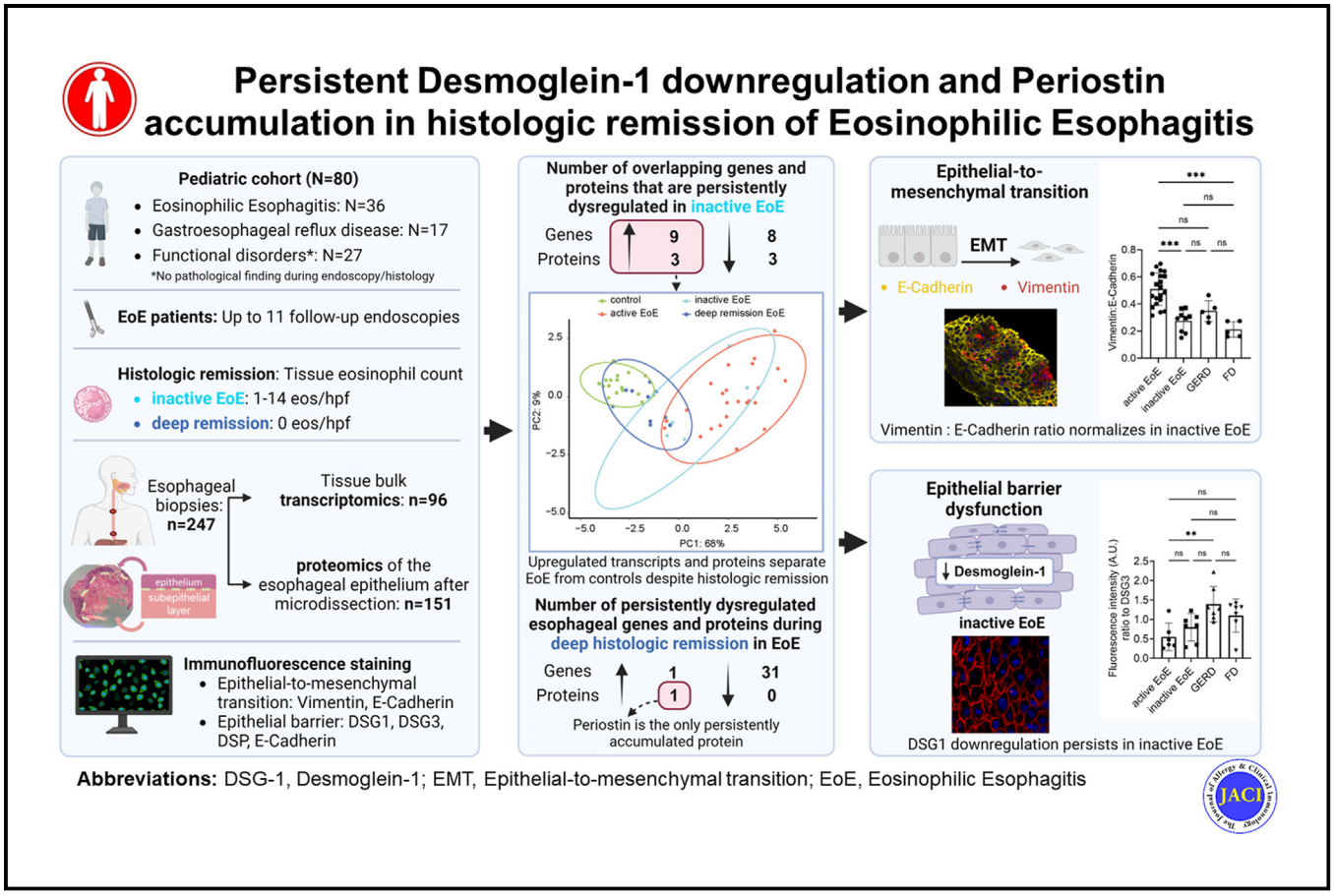
Persistent desmoglein-1 downregulation and periostin accumulation in histologic remission of eosinophilic esophagitis

Hannes Hoelz, MD, Tim Faro, Marie-Luise Frank, Ignasi Forné, PhD, Daniela Kugelmann, MD, Anja Jurk, et al

GRAPHICAL ABSTRACT



Capsule summary: A comparative analysis of transcripts and proteins in esophageal biopsy samples from pediatric patients with eosinophilic esophagitis (EoE) showed persistent molecular dysregulation despite histologic remission and identified a set of biomarkers distinguishing EoE in deep remission from controls.

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Persistent desmoglein-1 downregulation and periostin accumulation in histologic remission of eosinophilic esophagitis

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Background: Patients with eosinophilic esophagitis (EoE) require long-lasting resolution of inflammation to prevent fibrostenosis and dysphagia. However, the dissociation between symptoms and histologic improvement suggests persistent molecular drivers despite histologic remission.

Objective: We characterized persisting molecular alterations in pediatric patients with EoE using tissue transcriptomics and proteomics.

Methods: Esophageal biopsy samples (n = 247) collected prospectively during 189 endoscopies from pediatric patients with EoE (n = 36, up to 11 follow-up endoscopies) and pediatric controls (n = 44, single endoscopies) were subjected to bulk transcriptomics (n = 96) and proteomics (n = 151). Intercellular junctions (desmoglein-1/3, desmoplakin, E-cadherin) and epithelial-to-mesenchymal transition (vimentin:E-cadherin ratio) were assessed by immunofluorescence staining.

Results: Active EoE (≥15 eosinophils per high-power field [eos/hpf]), inactive EoE (<15 eos/hpf), and deep-remission EoE (0 eos/hpf) were diagnosed in 107 of 185, 78 of 185, and 41 of 185 biopsy samples, respectively. Among the dysregulated genes (up-/downregulated 310/112) and proteins (up-/downregulated 68/16) between active EoE and controls, 17 genes, and 6 proteins remained dysregulated in inactive EoE. Using persistently upregulated genes (n = 9) and proteins (n = 3) only, such as *ALOX15, CXCL1, CXCL6, CTSG, CDH26, PRRX1,* CLC, EPX, and periostin (POSTN), was sufficient to separate inactive EoE and deep-remission biopsy samples from control tissue. While 32 differentially expressed genes persisted in deep-remission EoE compared to controls, the proteome normalized except for persistently upregulated POSTN. Epithelial-to-mesenchymal transition normalized in inactive EoE, whereas desmosome recovery remained impaired as a result of desmoglein-1 downregulation.

Conclusion: The analysis of molecular changes shows persistent EoE-associated esophageal dysregulation despite histologic remission. These data expand our understanding of inflammatory processes and possible mechanisms that underlie tissue remodeling in EoE. (J Allergy Clin Immunol 2025;155:505-19.)

Key words: *Eosinophilic esophagitis, proteome, transcriptome, epithelial barrier dysfunction, fibrosis*

Eosinophilic esophagitis (EoE) is a chronic allergen-mediated disease with rising incidence and prevalence, particularly in the Western world.[1] Multiple cell types and mechanisms contribute to the EoE tissue pathology, such as eosinophils, mast cells, *ALOX15*-positive macrophages, T-helper type 2 cells, related cytokines, epithelial remodeling, and barrier disruption.[2-4] The treatment goal is to induce sustained resolution of inflammation because persistent EoE activity results in subepithelial fibrosis, leading to dysphagia and complications, such as stenotic strictures.[4] Until now, the cellular and molecular events that result in long-lasting resolution of inflammation without ongoing tissue remodeling are unknown.[5-7]

Remission in EoE is defined as improvement in histopathology, symptoms, and endoscopic appearance. Symptom control is considered the most important treatment goal for pediatric patients and their parents.[8] According to current guidelines, histologic remission is defined by a cutoff value of <15 eosinophils per high-power field (eos/hpf).[9-11] There is insufficient correlation between symptoms and histologic findings, and patients may experience persistent symptoms despite histologic remission, suggesting persistent molecular changes.[12,13]

We aimed in this study to describe the extent of molecular disease clearance and investigate pathophysiologic processes during active disease and after resolution of inflammation by transcriptomic and proteomic tissue analysis in esophageal

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Abbreviations used

CLC: Charcot-Leyden-Crystals
DAP: Differentially accumulated protein
DEG: Differentially expressed gene
DSG: Desmoglein
ED: Elimination diet
EDP: EoE diagnostic panel
EMT: Epithelial-to-mesenchymal transition
EoE: Eosinophilic esophagitis
eos: Eosinophils
EPX: Eosinophil peroxidase
EREFS: EoE endoscopic reference score
FC: Fold change
FD: Functional disorders
GERD: Gastroesophageal reflux disease
hpf: High-power field
PCA: Principal component analysis
PEC: Peak eosinophil count
POSTN: Periostin
PPI: Proton pump inhibitors
Vim/Ecad: Ratio of vimentin to E-cadherin

biopsy samples collected throughout the disease course. We identified persistent molecular alterations in deep histologic remission (no esophageal infiltrate; 0 eos/hpf), which may be related to persistent symptoms such as dysphagia due to fibrostenosis, and may represent biomarkers for treatment stratification or future targets for EoE therapy.

