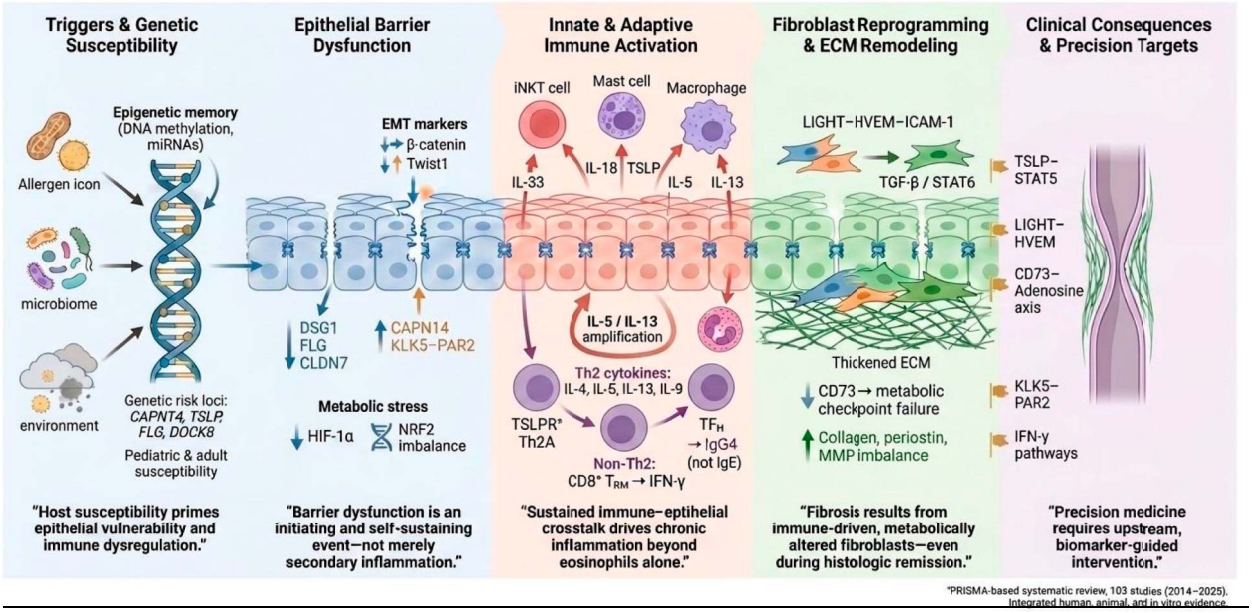
Pathogenesis of Eosinophilic Esophagitis: A PRISMA-Based Systematic Review of Genetic, Immunological, and Molecular Drivers

Joanna Lachowicz, PhD[1,2,*], Amalia Di Petrillo, PhD[1,*], Sonia Floris, PhD[1], Agnese Favale, PhD[1,3], Nicola Lutzu, MS[1], Sara Onali, PhD[1,3] and Massimo Claudio Fantini, MD, PhD[1,3]

INTRODUCTION: **Eosinophilic esophagitis (EoE) is a chronic, antigen-driven inflammatory disease characterized by esophageal dysfunction and tissue remodeling. Despite increasing prevalence, its pathogenesis remains incompletely understood. This review aims to systematically synthesize current evidence on the immunological, genetic, epithelial, and extracellular matrix mechanisms driving EoE. The goal is to identify upstream molecular targets and biomarkers to support precision medicine approaches.**

METHODS: **A PRISMA-based systematic literature search was conducted in PubMed, Web of Science, and Scopus for studies published between January 2014 and June 2025. Eligibility criteria were defined using the PECOS model, focusing on studies reporting quantitative biomolecular data. Risk of bias was assessed using validated tools for observational, *in vivo*, and *in vitro* studies. A total of 103 original research articles were included and categorized into 4 mechanistic domains.**

An Integrated Model of Eosinophilic Esophagitis Pathogenesis for a Precision Medicine Approach



Lachowicz et al. *Clin Trans Gastroenterol.* 2026. doi:10.14309/ctg.0000000000001052 Clinical and Translational GASTROENTEROLOGY

[1]Department of Medical Science and Public Health, University of Cagliari, Italy; [2]Department of Environmental Health, Occupational Medicine and Epidemiology, Wroclaw Medical University, Wroclaw, Poland; [3]Azienda Ospedaliero-Universitaria di Cagliari, Gastroenterology Unit, Monserrato, Italy. **Correspondence:** Massimo Claudio Fantini, MD, PhD. E-mail: massimoc.fantini@unica.it.
*Joanna Lachowicz and Amalia Di Petrillo contributed equally to this work.
Received December 11, 2025; accepted May 20, 2026; published online June 3, 2026
© 2026 The Author(s). Published by Wolters Kluwer Health, LLC. on behalf of The American College of Gastroenterology

