig 1, *A*, with type C being the most dominant type in both the EA+EoE+ and EA+EoE− cohorts. Consistent with recent studies,[10-12] approximately 18% of patients with EA had EoE (Fig 1, *D*).[3] This value contrasts with that of the reported prevalence of EoE in approximately 0.05% of the general pediatric population,[3] resulting in a 364-fold enrichment of EoE in patients with EA compared with the general pediatric population (odds ratio, 444; 95% CI, 59-3347; $P < .001$).

Characterizing EoE in patients with EA

The histologic features of EoE of the EA+EoE+ and EA−EoE+ cohorts were similar, as noted based on tissue eosinophilia, spongiosis, basal layer hyperplasia, parakeratosis, and subepithelial hyalinization. A representative photomicrograph of EA+EoE+ is shown in Fig 1, *B*. The EA+EoE+ cohort was 63% male, 82% white, 72% atopic, and 36% food allergic, whereas the EA−EoE+ cohort was 69% male, 92% white, 46% atopic, and 38% food allergic (Fig 1, *C*). Tissue eosinophil counts were indistinguishable between the EA+EoE+ and EA−EoE+ cohorts (Fig 1, *E*). Patients of the EA+EoE+ cohort were given a diagnosis of EoE at 3.9 ± 2.5 years of age (mean ± SD), which was comparable with 6.6 ± 5.0 years of age for patients of the EA−EoE+ cohort ($P = .80$; Fig 1, *F*), suggesting a similar natural history for EoE with or without EA.

Patients with EoE with or without EA had significantly more reflux symptoms than NL subjects (EA+EoE+ and EA−EoE+, $P = .03$ and .003 vs NL subjects, respectively; see Table E1 in this article's Online Repository at www.jacionline.org). A significantly greater percentage of patients with EA+EoE+ than those with EA alone (EA+EoE−) or EoE alone (EA−EoE+) complained of dysphagia (EA+EoE+ vs EA+EoE− or EA−EoE+: $P = .02$ and .03, respectively). Significantly more patients with EA+EoE+ than NL subjects had dysphagia ($P = .0003$). Significantly more patients with EA+EoE+ had episodes of food bolus impactions when compared with the EA−EoE+ ($P = .023$), EA+EoE− ($P = .012$), and NL cohorts ($P = .012$, see Table E1). Significantly more patients in the EA+EoE+ cohort had strictures and needed dilatations than did patients in the EA−EoE+ cohort ($P = .001$ for strictures and $P = .0011$ for dilatations, see Table E1).

Comparative EDP analysis of EoE with and without EA

The EDP molecular signature of the EA+EoE+ and EA−EoE+ cohorts have comparable profiles relative to their EA+EoE− and NL control subjects, respectively (Fig 2, *A* and *B*).[21] The EA+EoE+ and EA−EoE+ cohorts achieved a mean EoE score of 543 (95% CI, 502-584) and 532 (95% CI, 491-573), respectively. One-way ANOVA with a Tukey *post hoc* test on all cohorts with *P* values and 95% CIs is illustrated for comparison purposes (Fig 2, *B*). There was a significant difference ($P < .001$) in the EDP-derived EoE score for patients with EA+EoE+ versus NL subjects, patients with EA+EoE−, and patients with EA+EoE+ remission and for patients with EA−EoE+ versus NL subjects and patients with EA−EoE+ remission (see Table E2 in this article's Online Repository at www.jacionline.org). The EDP-derived EoE score showed no significant differences between patients with EA+EoE− and NL subjects or patients with EA+EoE+ and those with EA−EoE+. Furthermore, PCA (Fig 2, *C*) demonstrated that the EA+EoE+ and EA−EoE+ cohorts exhibited a common expression pattern, whereas the other 4 cohorts had dissimilar expression patterns, as indicated by the quadrant positions.

EoE remission pattern is similar with or without EA

We next used a paired design to further elucidate the EoE molecular remission pattern after treatment (largely topical glucocorticoid with the rest being diet). The EA+EoE+ transcriptome was reversible after successful treatment and comparable with the EA−EoE+ remission transcriptome, as shown by reversal of the heat diagram pattern (Fig 2, *A*). As expected, both EoE scores and *CCL26*/eotaxin 3 levels were significantly ameliorated after EoE treatment ($P < .001$ for both comparisons; Fig 3, *A*). A similar remission pattern in the EA+EoE+ and EA−EoE+ cohorts after treatment of EoE was observed; for instance, the mean posttreatment EoE scores were 662 (95% CI, 648-676) for EA+EoE+ versus 654 (95% CI, 631-671) for EA−EoE+, which were accompanied by a similar *CCL26* mRNA level reduction of 85% ± 27% versus 91% ± 12% (mean ± SD; Fig 3, *B*), respectively. A comparable disease status (active to remission) in the EA+EoE+ and EA−EoE+ cohorts was also revealed by using PCA before and after EoE treatment (Fig 3, *C*).