METHODS

Patient population and study design

In this monocentric study, pediatric patients with suspected or established EoE were recruited between April 15, 2020, and September 3, 2021, at Dr von Hauner Children's Hospital and followed prospectively. Written informed consent was obtained from parents/legal guardians, and children older than 6 years signed a statement of assent. The study was approved by the local institutional review board (ethical approval LMU Munich 02/13/2020, approval no. 19-777). Children (3-17 years of age) were enrolled if they were scheduled for endoscopy as part of their routine clinical care to evaluate upper gastrointestinal symptoms. All participants were diagnosed and treated according to current guidelines.[9-11] Active EoE was diagnosed if patients had symptomatic esophageal dysfunction and a peak eosinophil count (PEC) of ≥15 eos/hpf, corresponding to ≥60 eos/mm.[9-11] To detect possible residual changes in the transcriptome and proteome in deep histologic remission, we used 0 eos/hpf as a further cutoff in addition to the guideline-based definition of histologic remission (PEC <15 eos/hpf). Proton pump inhibitors (PPI), elimination diet (ED), or topical corticosteroid therapy were used alone or in combination to induce and maintain histologic remission.

Patients with gastroesophageal reflux disease (GERD), defined by the presence of troublesome GERD symptoms (eg, heartburn, regurgitation) and endoscopic and/or histologic findings attributed to active reflux esophagitis (eg, erosions, microscopic evidence of inflammation, basal cell hyperplasia) but without esophageal eosinophilia (0 eos/hpf) served as inflammatory controls.[14] Patients in whom endoscopy for upper gastrointestinal symptoms did not reveal any endoscopic or histologic abnormalities (no evidence of inflammation, 0 eos/hpf) served as noninflammatory controls, and their disease was termed functional disorder (FD). For the control groups, one biopsy per patient with GERD or FD was included.

We analyzed clinical, endoscopic, histologic, and molecular features. Esophageal biopsy collection and preparation are described in the Methods section in this article's Online Repository available at www.jacionline.org. Sex was considered as a biologic variable in the transcriptomics and proteomics analysis.

Microdissection and proteomics

Mass spectrometry may be biased toward the detection of abundant proteins.[15] To reduce bias through extracellular matrix proteins such as collagens, elastins, fibronectins, and laminins, the esophageal mucosa of the epithelium was isolated from the surrounding tissue layers by microdissection.[16] This targeted proteomic analysis allows a better assignment of protein dysregulation to epithelial cells and infiltrating immune cells due to the homogeneous starting tissue. Formalin-fixed, paraffin-embedded tissue biopsy samples were cut into 10 μm scrolls, the paraffin removed, and mounted on glass slides. The epithelium was scratched from the glass slide using an optical light microscope and corresponding hematoxylin and eosin staining (see Fig E2, *B,* in the Online Repository available at www.jacionline.org). Label-free proteomic analysis was performed by liquid chromatography–tandem mass spectrometry and MaxQuant analysis, as previously described.[17] Proteomic analysis was controlled for total ion intensity, and individual samples were reanalyzed if necessary. Only the analysis with the higher total ion intensity was considered for further analysis. Details of the microdissection, protein extraction, and proteomic analysis are provided in the Methods section of the Online Repository.

Bulk transcriptomics

RNA isolation was performed from esophageal biopsy samples, which were stored in an RNA stabilizing solution and processed for RNA extraction. RNA was isolated using standard techniques, and RNA sequencing and downstream transcriptomics analysis were performed as described in the Methods section of the Online Repository.

Immunostaining

Immunostaining was performed on formalin-fixed, paraffin-embedded esophageal biopsy samples as well as snap-frozen biopsy samples. Staining protocols, image acquisition, and analysis are specified in the Methods section of the Online Repository. The antibodies and dyes we used are specified in Tables E3 and E4, also available in the Online Repository available at www.jacionline.org.

Statistical analysis

Descriptive statistics were used to describe the demographics and disease characteristics of the pediatric EoE cohort at baseline. One-way ANOVA (Kruskal-Wallis test) with Dunn multiple comparison test and 2-way ANOVA with Bonferroni multiple comparisons test were used to analyze multiple groups for statistical significance. For statistical analysis, GraphPad Prism v10.5.0 (GraphPad Software, Boston, Mass) and R v4.3.1 (R Project;

TABLE I. Baseline cohort patient characteristics

Characteristic	Total (N = 80)	EoE (N = 36)	Controls	
			GERD (N = 17)	FD (N = 27)
Sex				
Male	52 (65)	29 (81)*	23 (52)*	
Female	28 (35)	7 (19)*	21 (48)*	
Age (years) at diagnosis	10.0 ± 4.4	9.1 ± 4.8	10.8 ± 4.0	
Age (years) at study inclusion	10.1 ± 4.5	9.7 ± 4.6	10.4 ± 4.3	
Newly diagnosed	68 (85)	24 (67)	17 (100)	27 (100)
Therapy at baseline				
No therapy	58 (73)	22 (61)*	36 (82)*	
PPI	12 (15)	7 (19)	5 (11)	
ED (±PPI)	6 (8)	6 (17)**	0**	
Topcial corticosteroid (±PPI±ED)	1 (1)	1 (3)	0	
Therapy during follow-up				
PPI		33 (92)		
ED (±PPI)		24 (67)		
Topcial corticosteroid (±PPI±ED)		8 (22)		
Histology (n = 145)				
Peak eosinophil count (eos/hpf), median (IQR)	—	21 (2-45)	—	—
Endoscopies and histologic disease activity	189	145	17	27
Active EoE (≥15 eos/hpf)	NA	83	NA	NA
Inactive EoE (<15 eos/hpf)	NA	62	NA	NA
Deep remission (0 eos/hpf)	NA	31	NA	NA

Data are presented as nos. (%), means ± standard deviations, or medians (IQR, 25th to 75th percentile).
EREFS, EoE endoscopic reference score;[19,20] *IQR,* interquartile range; *NA,* not applicable; *N,* individual patients; *n,* biopsies/samples.
*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$, respectively; significant differences between EoE and controls were calculated by Fisher exact test and Mann-Whitney test.

www.r-project.org) were used. Corrected *P* values for multiple comparison of *<.05, **<.01, ***<.001, and ****<.0001 were considered statistically significant.

RESULTS

Patient characteristics

Detailed patient characteristics are listed in Table I and Table E1 in the Online Repository available at www.jacionline.org. Proteomic and transcriptomic analyses were performed in 247 biopsy samples acquired during 189 endoscopies from 80 patients in total (Fig 1, *A*). Among those, 36 patients with EoE were included, from whom biopsy samples of 145 endoscopies were analyzed (Fig 1, *A*). Age distribution and disease duration of the EoE cohort are illustrated in Fig E2, *A*. As controls, we analyzed biopsy samples from 17 patients with GERD and 27 patients with FD (1 biopsy/endoscopy time point per patient). Of patients with EoE, 1 baseline sample and up to 11 follow-up biopsy samples were included (maximum follow-up time of 41 months; no follow-up available in 4 patients, Fig E2, *C*). The grouping of disease activity was based solely on histologic PEC as a result of poor correlation between PEC and clinical symptoms (see Fig E1 in the Online Repository), which was also described previously.[18] Active EoE (≥15 eos/hpf) was diagnosed in 107 (58%) of 185 biopsy samples and inactive EoE (<15 eos/hpf) in 78 (42%) of 185 biopsy samples, respectively. Of those, deep histologic remission of EoE (0 eos/hpf) was observed in 41 (22%) of 185 biopsy samples. Active, inactive, and deep-remission EoE biopsy samples were derived from 34, 19, and 22 patients of the EoE cohort, respectively. Among 24 newly diagnosed patients, 3 (12.5%) were pretreated with PPI because of symptoms suggestive of gastroesophageal reflux. Among 12 patients with established EoE, 11 (92%) of 12 were receiving typical treatment with PPI (n = 4), ED (±PPI) (n = 6), or topical corticosteroid (n = 1) at the time of the first available biopsy sample, and 4 (36%) of 11 patients had persistently active disease (2 with PPI and 2 with PPI + ED). Symptoms despite histologic remission were present in 26 (72%) of 36 patients with EoE (Fig E2, *C*).