REVIEW ARTICLE

RESULTS: **The review identified epithelial barrier dysfunction, type 2 inflammation, and fibroblast activation as central features of EoE. Key cytokines (IL-4, IL-5, IL-13, TSLP) and transcriptional regulators (e.g., GATA-binding protein 3, Krüppel-like factor 5, resistin-like molecule alpha) were implicated in disease progression. Genetic and epigenetic analyses revealed susceptibility loci and persistent molecular alterations. Novel therapeutic targets and biomarkers were proposed, including TSLPR$^+$ pathogenic memory T-helper type 2A (Th2A) cells, lymphotoxin-like exhibits inducible expression competes with herpes simplex virus glycoprotein D for herpesvirus entry mediator a receptor expressed by T lymphocytes–herpesvirus entry mediator (LIGHT–HVEM) axis, and cluster of differentiation 73/ecto-5′-nucleotidase metabolic checkpoints.**

DISCUSSION: **EoE is a multifactorial disease involving complex immune–epithelial–stromal interactions. This review highlights emerging molecular pathways and supports a shift toward biomarker-guided, stratified treatment strategies. Integration of multiomics data and age-stratified analyses enhances understanding of disease heterogeneity. Future research should focus on longitudinal studies, pediatric cohorts, and validation of noninvasive biomarkers.**

KEYWORDS: eosinophilic esophagitis; pathogenesis; precision medicine

ABBREVIATIONS: AGREE, A Working Group on Proton Pump Inhibitor-Responsive Esophageal Eosinophilia; ALOX15, arachidonate 15-lipoxygenase; APOBEC3A, apolipoprotein B mRNA editing enzyme catalytic subunit 3A; ATP12A, ATPase H+/K+ transporting non-gastric alpha-2 subunit; BMP, bone morphogenetic protein; C3M, matrix metalloproteinase-generated fragment of type III collagen; C4M, matrix metalloproteinase-generated fragment of type IV collagen; CAPN14, calpain 14; CCL24, C-C motif chemokine ligand 24; CCL26, C-C motif chemokine ligand 26; CD73, cluster of differentiation 73/ecto-5′-nucleotidase; CDH1, cadherin 1; CIITA, class II major histocompatibility complex transactivator; CLDN7, claudin 7; CLEC16A, C-type lectin domain family 16 member A; COL1A2, collagen type I alpha-2 chain; CPA3, carboxypeptidase A3; CRISP3, cysteine-rich secretory protein 3; CRTH2, chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells; CSF-1, colony-stimulating factor 1; CTGF, connective tissue growth factor; CXCL8, C-X-C motif chemokine ligand 8; CXCR2, C-X-C motif chemokine receptor 2; CXCR4, C-X-C motif chemokine receptor 4; DEG, differentially expressed gene; DEXI, dexamethasone-induced protein; DHTKD1, dehydrogenase E1 and transketolase domain-containing protein 1; DNA, deoxyribonucleic acid; DOCK8, dedicator of cytokinesis 8; DSG1, desmoglein 1; ECM, extracellular matrix; EGR1, early growth response 1; EMT, epithelial–mesenchymal transition; EndoFLIP, endoluminal functional lumen imaging probe; EoE, eosinophilic esophagitis; EPC2, esophageal epithelial cell line 2; EPPK1, epiplakin 1; EPX, eosinophil peroxidase; F2, coagulation factor II; FcεRII, low-affinity immunoglobulin E receptor II; FLG, filaggrin; FOXP3, forkhead box P3; GATA-3, GATA-binding protein 3; GERD, gastroesophageal reflux disease; GM-CSF, granulocyte–macrophage colony-stimulating factor; GWAS, genome-wide association study; GzmB, granzyme B; HIF-1α, hypoxia-inducible factor 1-alpha; HVEM, herpesvirus entry mediator; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon gamma; IgE, immunoglobulin E; IgG4, immunoglobulin G4; IL, interleukin; ILC2, group 2 innate lymphoid cell; IL-13Rα1, interleukin-13 receptor alpha 1; iNKT, invariant natural killer T cell; JAK, Janus kinase; JCHAIN, joining chain of multimeric immunoglobulins; KCNJ2, potassium inwardly rectifying channel subfamily J member 2; KLF4, Krüppel-like factor 4; KLF5, Krüppel-like factor 5; KLK5, kallikrein-related peptidase 5; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LIGHT, lymphotoxin-like, exhibits inducible expression, competes with herpes simplex virus glycoprotein D for herpesvirus entry mediator, a receptor expressed by T lymphocytes; LOX, lysyl oxidase; LRRC31, leucine-rich repeat-containing protein 31; LRRC32, leucine-rich repeat-containing protein 32; MBP, major basic protein; MC, mast cell; MIF, macrophage migration inhibitory factor; miRNA, microRNA; MMP, matrix metalloproteinase; MYD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor kappa B; NLRP3, NLR family pyrin domain-containing protein 3; NOS, Newcastle–Ottawa Scale; NRF2, nuclear factor erythroid 2-related factor 2; OGDHL, oxoglutarate dehydrogenase-like protein; OSM, oncostatin M; PAI-1, plasminogen activator inhibitor 1; PAR2, protease-activated receptor 2; PCR, polymerase chain reaction; PECOS, Population, Exposure, Comparator, Outcome, and Study design; PIR-B, paired immunoglobulin-like receptor B; POSTN, periostin; PPI, proton pump inhibitor; PPI-REE, proton pump inhibitor-responsive esophageal eosinophilia; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PRO-C3, N-terminal propeptide of type III collagen; PRO-C6, C-terminal propeptide of type VI collagen; RELM-α, resistin-like molecule alpha; RHOF, Ras homolog family member F; RNA-seq, RNA sequencing; SCF, stem cell factor; scRNA-seq, single-cell RNA sequencing; SFRP1, secreted frizzled-related protein 1; Siglec-8, sialic acid-binding immunoglobulin-like lectin 8; SLC9A3, solute carrier family 9 member A3; SMAD, suppressor of mothers against decapentaplegic; SOX2, SRY-box transcription factor 2; SPINK7, serine peptidase inhibitor Kazal type 7; STAT, signal transducer and activator of transcription; ST2, suppression of tumorigenicity 2/interleukin-33 receptor; SYNPO, synaptopodin; TFH, T follicular helper cell; TGF-β, transforming growth factor beta; Th1, T-helper type 1; Th2, T-helper type 2; Th2A, pathogenic memory T-helper type 2A cell; TIMP-1, tissue inhibitor of metalloproteinases 1; TLR3, Toll-like receptor 3; TNFSF14, tumor necrosis factor superfamily member 14; Treg, regulatory T cell; TSP-1, thrombospondin 1; TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor; TYK2, tyrosine kinase 2; VIP, vasoactive intestinal peptide; ZO-1, zonula occludens 1