Basal EDP gene dysregulation in patients with EA identifies EoE susceptibility gene candidates

To identify dysregulated genes in patients with EA at baseline (before EoE onset), we compared the NL and EA+EoE− cohorts

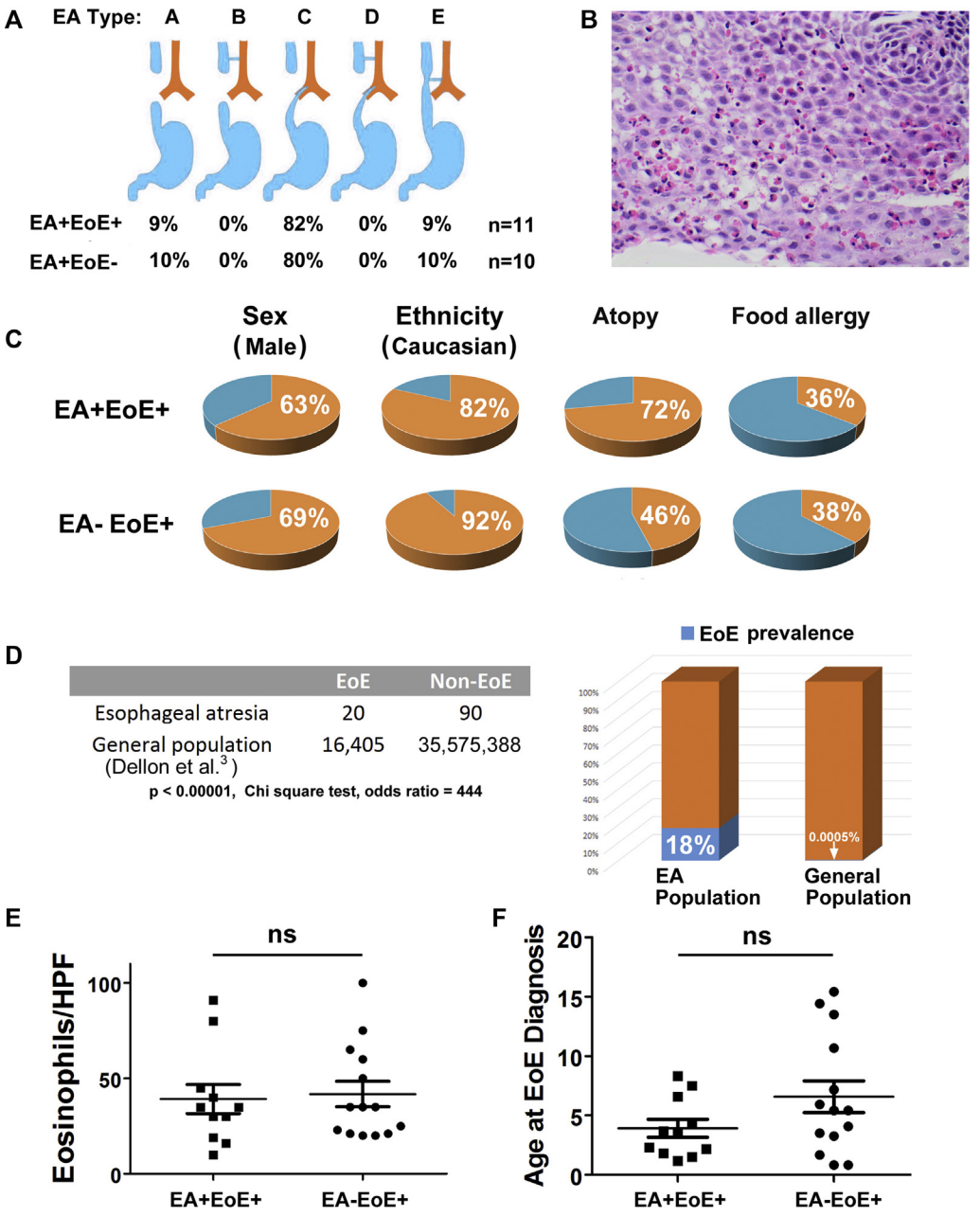

FIG 1. Clinical and demographic features of patients with EA and EoE. **A,** Schematic summary of the surgical and anatomic classification of the patients with EA involved in this study. **B,** High-power (×40 magnification) micrograph showing a 5-μm hematoxylin and eosin–stained biopsy section from a patient representative of the EA+EoE+ cohort, demonstrating extensive tissue eosinophilia. **C,** Percentage pie charts of the EA+EoE+ and EA−EoE+ study cohorts, illustrating largely comparable frequencies of male sex, white ethnicity, atopy, and food allergy. **D,** Incidence of patients with EoE, EA, or both in SCH. Twenty patients with EA had EoE 4 years after postnatal surgical intervention. The EoE incidence in the general population (reported by Dellon et al[3]) is juxtaposed for comparison. A χ^2 test indicates a highly significant difference in EoE prevalence between the population with EA and the general population. **E,** Tissue eosinophilia levels between the EA+EoE+ and EA−EoE+ cohorts were compared. **F,** Based on patients with EA who received postnatal surgical intervention, initial ages of EoE diagnosis were compared between the EA+EoE+ and EA−EoE+ cohorts. *ns*, Nonsignificant, as determined by using a 2-tailed *t* test. In Fig 1, *E* and *F*, each *symbol* represents an independent subject. Bar chart data are presented as means ± SEMs.

using a 2-tailed *t* test within the scope of the EDP. Notably, a baseline expression difference, with increased expression of several genes, was observed between the NL and EA+EoE− cohorts (Fig 2, *A*, heat diagram). Comparing NL subjects with patients with EA+EoE− at baseline, there were 19 genes significantly different ($P < .05$) between the 2 cohorts (Fig 4, *A*). Among the 19 significant genes, 17 were upregulated and 2 were downregulated in patients with EA+EoE− versus NL subjects, which was