Unbiased proteomic and transcriptomic profiling of esophageal biopsy samples

To investigate molecular differences between active and inactive EoE and controls, we performed transcriptome and proteome profiling in 54 and 131 longitudinally collected EoE biopsy samples and 42 and 20 control biopsy samples, respectively. Patient and sample overlap between both studies is illustrated in Fig 1, *A*. Histologic disease activity and the number of follow-up visits from patients with EoE are shown separately for transcriptomic and proteomic analyses (Fig 1, *B*).

As a result of the varying number of follow-up endoscopies per patient with EoE during the observation period, the analyses based on all available biopsy samples are unbalanced because patients with many follow-up visits are overrepresented. To confirm that the results are not skewed by the unequal patient weighting, balanced analyses, based on the same number of biopsy samples per patient, were conducted. As shown in the Online Repository available at www.jacionline.org (especially in Table E2, Fig E3, and Fig E14), only minor differences in

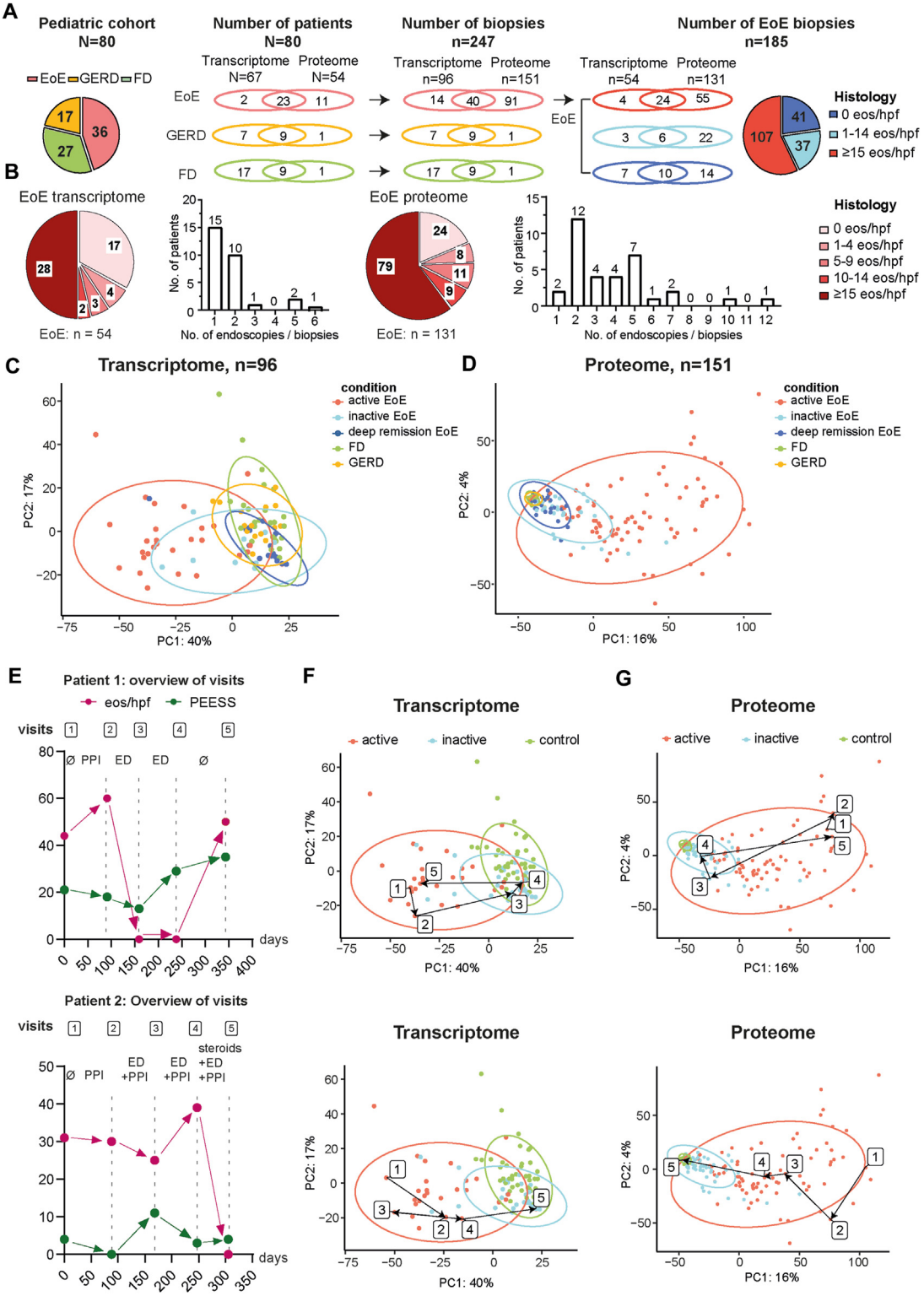

FIG 1. Patient cohort and expression profiles of transcripts and proteins in EoE and controls. **A,** Overview of patient cohort. Overlapping samples and patients between tissue transcriptome and proteome are depicted. **B,** Distribution of histologic disease activity and number of endoscopies for EoE patients is shown. Only 1 biopsy sample was analyzed per time point. **C** and **D,** Unsupervised clustering of expression profiles from esophageal biopsy samples is shown in PCA (after batch correction) for both *(C)* transcripts and *(D)* proteins. PCAs were created using standard DESeq2 function plotPCA() selecting top 500 genes that show highest variance across all biopsy samples.[21] Number of follow-up biopsy samples of EoE patients used for PCA is depicted in *(A)*. **E,** Histologic assessment of eos/hpf and clinical symptoms assessed by PEESS v2.0, are illustrated for 2 exemplary patients over treatment course. Individual changes in **(F)** transcriptome and **(G)** proteome during disease course for these patients are highlighted in PCA, showing timeline of visits *(arrows)*. *PEESS,* Pediatric Eosinophilic Esophagitis Symptom Score.

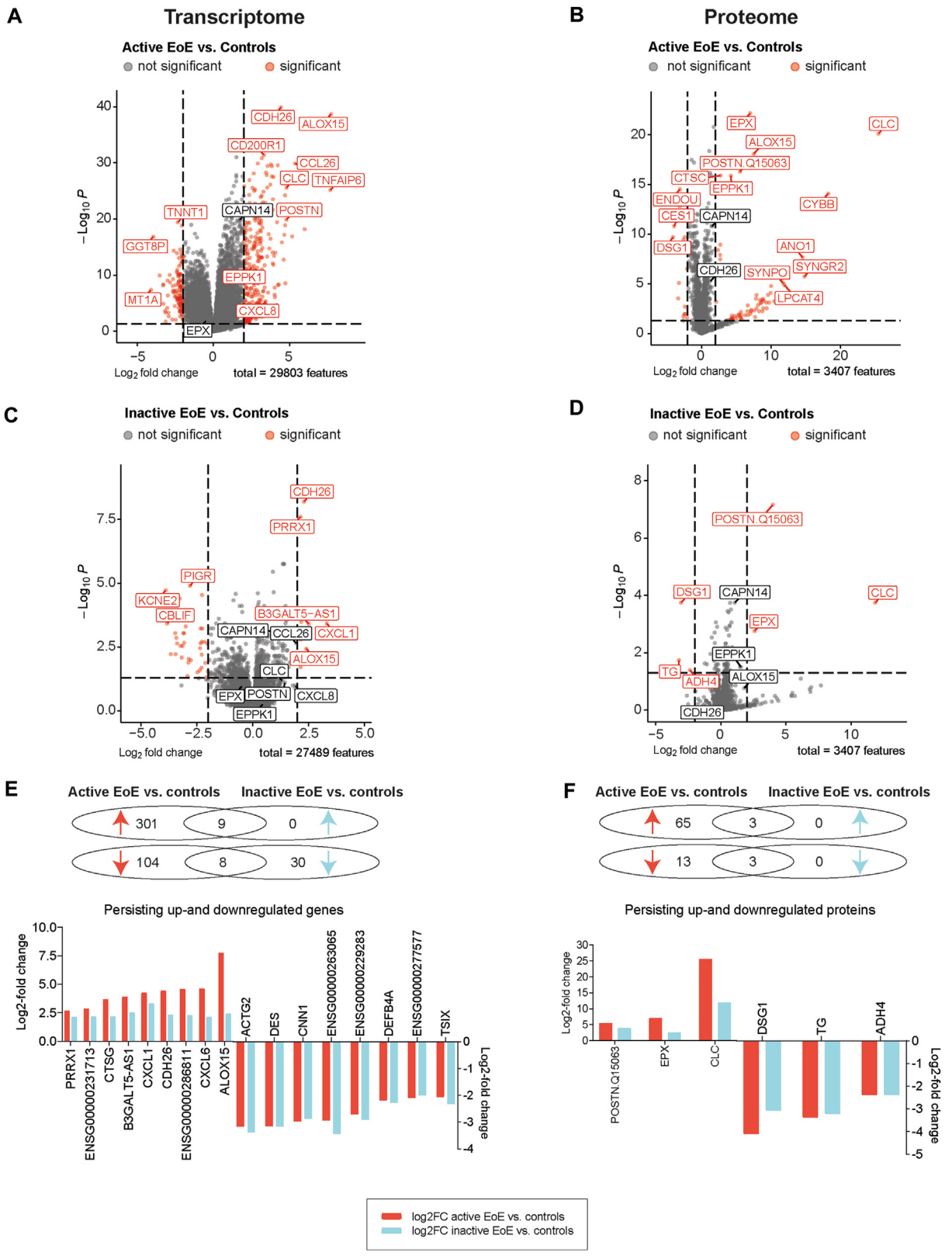

FIG 2. Differentially expressed transcripts and proteins in EoE and controls. Identification of up- and downregulated genes in EoE compared to controls based on all available analyzed biopsy samples in transcriptome and proteome as depicted in Fig 1, *A*. **A-D,** Volcano plot representation of differential expression analysis of (*A* and *C*) transcripts and (*B* and *D*) proteins. Log_2FC is represented on x-axis and $-log_{10}$ of Benjamin-Hochberg–corrected *P* values on y-axis. Significantly accumulated transcripts and proteins (*A* and *B*) of active EoE compared to controls or (*C* and *D*) inactive EoE compared to controls appear in *red.*

differentially expressed genes (DEGs) or accumulated proteins between the analyses based on all available samples and the filtered sample set with the same number of samples per patient were observed. Therefore, the remainder of results are shown for the entire biopsy dataset.

For a targeted proteomic analysis of the esophageal epithelium, a microdissection was performed (Fig E2, *B*). We identified 29,828 transcripts and 3,407 proteins with Ensemble IDs, of which 3,165 transcripts and proteins overlapped. In a principal component analysis (PCA), biopsy samples from active EoE clustered separately from samples of patients with FD and GERD (Fig 1, *B* and *C*; Fig E3). Most samples from patients with deep remission and inactive EoE did not cluster differently from patients with FD and GERD. A few biopsy samples clustered together with active EoE, suggesting residual inflammation despite histologic remission (Fig 1, *B* and *C*). Clustering according to PPI-, ED-, or topical corticosteroid–induced remission showed no treatment-dependent pattern (see Fig E4 in the Online Repository available at www.jacionline.org). Although GERD is associated with distal esophageal inflammation, patients with GERD did not separate from noninflammatory FD patients. However, most of our patients with GERD had only mild endoscopic (mostly Los Angeles classification A and B) or histologic disease but still experienced typical reflux symptoms. Because patients with GERD and FD showed a similar gene and protein expression profile (Fig 1, *B* and *C*), both groups were combined for subsequent analyses.

To illustrate the validity of an omics-based separation of disease activities, we highlighted the individual disease courses in 2 representative patients (Fig 1, *E* and *F*). The expression profiles of transcripts (Fig 1, *E*) and proteins (Fig 1, *F*) correlated well with histologic disease activity but correlated poorly with the Pediatric Eosinophilic Esophagitis Symptom Score's activity score (Fig 1, *D*).

Differentially expressed transcripts and proteins between active EoE, inactive EoE, and controls

To identify DEGs and differentially accumulated proteins (DAPs) that persist in mucosal tissue despite histologic remission, we compared results from active EoE versus controls and inactive EoE versus controls, and identified overlapping genes and proteins (Fig 2). In active EoE compared to controls, 310 genes and 68 proteins were significantly upregulated, and 112 genes and 16 proteins were downregulated (Fig 2, *A* and *B;* see Tables E5 and E6 in the Online Repository available at www.jacionline.org). Comparison of inactive EoE with controls resulted in 9 up- and 38 downregulated DEGs and 3 up- and 3 downregulated DAPs (Fig 2, *C* and *D;* see Tables E9 and E10 in the Online Repository).

The fold change (FC) of eosinophilic peroxidase (EPX) protein level was more pronounced in active EoE versus control (log_2FC, 7.02 [~129.8-fold increase], $P_{adj} < .001$) than in inactive EoE versus control versus (log_2FC, 2.59 [~6-fold increase]; $P_{adj} = .002$; Tables E6 and E10). To validate our findings of upregulated proteins like EPX, we performed a correlation analysis of protein expression with histologic disease activity, in which a significantly positive association (with $P < .001$ each) of EPX ($r = 0.8776$), ALOX15 ($r = 0.7843$), Charcot-Leyden-Crystals (CLC) ($r = 0.8090$), and POSTN ($r = 0.5074$) with increasing numbers of eosinophils was shown (see Fig E5 in the Online Repository available at www.jacionline.org).

POSTN, which encodes for the extracellular matrix protein periostin, is involved in esophageal remodeling.[22] At the protein level, we detected 2 upregulated isoforms of POSTN, but only one of these was present in all patients. (POSTN.B1ALD9 was expressed in 39 of 151 proteome samples, comprising 36 active EoE samples and 3 inactive EoE samples, and therefore has an imputation rate of 74%, whereas POSTN.Q15063 was expressed in all patients.) Therefore, subsequent analyses were based on POSTN.Q15063 (Table E6).

Analysis of persisting molecular dysregulation in inactive EoE resulted in 9 up- and 8 downregulated DEGs as well as 3 up- and 3 downregulated DAPs (Fig 2, *E* and *F;* see Tables E13 and E14 in the Online Repository available at www.jacionline.org). There was no match of overlapping genes and proteins from the comparison of active EoE versus control and inactive EoE versus control. Persistently upregulated transcripts were functionally related to inflammatory processes *(ALOX15, CXCL1, CXCL6, CTSG),* cell adhesion *(CDH26),* and fibrosis *(PRRX1).* Upregulated overlapping proteins have a functional role in eosinophilia (CLC, EPX) and cellular remodeling (POSTN).

To analyze which molecular changes occur depending on disease activity and which may be influenced by established therapies, we analyzed DEGs and proteins in active compared to inactive EoE (see Fig E6, Table E17, and Table E18 in the Online Repository available at www.jacionline.org). Among the top upregulated DEGs (n = 241) were *ALOX15* and *TNFAIP6,* while CLC and POSTN were among the most upregulated DAPs (n = 73).

Identified DEGs and DAPs of the comparisons of active and inactive EoE with controls and active with inactive EoE were compared to the EoE diagnostic panel (EDP), a 96-gene–containing PCR array from formalin-fixed, paraffin-embedded tissue RNA or fresh RNA that is described to identify EoE patients with ~96% sensitivity and ~98% specificity and that discriminates between EoE patients in remission and healthy controls as well as EoE and reflux esophagitis.[23] Among 96 EDP genes, 48 corresponding transcripts (active vs control, n = 46; inactive vs control, n = 5; active vs inactive, n = 26) and 12 corresponding proteins (active vs control, n = 11; inactive vs control, n = 3; active vs inactive, n = 10) were identified (see Tables E21 and E22 in the Online Repository available at www.jacionline.org).

In addition to the EDP, identified DEGs from the comparison of active EoE with controls were compared to a recently published

Differential expression criteria are adjusted $P \leq .05$ and log_2FC >2. **E** and **F,** Venn diagrams summarizing overlap of *(E)* DEGs and *(F)* accumulated proteins (DAPs) in active EoE versus controls as well as inactive disease versus controls. Persisting DEGs and DAPs shared between active EoE (*red, A* and *B*) and inactive EoE (*blue, C* and *D*) compared to controls are shown as bar plots. These genes and proteins were significantly differentially expressed in active EoE (≥15 eos/hpf) compared to controls and remained differentially expressed in inactive EoE (<15 eos/hpf) compared to controls.