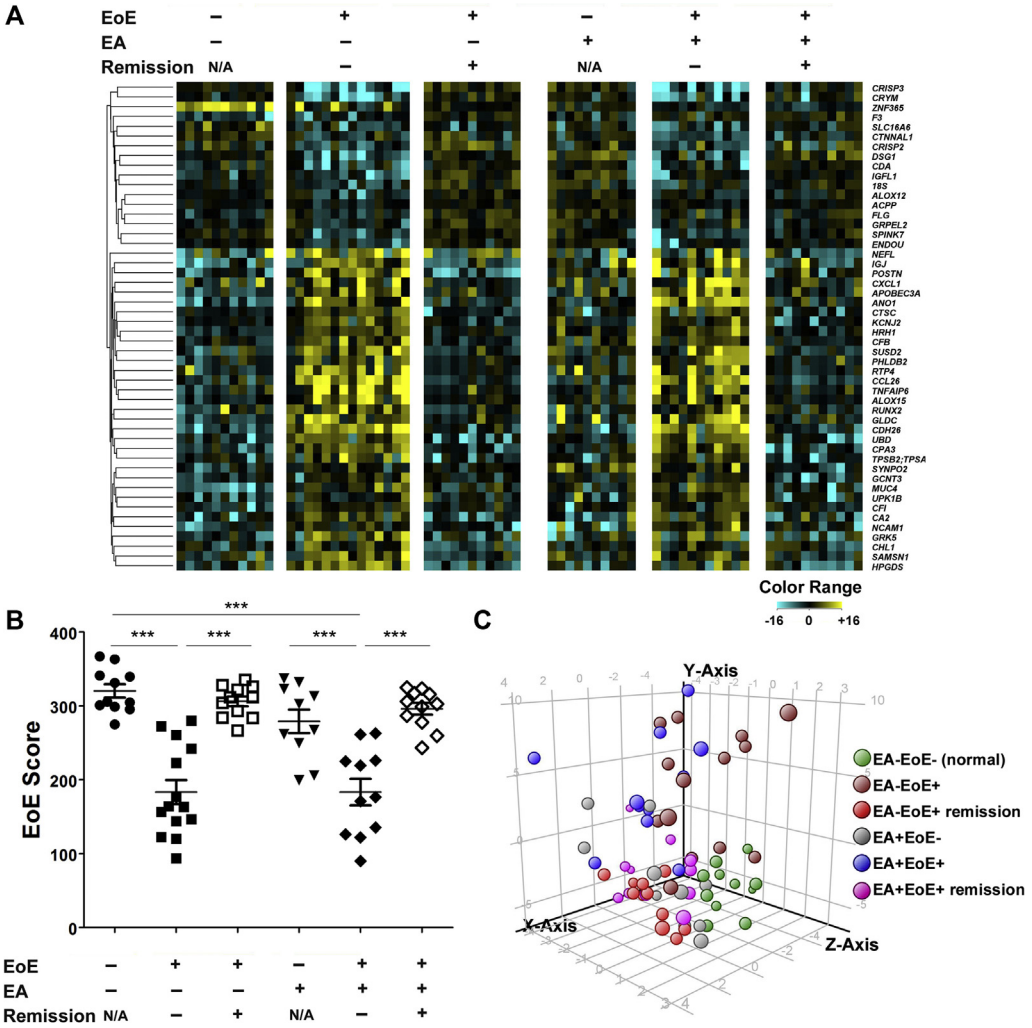

FIG 2. EA and EoE transcriptomic analysis using EDP. **A,** By using the EDP, the transcriptome of each patient group is shown in the form of a heat diagram in the context of 50 significant EDP genes ($P < .05$, fold change > 2.0, EA−EoE− [NL] vs EA−EoE+ cohorts). Remission status shown with + and − indicates EoE remission and active disease status, respectively. *N/A*, Not applicable. **B,** EDP gene expression profiles were converted into an "EoE score," as previously described.[21] These integers directly reflect the degree of gene dysregulation during allergic inflammation in patients with EoE. Each *symbol* represents an individual patient: *solid circle*, NL subjects, n = 11; *solid squares*, patients with EA−EoE+, n = 14; *open squares*, patients with EA−EoE+ remission, n = 11; *solid triangles*, patients with EA+EoE−, n = 10; *solid diamonds*, patients with EA+EoE+, n = 11; *open diamonds*, patients with EA+EoE+ remission, n = 11. ***$P < .001$, 1-way ANOVA plus Tukey *post hoc* test. **C,** Multidimensional expression matrix was reduced to 3 major variation-contributing factors by using principle component analysis. The relative position of the 6 involved patient groups is summarized and visualized in a 3-dimensional diagram, with each of the axes representing one of the 3 major variation components. Each *symbol* represents an individual patient. Bar chart data are presented as means ± SEMs.

the same direction as observed in patients with EA−EoE+ versus NL subjects. Gene ontology analysis of these 19 differential genes found functional nodes that were enriched in pathways involved in human type 2 disorders and food intolerance (Fig 4, *B*). Interestingly, the upregulated genes in the EA+EoE− cohort at baseline were also increased in patients with EA−EoE+ compared with NL subjects; expression of these genes was typically further increased in patients with EoE+EA+ (Fig 4, *C*). Summation scores based on the EoE score algorithm (described previously[21]) of these 19 genes were different between the EA+EoE− and NL cohorts (Fig 4, *D*). Moreover, by using 2-way ANOVA interaction (±EA vs ±EoE), a gene dosage interaction was seen between the EA and EoE factors ($P = .001$; Fig 4, *D*). The EoE score was greater at baseline in patients with EA compared with NL subjects. There was dysregulated expression of 3 epithelial genes known to be involved in allergic inflammation (ie, *FLG*, *MUC4*, and *SYNPO2*; $P < .01$ for all); these genes were more highly expressed in patients with EA+EoE− compared with NL subjects (2-tailed Student *t* test). These 3 genes passed the false discovery rate filter when comparing NL subjects and patients with EA+EoE− at baseline and were replicated in 2 biologically independent cohorts.