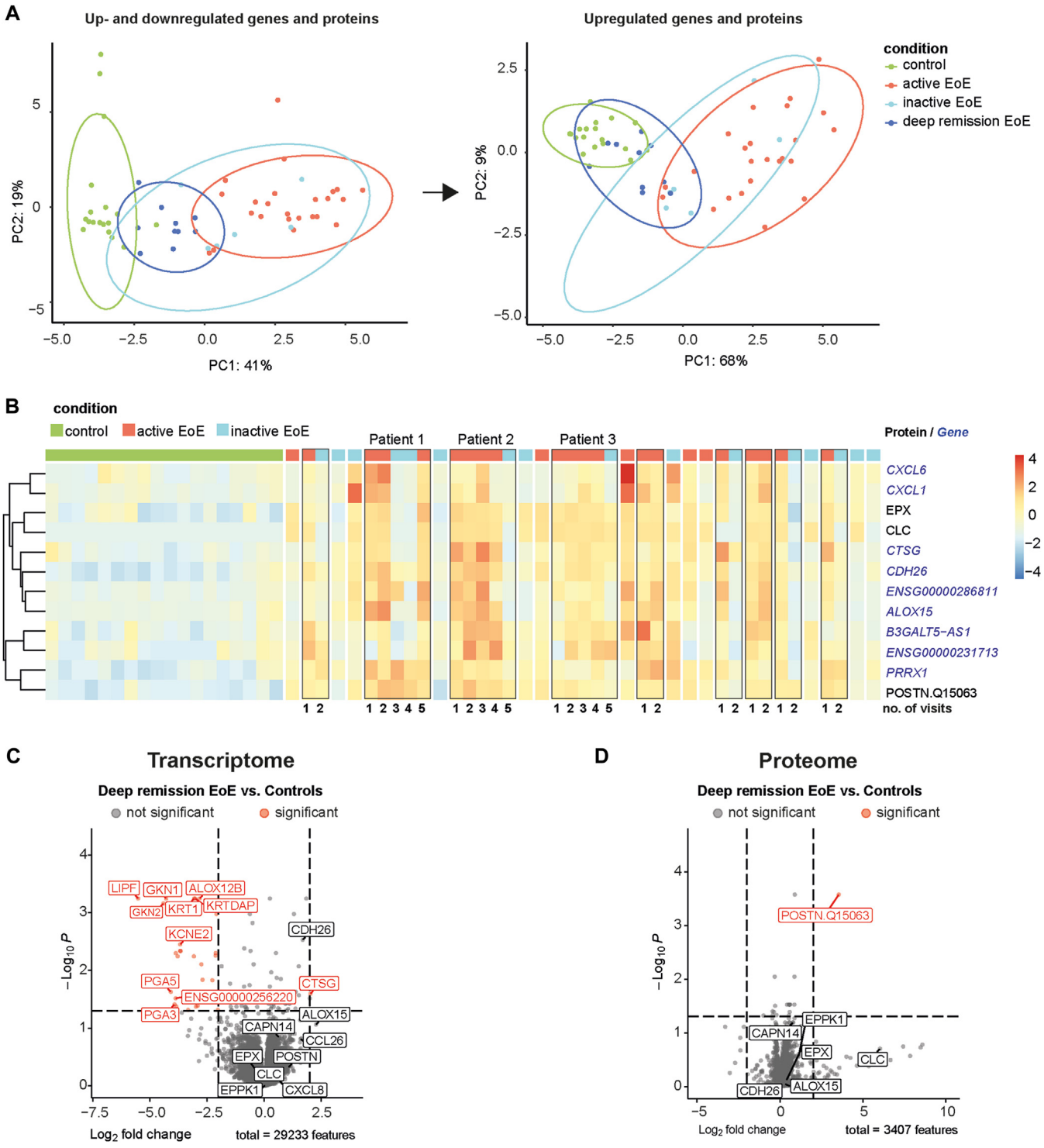

FIG 3. Histologic deep remission of EoE shows molecular differences to controls. **A,** PCA showing unsupervised clustering based on persistently up- and downregulated genes (n = 17) and proteins (n = 6), as well as only persistently upregulated genes (n = 9) and proteins (n = 3) that overlapped when comparing DEGs and proteins of active EoE versus control and inactive EoE versus control. Shown are esophageal biopsy samples from 58 endoscopies (EoE, 1 biopsy sample, N = 13; 2 biopsy samples, N = 6; 5 biopsy samples, N = 3; and 18 controls with 1 biopsy sample each). **B,** Heat map showing *z* score–scaled expression of DEGs and DAPs used for clustering in *(A)* (Benjamini-Hochberg–adjusted $P < .05$, FC >2). Dendrogram illustrates hierarchical clustering using Euclidean distance. Patients 1, 2, and 3 are highlighted because additional information for these patients is provided in Fig 1, *E* (patients 1 and 2), and Fig 4, *F* (patients 1 and 3). **C** and **D,** Volcano plot representation of differential expression analysis. Log_2FC is represented on x-axis and $-log_{10}$ of Benjamin-Hochberg–corrected *P* values on y-axis. Significantly accumulated *(C)* transcripts and *(D)* proteins in deep remission versus controls are depicted. Differential expression criteria are adjusted $P \leq .05$ and $log_2FC > 2$. *N* indicates individual patients, and *n* indicates proteins or genes.

transcriptome meta-analysis by Jacobse et al[24] based on 7 different studies (Table E10). Using the same FC of 2 as in our study, 468 DEGs are identified, of which 179 DEGs overlap with our 422 identified DEGs (see Table E23 in the Online Repository available at www.jacionline.org).

The 84 identified DAPs between active EoE and controls were compared to the proteome dataset by Molina-Jiménez et al[25] (Table E2). Using a FC greater than 1.5, the authors identified 363 DAPs in inflamed samples of patients with EoE compared to controls, of which 17 proteins overlapped. Assuming a FC of 2 in the data from Molina-Jiménez et al, only 7 proteins are still differentially accumulated, with an overlap of 4 proteins (POSTN, RNASE3, CRNN, PRG3) compared to the proteins in our study (Table E23).

Histologic remission of EoE shows molecular differences compared to controls

On the basis of the analysis of DEGs and accumulated proteins, we investigated, using biopsy samples obtained simultaneously for transcriptome and proteome analysis (40 biopsy samples from 23 patients with EoE; 18 controls), to what extent EoE differs from controls at the molecular level after resolution of inflammation (based on PEC). Persistently up- and downregulated DEGs and DAPs (Fig 2, *E* and *F*) separate inactive EoE and EoE in deep histologic remission (0 eos/hpf) from controls (Fig 3, *A*). Focusing only on the upregulated genes (n = 9) and proteins (n = 3) improves the separation between biopsy samples from patients with EoE in deep histologic remission and controls (Fig 3, *A*).

To understand the dynamics of these genes and proteins in the course of the disease depending on the disease activity, we investigated changes in the expression profiles in patients with EoE during several follow-up visits (Fig 3, *B;* see Fig E7 in the Online Repository available at www.jacionline.org). Genes and proteins associated with inflammation and eosinophilia (EPX, CLC, *CXCL1, CXCL6, CTSG, CDH26, ALOX15*) mostly show a clear gradient of expression between active and inactive EoE. In contrast, expression levels of genes and proteins functionally associated with epithelial-to-mesenchymal remodeling (*PRRX1,* POSTN) remained relatively constant with changing disease activity (Fig 3, *B*).

Even though most biopsy samples from patients with inactive EoE showed no different expression pattern from patients with deep histologic remission, there were individual exceptions. We investigated which genes and proteins are responsible for this differentiation by calculating DEGs and DAPs between inactive EoE and deep remission. The highest FC was observed for the upregulation of the gene *TNFSF18* (see Fig E8 and Table E24 in the Online Repository available at www.jacionline.org). *CLC, EPX,* and *ALOX15* were the upregulated proteins in inactive EoE versus deep remission (Fig E8; and see Table E25 in the Online Repository).

Next, we determined which DEGs, and DAPs differentiate between deep-remission EoE and controls (Fig 3, *C* and *D;* see Tables E28 and E29 in the Online Repository available at www.jacionline.org). In deep remission, 32 DEGs were present, of which *CTSG* remained the only upregulated transcript (Fig 3, *C*). Of note, *POSTN* remained the only upregulated DAP in deep-remission EoE, and there were no downregulated proteins (Fig 3, *D*). There was no overlap between DEGs and DAPs after complete resolution of inflammation.

Expression criteria are adjusted $P \leq .05$ and a $\log_2$FC value of >2.

Gene set enrichment analysis

To identify biological processes and signaling pathways relevant in EoE, we performed gene set enrichment analysis (see Figs E9 to E12 in the Online Repository available at www.jacionline.org). Persistently upregulated pathways of DEGs in inactive EoE versus controls were mainly associated with antigen recognition and immune response regulation, whereas suppressed pathways included filament cytoskeleton organization and epidermal cell differentiation (Fig E11). Functional pathway analysis of DAPs in active EoE versus controls and inactive versus controls showed suppression of pathways associated with suppressed epidermal cell differentiation (Figs E10 and E12).

Characterization and dynamics of dysregulated functional modules in EoE

To further evaluate the extent of disease clearance in EoE, we investigated the functional mechanisms of 2 distinct modules: tissue remodeling and epithelial barrier dysfunction.

Tissue remodeling. Genes and proteins involved in epithelial–stromal cross talk leading to epithelial-to-mesenchymal transition (EMT) were elevated in active EoE compared to controls, such as *POSTN, CLC,* and *CCL26* (Fig 2; Tables E5 and E6).[4,22] POSTN was the only persistently remaining protein in deep remission versus controls (Fig 3, *D*), and CLC was the top upregulated protein in inactive EoE versus controls (Fig 2, *D*). Prior data showed that luminal-captured *CLC* and *POSTN* levels correlate with fibrotic remodeling in EoE.[22] We verified this observation using our proteomic dataset (Fig 4, *A* and *B;* and see Fig E14 in the Online Repository available at www.jacionline.org). EMT was determined in a selection of biopsy samples (active, n = 18; inactive, n = 3; deep remission, n = 10; FD and GERD, each n = 5) examined in the proteome and transcriptome analyses using the ratio of vimentin and E-cadherin expression by immunofluorescence staining (Vim/Ecad ratio) (Fig 4, *C;* see Figs E13 and E14 in the Online Repository). Active EoE showed a statistically significant higher Vim/Ecad ratio compared to inactive EoE ($P < .001$) and FD ($P < .001$) (Fig 4, *D*). The proportion of vimentin-positive cells was significantly higher in active EoE versus FD ($P = .02$) (Fig 4, *E*). A positive correlation was present between the Vim/Ecad ratio and eosinophil count per high-power field ($r = 0.77$, $P < .001$; Fig E14, *C*). For 2 representative patients with EoE, a correlating trend of the Vim/Ecad ratio and the corresponding PEC in the respective esophageal biopsy is shown in Fig 4, *F.* To assess the predictive value of *POSTN* and *CLC* gene expression for the extent of EMT, a correlation analysis was performed (Fig 4, *G*). A statistically significant moderate correlation was found between *POSTN* (Spearman $r = 0.67$; $P < .001$) or CLC (Spearman $r = 0.64$; $P < .001$) and Vim/Ecad ratio. The correlation between *POSTN* or *CLC* gene and protein expression with the EoE endoscopic reference score (EREFS) showed a significant correlation, particularly at the transcript level (*POSTN:* Spearman

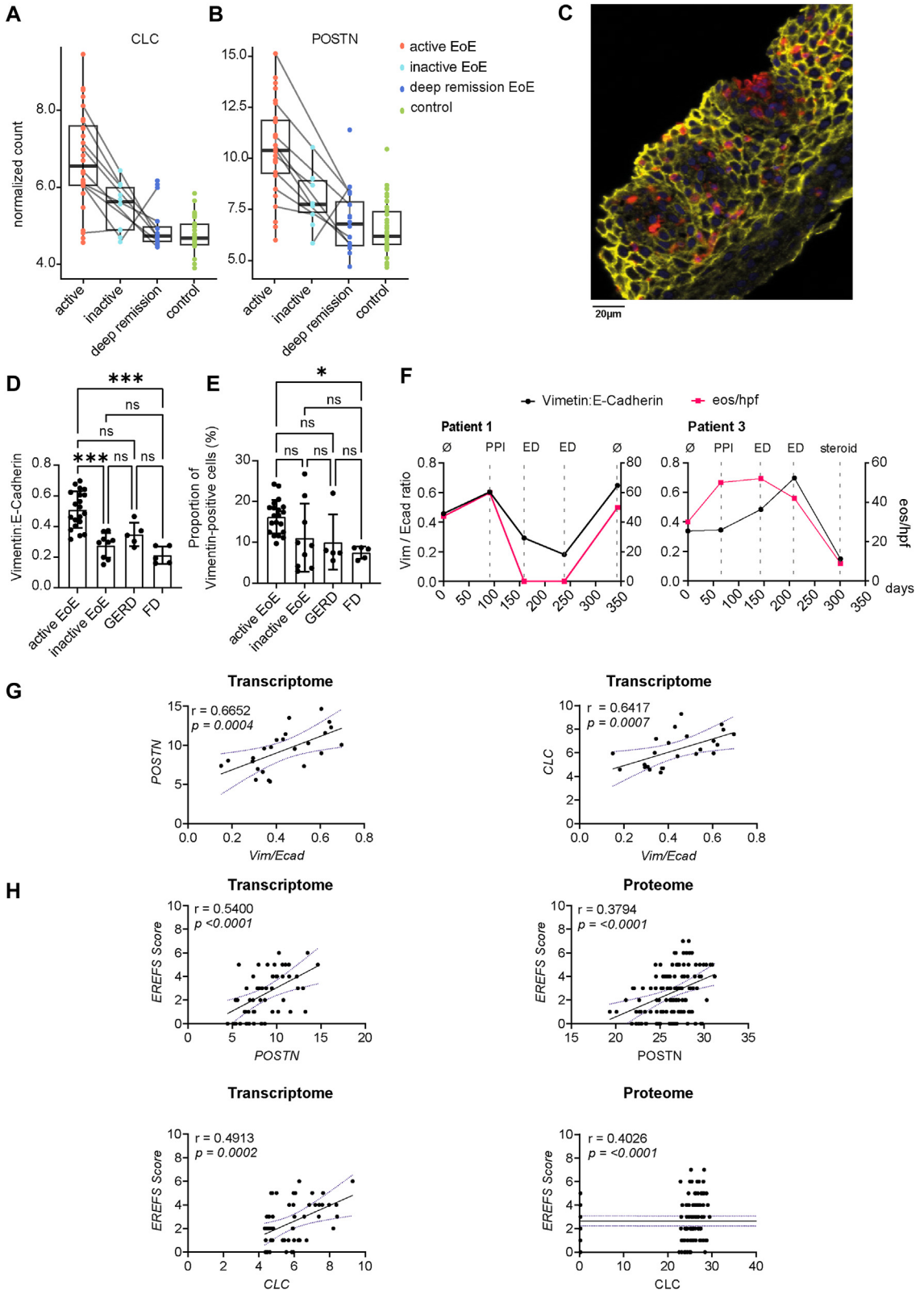

FIG 4. POSTN and CLC correlate with degree of epithelial to mesenchymal remodeling and EREFS. **A** and **B,** Differential gene expression of *(A) CLC* and *(B) POSTN*. Samples from same patient are connected. **C,** Opal staining with vimentin (*red,* Opal 650) and E-cadherin (*yellow,* Opal 570). **D** and **E,** Ratio of *(D)* Vim/Ecad expression and *(E)* proportion of vimentin-positive cells in esophageal biopsy samples from patients with EoE (N = 9), GERD (N = 5), and FD (N = 5). Samples from active EoE (4 and 3 samples, N = 2 each; 2 samples, N = 1; 1 sample, N = 4) and inactive EoE (2 samples, N = 1; 1 sample, N = 8) were included.

$r = 0.54$, $P < .001$; *CLC:* Spearman $r = 0.49$, $P < .001$) (Fig 4, *H*). In inactive EoE, there was no significant correlation between the gene expression of *POSTN* (Spearman $r = 0.30$, $P = .13$) or *CLC* (Spearman $r = 0.22$ $P = .26$) with the EREFS (see Fig E15 in the Online Repository).

Epithelial barrier dysfunction. Desmoglein (DSG)-1 was significantly downregulated in active EoE versus control (Fig 2, *B*) and inactive EoE versus control (Fig 2, *D,* and Fig 5, *A;* $\log_2$FC, −4.10 [~16.8-fold decrease] and −3.06 [~9.4-fold decrease], each $P_{adj} < .001$). To further investigate structural differences in desmosomal cadherins between EoE, GERD, and FD, we performed immunofluorescence staining. For the staining of E-cadherin, DSG1, DSG3, and desmoplakin from cryosections of esophageal biopsy samples, 1 sample per patient from at least 4 patients per group with active EoE, inactive EoE, GERD, and FD was included (Fig 5, *B-D,* and see Fig E16–E18 in the Online Repository available at www.jacionline.org).