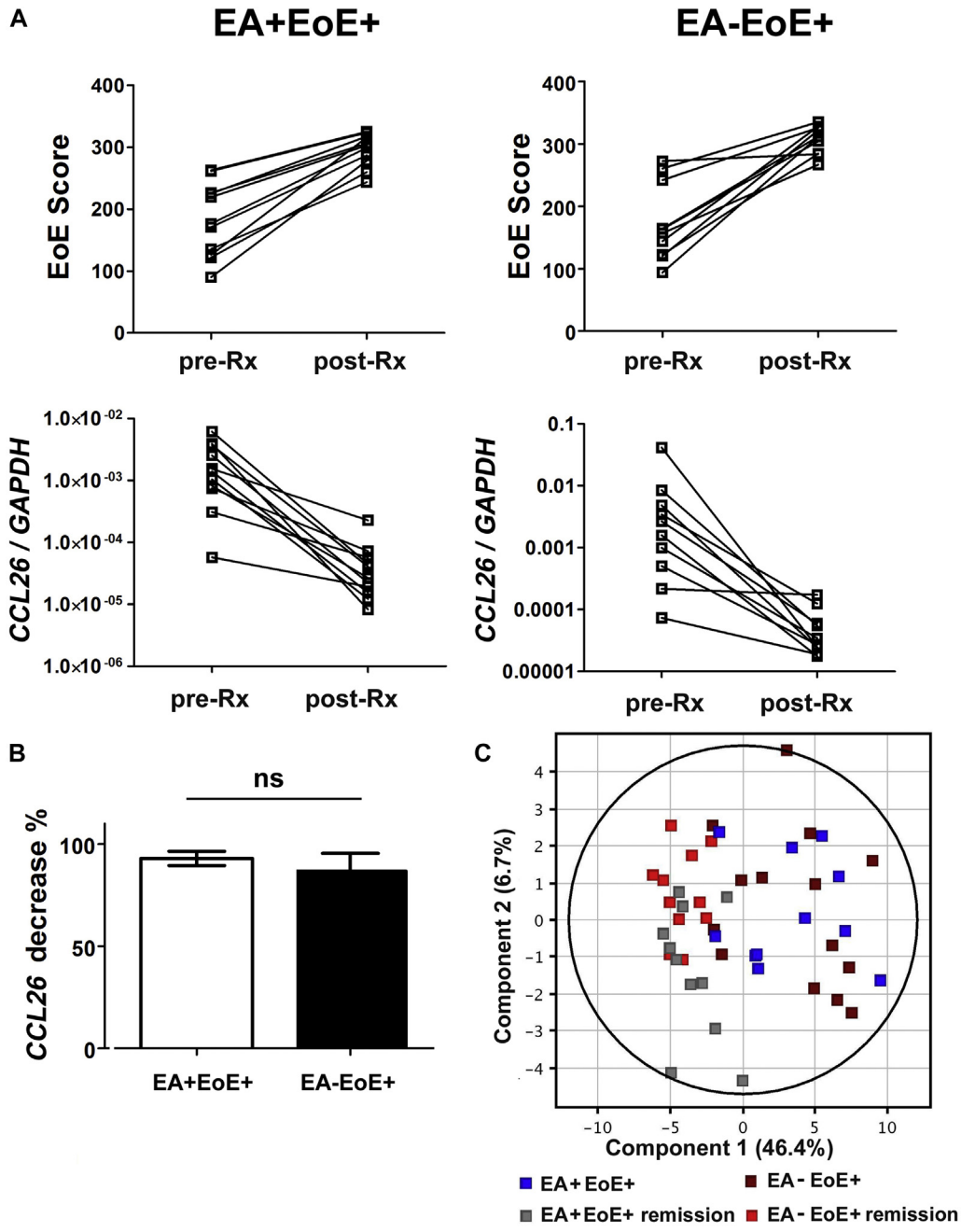

FIG 3. Transcriptome comparisons during remission in patients with EoE with and without EA. **A,** With the paired design in both the EA+EoE+ and EA−EoE+ cohorts, we characterized the treatment *(Rx)*–induced amelioration in EoE score *(upper panel)* and reduction in *CCL26* expression level *(lower panel)* for each patient recruited before and after EoE treatment. *Left*, EA+EoE+; *right*, EA−EoE+. Each *line* represents an individual patient before and after treatment. **B,** *CCL26* expression levels in the EA+EoE+ and EA−EoE+ cohorts. *ns,* Not significant. **C,** PCA reduces multidimensional data to 2 dimensions, with the *x-axis* and *y-axis* depicting the genetic components contributing to the top 2 variation factors. EA+EoE+ and EA−EoE+ expression patterns are illustrated before and after EoE treatment, with the distance between any 2 given data points in the 2-dimensional space summarizing their expression difference level in 50 representative EoE/EDP genes. Bar chart data are presented as means ± SEMs.

Molecular interpretation of the more severe EoE phenotype in patients with EA+EoE+

The EA+EoE+ cohort exhibited significantly more frequent dysphagia, food bolus impactions, stricture, and need for dilations than did patients with EA−EoE+ (see Table E1). To molecularly decipher this notable clinical phenotype, we aimed to identify those genes whose dysregulation could explain the more severe EoE-like phenotypes in the comorbidity. We first performed 3

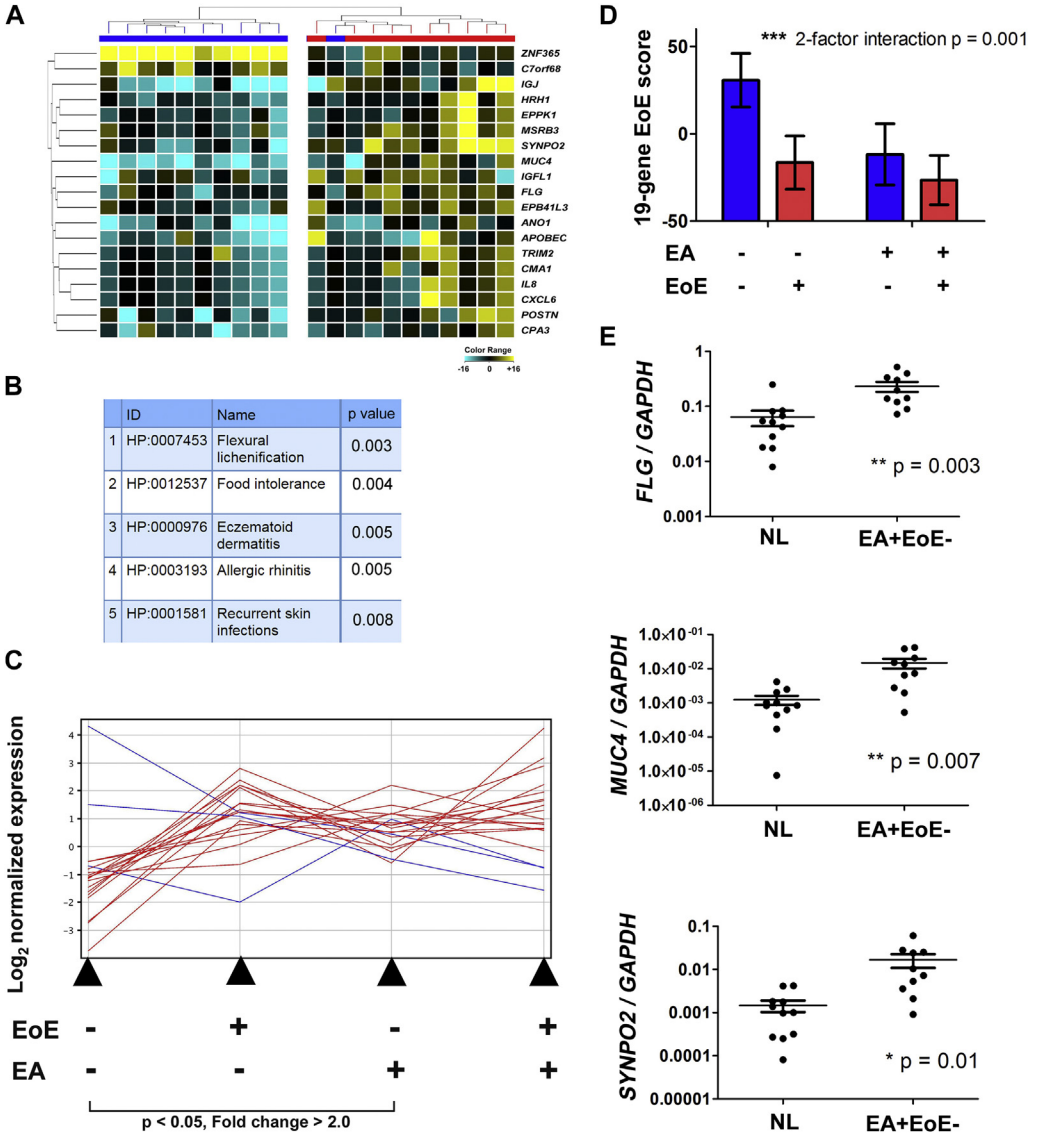

FIG 4. Identification of a cohort of EoE-susceptible genes in patients with EA at baseline. **A,** To identify potential genes expressed in patients with EA that predispose this population to EoE development, we compared the molecular signature between the EA+EoE− *(EA)* and EA−EoE− *(NL)* cohorts by using EDP analysis, resulting in 19 differentially expressed genes ($P < .05$, fold change > 2.0, 2-tailed *t* test). With unsupervised clustering based on these 19 genes, patients with EA *(red label)* and NL subjects *(blue label)* were well separated, as indicated by the colored hierarchy tree on top of the heat expression diagram. **B,** Gene ontology analysis illustrates the biofunction of these 19 significant genes. Molecular function and related human disorders are illustrated, with the *P* value for each category shown. **C,** Individual gene expression patterns across the 4 study cohorts were plotted with a $\log_2$-converted scale. **D,** EoE scores based on these 19 significant genes are plotted across all 4 study groups. **E,** Among the 19 significant genes, scattered dot plots are shown for a selected cohort of 3 genes, *FLG*, *MUC4*, and *SYNPO2*, that passed the false discovery rate filter. Each shape represents an individual patient. Bar chart data are presented as means ± SEMs.

independent moderated *t* tests in the context of the presence of dysphagia, food bolus impactions, strictures, and need for dilation in all studied subjects. In addition, we compared the EDP signatures of the EA+EoE+ and EA−EoE+ cohorts to identify those EA-specific EoE genes (volcano plots were shown in Fig 5, *A*). The above-mentioned 4 differential gene cohorts were Venn overlapped to identify the core genes potentially regulating all clinical and molecular features, with the screening strategy schematically summarized in Fig 5, *A*. Of note, *ANO1* and *CTNNAL1* were dysregulated in all 4 comparisons ($P < .05$, fold change > 2.0). *ANO1* and *CTNNAL1*, together with *UPK1B*, *ZNF365*, *HPGDS*, and *F3*, were collectively dysregulated in the EA+EoE+ cohort compared with the EA−EoE+ cohort (Fig 5, *A* and *B*). Given their potential etiological importance, the expression patterns of *ANO1* and *CTNNAL1* across all studied groups are shown in Fig 5, *B*.