Fluorescence intensity showed a significantly decreased DSG1 expression in active EoE compared to GERD ($P < .001$) (Fig 5, *B* and *C*). Because DSG3 expression did not show any significant differences between the disease groups (Fig E18), we used the fluorescence intensity ratio of DSG1/DSG3 to analyze the pooled expression levels between the disease groups. The fluorescence intensity DSG1/DSG3 ratio was significantly lower in active EoE versus GERD ($P = .006$), and there was a nonsignificant trend for inactive EoE versus GERD ($P = .06$; Fig 5, *D*).

DISCUSSION

In pediatric patients, we performed a proteomic analysis of microdissected esophageal epithelium and RNA bulk sequencing from entire esophageal biopsy samples. We demonstrated DEGs and accumulated proteins despite histologic remission. The identified panel of persistently upregulated, overlapping genes and proteins during inactive EoE separated EoE from noninflammatory controls despite deep histologic remission. Using persistently dysregulated proteins during histologic remission, we investigated cellular remodeling (CLC, POSTN) and epithelial barrier dysfunction (DSG1) as functionally relevant mechanisms to characterize the extent of inflammatory resolution. EMT normalized in inactive EoE despite persistent upregulation of POSTN during deep histologic remission, whereas desmosomal impairment persisted in inactive EoE as a result of downregulation of DSG1.

So far, mostly DNA- or RNA-based sequencing studies were performed in EoE.[23,26-30] To our knowledge, this is the first study assessing esophageal epithelial proteomic changes along with tissue transcriptomic alterations during the disease course in pediatric patients with EoE. Data on proteomic characterization of the esophageal epithelium are limited.[25,31,32] One study, performed in adult patients with EoE, assessed proteomic and transcriptomic changes as well.[25] In parallel to the study by Molina-Jiménez et al,[25] far more transcripts than proteins have been identified in our study, and protein abundance only partially correlated with transcript levels. These discrepancies could be partly explained by the extent of protein translation, protein half-life, and protein transport.[33,34] One restriction in the label-free proteomic analysis is that low-abundance proteins are not well detected, which limits the possibility of correlating downregulated proteins with downregulated genes. In addition, differences in the starting tissue for analysis existed: RNA was extracted from entire esophageal biopsy samples, while protein analysis was performed on the samples' microdissected esophageal epithelia. Nevertheless, histologic disease activity could be differentiated by both transcriptome and proteome profiling. Molina-Jiménez et al identified 363 DAPs in inflamed samples of patients with EoE compared to controls, while our study only identified 84 DAPs. The reasons for this difference in the amount of identified DAPs may be a different FC as a threshold for differential expression analysis (FC of 1.5 in the study by Molina-Jiménez et al vs FC of 2.0 in our study), differences in the patient cohorts (adult vs pediatric), and differences in the analyzed tissue (whole esophageal biopsy vs microdissection of the epithelium).

Many of the DEGs and accumulated proteins comparing active EoE with controls overlap with previous studies and the EDP.[23-26,35] A possible role in the pathomechanism of EoE has already been described for some of the overlapping genes, including *EPX,*[36] *CCL26,*[23] *CDH26,*[37] *POSTN,*[4,22] *SIGLEC6,*[38] *SLC9A3,*[39] *IL1RL1,*[40] *CLC,*[41] and *DSG1.*[42] Some genes, like *ALOX15*, *SLC26A4,* and *TNFAIP6,* have been described as differentially expressed in active EoE compared to controls,[24] but their functional relevance in the pathogenesis of EoE is still unclear.

We could not identify treatment-related differences in gene and protein expression, indicating that molecular changes driven by inflammation override treatment-specific changes. This assumption is supported by the fact that similar genes and proteins are differentially expressed in adult and pediatric populations despite different treatments.[25,27] However, combination therapies in our cohort, such as PPI with ED, limit the ability to identify treatment-related molecular changes.

In line with prior studies gene set enrichment analysis of biological processes, Gene Ontology (geneontology.org) terms showed activation of immune response-related pathways and downregulation of cornification and epithelial differentiation processes.[25,35]

Analogous to a recent study investigating persistent changes in EDP genes in histologic remission of EoE,[27] our data demonstrate persistent DEGs in patients with EoE compared to controls even with disease in deep histologic remission, stringently defined by 0 eos/hpf. Ruffner et al[27] found that in pediatric patients with EoE, *CDH26* was the top DEG. We identified *CDH26* as a persistently upregulated transcript in inactive EoE as well, but it was no

Kruskal-Wallis test with Dunn multiple comparisons test ($P < .05$) was used. **F,** Vim/Ecad ratio and eos/hpf throughout disease course are illustrated for 2 patients. **G** and **H,** Correlation analyses were performed with nonparametric Spearman rank correlation; $P < .05$ (2-tailed). Correlation coefficient *(r)* and simple linear regression with 99% confidence interval (CI) bands are shown. Each point represents 1 patient sample. POSTN and CLC expression were calculated relative to controls. **G,** Correlation between Vim/Ecad ratio and POSTN/CLC expression in 24 paired measurements from 9 EoE patients (5 biopsy samples, N = 2; 4 biopsy samples, N = 1; 2 biopsy samples, N = 4; 1 biopsy sample, N = 2). **H,** Correlation between POSTN/CLC expression and EREFS in 54 paired measurements from 29 EoE patients. *N* indicates individual patients.