Based on those 6 dysregulated genes between the EA+EoE+ and EA−EoE+ cohorts, we further carried out a gene ontology

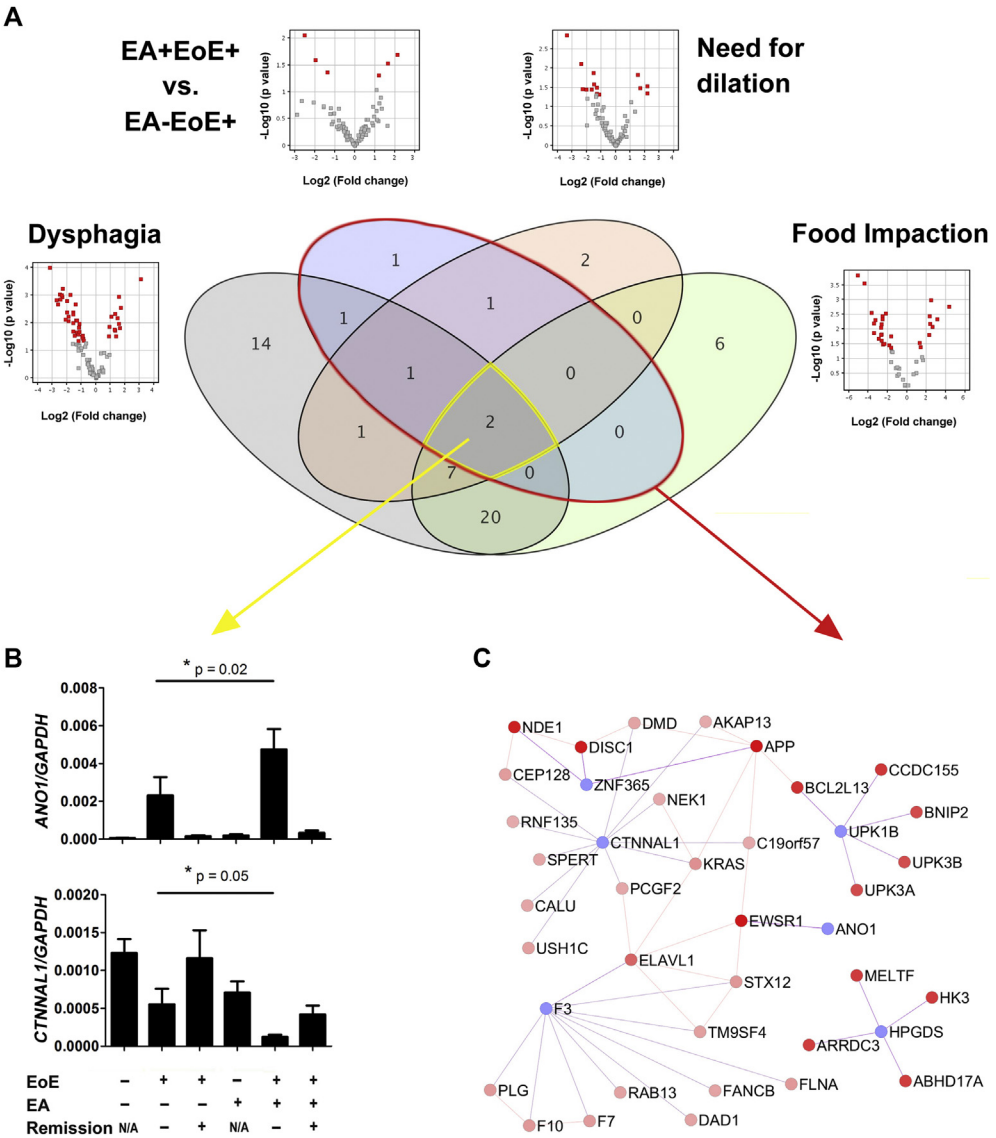

FIG 5. Severe symptoms in the EA+EoE+ cohort explained by molecular characterization. **A,** Within the scope of the EDP, differentially expressed genes related to symptoms of dysphagia, presence of strictures, need for dilation of strictures in patients with EA+EoE+ versus those with EA−EoE+ were identified by using a moderated 2-tailed *t* test. Multiscreening results are individually summarized by using the volcano plots in the *upper panel*, with the *x-axis* being the fold change and the *y-axis* being the negative-logarithm-based *P* values. Overlap among these 4 cohorts of genes are exhibited in the Venn diagram in the lower panel. **B,** As the quad-overlapping genes, the expression pattern of *ANO1* and *CTNNAL1* among all studied groups is illustrated as the mean ± SEM. **P* ≤ .05, 2-tailed moderated *t* test. **C,** On the basis of the 6 differentially expressed genes between patients with EA+EoE+ and those with EA−EoE+ (enclosed in the *red circle* in the Venn diagram in Fig 5, *A*), we performed a protein-protein interaction network analysis to deduce the underlying pathogenic pathways, resulting in a hypothetical interaction network originating from the 6 original training genes in blue nodes and extended to 53 interactions (red nodes, with darker color indicating stronger interaction; ToppGene modules, https://toppgene.cchmc.org/).

analysis focusing on protein-protein interaction networks in human esophageal tissue (Fig 5, *C*). By elucidating protein-protein interactions potentially originated and orchestrated by these 6 EA-EoE differential genes, we initially identified the potential molecular etiological differences between the EA+EoE+ and EA−EoE+ cohorts, which might partially explain the more severe symptom phenotype in the former.

DISCUSSION

We report a strikingly high prevalence (18%) of EoE in the population with EA, extending previous preliminary observations with smaller cohorts.[10-17] Patients with EA+EoE+ had significantly greater rates of dysphagia and episodes of food bolus impactions and had strictures requiring dilations more often than did patients with EA−EoE+, indicating a more severe phenotype

of EA+EoE+; meanwhile, patients with EA+EoE+ also had significantly greater rates of dysphagia and episodes of food bolus impactions than did patients with EA+EoE−, underscoring the clinical and pathologic significance of EoE in patients with EA and the potential importance of timely diagnosis and treatment of EoE.