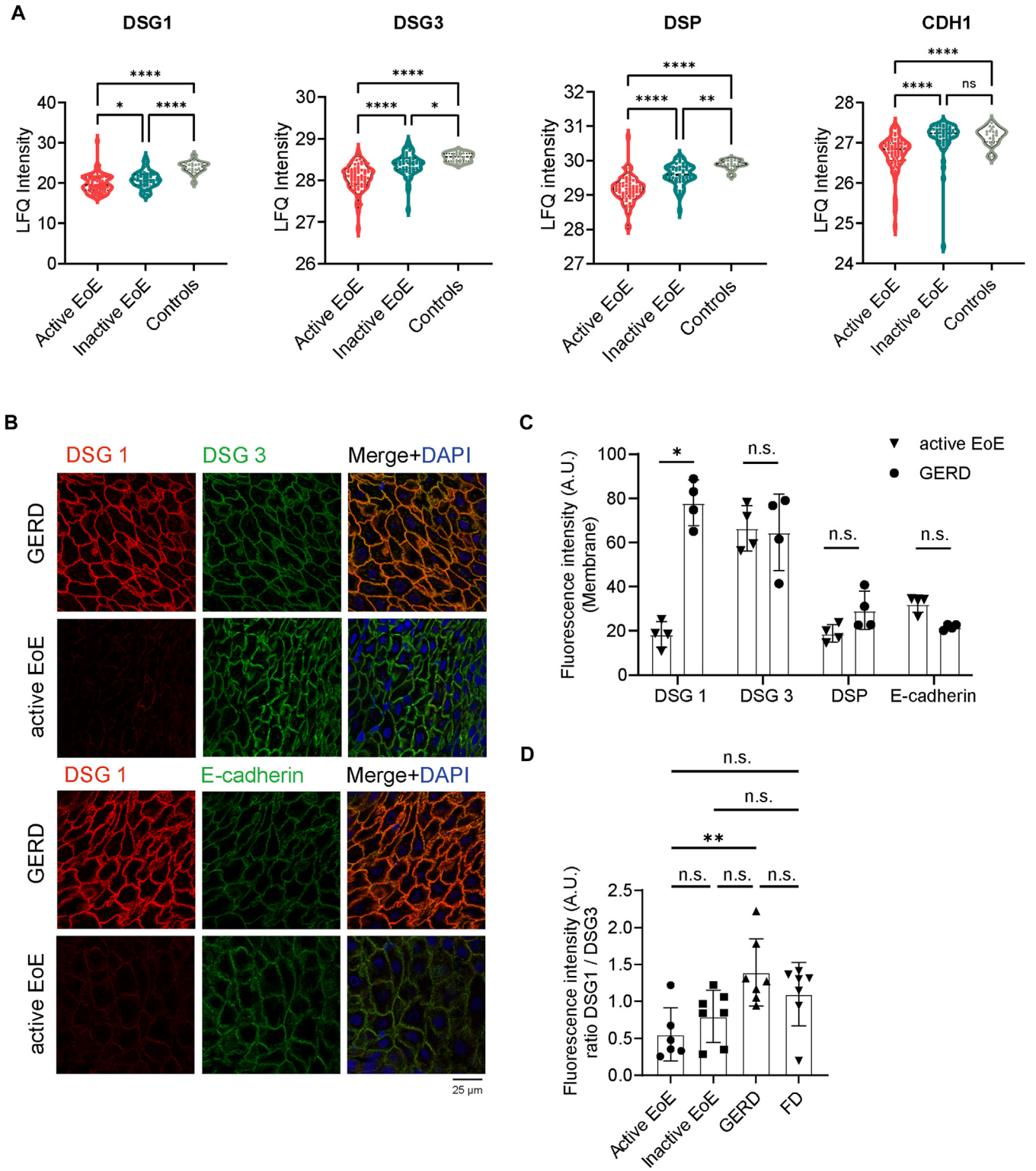

FIG 5. Desmosomal impairment in patients with EoE compared to GERD and FD. **A,** Box plots showing protein expression according to proteomics from esophageal epithelium in all analyzed biopsy samples (Fig 1, *A*) of selected structurally important proteins of epithelial barrier. **B,** Representative images of confocal microscopy showing immunofluorescence staining of E-cadherin, DSG1, DSG3, and desmoplakin (DSP) in a cryosection of patient with active EoE and GERD, respectively. **C,** Comparison of fluorescence intensity (AU) of membrane for E-cadherin, DSG1, DSG3, and DSP in 4 biopsy samples each of 4 patients with active EoE and GERD. **D,** AU ratio of DSG1/DSG3 is shown in 6 samples of patients with active EoE and 7 samples each of patients with inactive EoE, GERD, and FD, with 1 biopsy sample per patient. Statistical testing was performed by 2-way *(A-C)* or 1-way *(D)* ANOVA with Bonferroni multiple comparisons test with significance level of $P < .05$.

longer differentially expressed when we used the more stringent cutoff of 0 eos/hpf. The mesenchymal transcription factor *PRRX1* was one of the persisting upregulated transcripts, which separated inactive EoE as well as deep-remission biopsy samples from control tissue. In deep-remission EoE, *PRRX1* remained upregulated compared to controls (log_2FC, 1.84, $P_{adj} < .001$). *PRRX1* was identified as differentially expressed in active EoE compared to controls in prior studies.[24,25] *PRRX1* is expressed in all known fibroblast subtypes and modulates fibrosis by orchestrating the functional drift of fibroblasts into myofibroblast phenotype via TGF-β signaling.[43] The relevance and function of *PRRX1* in EoE has not yet been investigated. In the mouse ventral dermis PRRX1-positive fibroblasts were shown to contribute to scar formation in acute and chronic fibrosis.[44] Cardiac fibrosis is promoted by PRRX1 via the Twist1–PRRX1–tenascin–C loop.[45] PRRX1 was found to be a key mesenchymal transcription factor in idiopathic pulmonary fibrosis, and targeted inhibition of PRRX1 attenuates fibrotic remodeling *in vivo* in humans.[46] Whether PRRX1 is also an important transcription factor for fibrosis in EoE remains to be functionally demonstrated.

CTSG was significantly upregulated in deep remission compared to control (log_2FC, 2.02, $P_{adj} = .02$). CTSG encodes for protease cathepsin G, and single-cell RNA sequencing of mast cells in EoE showed a $CMA1^{high}CTSG^{high}$ mast cell population, which was detected in disease remission of EoE, where it was maintained in an activated state.[47]

POSTN remained the only upregulated DAP in deep-remission EoE, and CLC was the top upregulated persistent protein in inactive EoE. In a prior study, esophageal luminal POSTN and CLC protein levels correlated with endoscopic and histologic appearance (eosinophil density and basal zone hyperplasia, markers of EMT).[22] In line with this study, transcripts of *POSTN* and *CLC* showed a moderate correlation with the Vim/Ecad ratio, representing the degree of EMT. As previously shown, EMT decreases in treated children with reduced esophageal eosinophil load.[48] *POSTN* and *CLC* correlated with the EREFS at both the gene and protein level, but there was no correlation when focusing on inactive EoE. Previous data in an adult cohort showed a correlation between *POSTN* expression and EREFS ($r = 0.37$, $P = .055$), even in deep remission (0 eos/hpf).[27] A possible explanation for the positive correlation in adults could be the longer disease activity leading to higher EREFS despite the absence of an eosinophil infiltrate. *POSTN* is not only highly expressed in the extracellular matrix but can also be detected in the blood as a result of cellular secretion under IL-13 stimulation,[49] making it a possible serum biomarker. However, a previous study investigating the diagnostic value as a serum biomarker did not show a difference in serum *POSTN* levels between patients with EoE (n = 61) and controls (n = 87).[50] Currently, preclinical studies are evaluating the therapeutic potential of anti-periostin–directed therapies.[51]

A pathogenic role for desmosomal dysfunction is described in EoE and *DSG1* was shown to be dysregulated by IL-13, resulting in a reduced esophageal epithelial integrity.[42] Downregulation of the *DSG1* protein level was maintained in inactive EoE and was validated by immunofluorescence staining. Prior data showed that in adult patients with no esophageal eosinophilia, the transcript DSG1 was persistently downregulated,[27] whereas in our dataset, DSG1 was not differentially accumulated in deep remission. A better understanding of the regulatory mechanisms for desmosomal impairment would be desirable for potential therapeutic modulation. The current clinical challenge is that despite histologic remission, patients may experience persistent symptoms as a result of the lack of correlation between endoscopic findings and symptoms. It may be possible to obtain improved symptom resolution by targeting the persistent molecular changes.

A particular strength of this study is the long observation period, with many follow-ups, which allows the dynamics of molecular changes to be studied intra- and interindividually. Microdissection offered the possibility to focus on epithelial changes in protein expression. A large pediatric cohort with "inflammatory" (GERD) and "noninflammatory" (normal pathology report; termed FD) controls served as comparators of comparable age. By performing transcriptomic and proteomic analyses in matching esophageal biopsy samples derived from the same endoscopy procedure, it is possible to compare differences in transcription and translation. One important limitation of our study is that the Eosinophilic Esophagitis Histology Scoring System score was only available in a subset of biopsy samples, which is why no other histologic characteristics besides eos/hpf could be evaluated.

By studying a combination of differentially upregulated and persistent genes and proteins, better molecular discrimination between EoE and controls was possible, even in those with disease in deep remission, than by studying the transcriptome or proteome alone. This shows that DAPs substantially extend the mRNA-based molecular signature of EoE, improving our understanding of its pathophysiology and allowing treatment toward molecular remission in the future. Because the protein signature is closer to clinical characteristics, there is the potential to identify new biomarkers on the basis of this work, which may be helpful in diagnostics, therapy stratification, or monitoring.

DISCLOSURE STATEMENT

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All data supporting our findings are available here and in the Online Repository. The mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE partner repository with dataset identifier PXD052250.48 (www.ebi.ac.uk/pride/archive/projects/PXD052250). Raw RNA sequencing data are available on reasonable request from the corresponding author. The data are protected and not publicly available because the research participants are children.

Disclosure of potential conflict of interest: T. Schwerd reports receipt of received lecture honoraria from Nutricia and MSD, personal honorarium as member of an advisory board from AstraZeneca, and travel support from AbbVie and Ferring outside the submitted work. H. Hoelz reports receipt of lecture honoraria from Nutricia outside the submitted work. S. Koletzko reports receipt of fees as a member of advisory boards or as speaker from AbbVie, AstraZeneca, Danone, Janssen, Mead Johnson, Nestlé Nutrition, Pfizer, Sanofi, and Takeda outside the submitted work. M. List reports consulting for mbiomics GmbH. The rest of the authors declare that they have no relevant conflicts of interest.

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Key messages

- **Proteomic and transcriptomic profiling of pediatric patients with EoE extends our understanding of molecular changes during allergic inflammation and histologic remission in EoE.**
- **Persistently dysregulated genes and proteins during histologic remission in EoE underscore the relevance of functional modules such as desmosomal impairment and EMT, offering new potential biomarkers for diagnosis or treatment stratification.**
- **By identifying molecular changes that distinguish EoE in deep histologic remission from noninflammatory controls, it is possible to work toward a future goal of molecular remission.**

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