We have determined that the EA+EoE+ and EA−EoE+ cohorts share similar pathomolecular profiles, responsiveness to treatment, and remission characteristics. The largely overlapping signature and similar response to treatment suggest a common molecular pathogenesis, likely induced by food allergy.[1] The molecular similarity is also underscored by demographic and clinical data demonstrating similar percentages of white ethnicity, male sex, and food allergy status, supporting that EA/EoE comorbidity largely overlaps with conventional EoE. Although the age of EoE diagnosis was not significantly different in the EA+EoE+ and EA−EoE+ cohorts, children with EA who had EoE were given a diagnosis at an earlier age than those with EoE alone, and the lack of statistical significance could have been due the small sample size in this study. We believe that clinicians looking after symptomatic patients with EA need to be aware of the risk of EoE and perform diagnostic endoscopies with sufficient numbers of biopsies at multiple levels, irrespective of the age of the patients.[2]

Although not statistically significant, a higher percentage of the EA+EoE+ cohort were atopic (72%) when compared with the EA−EoE+ cohort (46%). This was likely secondary to the higher incidence of asthma in the EA+EoE+ cohort (64%) than the EA−EoE+ cohort (38%). The high incidence of asthma in the EA cohort has previously been described in literature consistent with our report.[22-27] The high rate of atopy in the EA+EoE+ cohort in our study (72%) also corroborates previous literature.[10,11,14,26,27]

A plausible explanation for the high prevalence of EoE in the population with EA is the dysregulated expression of epithelial genes known to be involved in allergic inflammation in patients with EA at baseline, rendering the affected esophagus more prone to EoE development. Genes related to esophageal epithelial type 2 inflammation (ie, *MUC4*, a specific mucin in response to T_H2 cytokines[21,28]; *SYNPO2*, a gene upregulated in EoE mucosa[6,21]; and *FLG*, a membranal barrier molecule downregulated in patients with EoE and atopic dermatitis[29]) were dysregulated in the EA+EoE− cohort at baseline compared with that seen in NL subjects, suggesting molecular predilection for EoE in this cohort. Although prospective longitudinal studies are needed to confirm whether these patients with EA (without EoE at the time) with dysregulated EoE-predisposing genes at baseline would have EoE in the future, our study is the first to document that the EA cohort is an "at-risk population" for the development of EoE. Notably, gene ontology analysis (Fig 4, *B*) based on the 19 EoE-related genes dysregulated in patients with EA suggest that food intolerance, allergic responses, and skin barrier impairment might be underlying predisposition processes in patients with EA.

A high prevalence of EoE in the EA population has been reported recently,[10-17] but the underlying pathogenesis has not been explored. The greater incidence of EoE in the EA population has been presumed to be caused by impairment of esophageal mucosal barrier function by acid refluxate and prolonged exposure to acid-suppressive medication. Esophageal dysmotility as a result of EA repair can also prolong contact between food antigens and the esophageal mucosa, thereby predisposing these patients to EoE.[17]

Microdeletions encompassing the forkhead box transcription factor gene cluster, specifically the *FOXF1* gene, have recently been shown to be associated with EA. Binding sites for FOXF1 protein include not only the promoter region of genes critical for mesenchyme proliferation but also that of genes for inflammation, including those for the eotaxins and IL-8.[14] Expression of eotaxin 3, a chemoattractant and activating factor for eosinophils, has been shown to be increased 53-fold above normal levels in patients with EoE.[6] It could be postulated that mutations in the FOX gene cluster not only lead to congenital malformations of both the lung and esophagus but also predispose toward EoE through transcriptional linkage to eotaxin.

The finding that the EA+EoE+ cohort has more severe EoE symptoms than the EA−EoE+ cohort is noteworthy and likely clinically relevant. To our knowledge, this is the first study to compare EoE in the EA cohort (EA+EoE+) with EoE alone (EA−EoE+). Interestingly, a previous study had compared patients with EA with and without EoE (EA+EoE+ vs EA+EoE−) and found that patients with EA with EoE had significantly more reflux symptoms and dysphagia than those without.[12] Using bioinformatic screening, we found that there were 6 genes differentially expressed between the 2 entities, with 2 of them (*ANO1* an *CTNNAL1*) associated with the exaggerated dysphagia and stricture development and need for dilations in patients with EA+EoE+. Intriguingly, *ANO1* is specifically expressed by the interstitial cells of Cajal in the human and mouse gastrointestinal tracts[30,31] and is a calcium-activated chloride channel governing gastrointestinal smooth muscle contraction rhythms,[30,32] which might be associated with dysphagia, food bolus impaction, and stricture development phenotypes observed in patients with EA+EoE+ (*ANO1*-high). In addition, *ANO1*, as an EoE-upregulated gene itself,[21] represents an esophageal cancer marker.[33] Notably, patients with EA are susceptible to esophageal squamous cell carcinoma development.

These findings might open the avenue for *ANO1* serving as the molecular marker for a malignant EA prognosis, EoE symptom monitoring, and esophageal cancer prognosis. Although our cohort size is not large enough to reveal a more pronounced molecular dysregulation, our initial findings call for future prospectively designed studies substantiating the implied central role of *ANO1* in the EA-EoE-esophageal cancer triangular relationship.

Despite our unique findings, this study has the limitations of retrospective design and the relatively narrow scope of the EDP compared with an unbiased genome-wide approach. Despite these limitations, our study supports that EoE+EA+ is similar to traditional EoE. Our findings need to be further corroborated with a prospectively designed study to substantiate the pathogenesis of EoE/EA comorbidity at genome-wide levels. Likewise, the full natural history and prognosis of EA+EoE+ is beyond the scope of this report and should be addressed by subsequent studies.

In summary, we report a high prevalence of EoE in an pediatric population with EA using the largest reported cohorts for these conditions to date.[10-17] Phenotypically, the EA+EoE+ and EA−EoE+ cohorts are similar in terms of male dominance, white predisposition, high rate of atopy and food allergy, early age of onset, and overall molecular signatures. However, patients with EA+EoE+ have a significantly greater incidence of

dysphagia and strictures and need for dilation compared with patients with EA−EoE+. Notably, EoE can be part of other congenital inborn genetic diseases, including hypermobility syndrome (eg, Loeys–Dietz syndrome)[8] and phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome,[34] and we now substantiate EoE association with EA. We report that although EA+EoE+ and EA−EoE+ molecular signatures are largely comparable and that their molecular responses to treatment are similar, there is a set of genes related to type 2 inflammation and barrier function that are abnormally expressed in patients with EA (EA+EoE−) at baseline, potentially rendering the population with EA more susceptible to EoE.

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Key messages

- **There is a 364-fold enrichment of EoE in patients with EA compared with the general pediatric population.**
- **There is a shared molecular cause between patients with EoE and patients with EoE and EA, as defined by a similar esophageal transcript profile.**
- **A panel of genes related to type 2 inflammation and barrier function are abnormally expressed in patients with EA at baseline.**

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TABLE E1. Clinical characteristics and patient demographics

Groups	No.	Age* (y [range])	Male sex, % (no.)	White ethnicity, % (no.)	Comorbidity	Atopy, % (no.)	Food allergy,† % (no.)	Reflux symptoms, % (no.)	Dysphagia, % (no.)	Food impaction, % (no.)	Strictures, % (no.)	Dilatations‡	Endoscopic appearance,§ % (no.)	EoE treatment, % (n)
EA−EoE− (NL)	10	8.4 (2.67-18)	30% (3)	80% (1)	1 FAP 1 CP	30% (3)	10% (1)	20% (2)	10% (1)	0% (0)	0% (0)	0	0% (0)	NA
EA+EoE+	11	3.9 (1.17-8.33)	63% (7)	82% (9)	1 CHARGE	72% (8)	36% (4)	73% (8)	91% (10)‖	56% (6)¶	67% (7)#	5**	64% (7)	91% (10) Steroid 9% (1) Diet
EA−EoE+	13	6.6 (1.92-14.42)	69% (9)	92% (12)	1 CP	46% (6)	38% (5)	85% (11)	46% (6)	8% (1)	0% (0)	0	92% (12)	85% (11) Steroid 15% (2) Diet
EA+EoE−	10	7.6 (3.75-16.33)	30% (3)	70% (7)	4 VACTERL	30% (3)	0% (0)	70% (7)	40% (4)	0% (0)	40% (4)	3.75	0% (0)	NA

CHARGE, Coloboma, heart defect, atresia choanae, retarded growth and development, genital abnormality, ear abnormality; *CP*, cerebral palsy; *FAP*, familial adenomatous polyposis; *NA*, not applicable; *VACTERL*, vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, limb abnormalities (having at least 3 of these characteristic features).

Statistical significance indicated at a P value of less than .05 (‖P = .033, ¶P = .023, #P = .001, and **P = .010) when comparing the EA+EoE+ cohort versus the EA−EoE+ cohort.

*Years of age at diagnosis of EoE.

†Food allergy criteria: positive RAST result and skin prick test response.

‡Average number of dilatations per patient before diagnosis and treatment of EoE.

§Endoscopic appearance of EoE: presence of exudate, rings, edema, furrows, stricture.

TABLE E2. EDP score comparison amongst study cohorts

Tukey multiple comparison test	Mean difference	*q* Value	*P* < .05	Summary	95% CI of difference
NL vs EA−EoE+	137	10.61	Yes	*	83.25 to 190.7
NL vs EA−EoE+ remission	14.23	1.041	No	NS	−42.64 to 71.10
NL vs EA+EoE−	41.04	2.93	No	NS	−17.24 to 99.31
NL vs EA+EoE+	137	10.02	Yes	*	80.13 to 193.9
NL vs EA+EoE+ remission	24.2	1.77	No	NS	−32.67 to 81.07
EA−EoE+ vs EA−EoE+ remission	−122.8	9.505	Yes	*	−176.5 to −69.02
EA−EoE+ vs EA+EoE−	−95.96	7.23	Yes	*	−151.2 to −40.74
EA−EoE+ vs EA+EoE+	0.01033	0.0007998	No	NS	−53.73 to 53.75
EA−EoE+ vs EA+EoE+ remission	−112.8	8.733	Yes	*	−166.5 to −59.06
EA−EoE+ remission vs EA+EoE−	26.81	1.914	No	NS	−31.47 to 85.08
EA−EoE+ remission vs EA+EoE+	122.8	8.982	Yes	*	65.90 to 179.6
EA−EoE+ remission vs EA+EoE+ remission	9.968	0.7293	No	NS	−46.90 to 66.84
EA+EoE− vs EA+EoE+	95.97	6.852	Yes	*	37.69 to 154.2
EA+EoE− vs EA+EoE+ remission	−16.84	1.202	No	NS	−75.11 to 41.44
EA+EoE+ vs EA+EoE+ remission	−112.8	8.253	Yes	*	−169.7 to −55.93

The Tukey multiple comparison test was used to compare all possible pairs.
ns, Not significant.
*$P < .001$.