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/B535, http://links.lww.com/CTG/B536

Clinical and Translational Gastroenterology 2026;17:e01052. https://doi.org/10.14309/ctg.0000000000001052

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic, antigen-driven, Th2-inflammatory disease, presenting with symptoms of esophageal dysfunction and dismotility such as dysphagia and food impaction (1). Over the last decades, the epidemiology of EoE has shown an increasing trend, with current prevalence estimated of up to 1 of 1,000 individuals in Western countries, with higher incidence peaks in male patients aged 35–39 years (2). EoE is considered an atopy-associated disorder, accordingly, more than half of patients with EoE exhibit at least one TH2-mediated allergic comorbidity (1).

Esophageal eosinophilia is the hallmark of EoE; indeed, current diagnostic criteria include an intraepithelial eosinophil peak count greater than or equal to 15 per high power fields in association with symptoms suggestive of EoE (2,3).

Management of EoE includes elimination diets, orodispersible budesonide, and the off-label use of proton pump inhibitors (PPIs), as well as dupilumab—the only biologic therapy currently approved for EoE—alongside endoscopic dilation when fibrostenotic complications are present (2).

Recent evidence has contributed to significant advances in the understanding of the pathophysiology of EoE. Nevertheless, significant gaps still remain (4). Genetic susceptibility plays a pivotal role in the pathogenesis of EoE and impaired epithelial barrier function, which results in enhanced permeability to antigens that trigger the inflammatory response (5,6). The downstream self-maintaining inflammatory cascade is driven by Th2 response characterized by cytokine milieu that promote eosinophilic infiltration and tissue remodeling, which can ultimately lead to disease progression toward fibrostenotic phenotype (7,8). Despite the central role of eosinophils, accumulating evidence suggest that other immune cells, both innate and adaptive, and eosinophils-independent molecular pathways also contribute to the pathogenesis of the disease (4). Consistently, multiple biologic agents targeting eosinophils—anti IL-5 and Sialic acid-binding immunoglobulin-like lectin 8—have proven efficacy in reducing tissue eosinophilia. However, they failed to achieve significant symptomatic improvement (9–11). This finding suggests that suppression of eosinophilic inflammation alone may be insufficient to achieve adequate disease control. Accordingly, dupilumab, the only biologic agent approved for the treatment of EoE, targets IL-4Rα, thus blocking both eosinophils-dependent and -independent IL-4- and IL-13–related pathways (12). Another critical issue is the absence of validated biomarker for disease monitoring, along with the lack of predictive factors for treatment response or failure. Therefore, in-depth exploration of EoE immunopathogenetic mechanism is crucial because it offers the possibility of identifying novel therapeutic targets located upstream in the pathogenetic mechanism and reliable biomarkers and predictive indicators, ultimately leading toward precision medicine.

METHODS

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (13). The protocol was developed based on standards for mechanistic reviews addressing disease pathogenesis.

Eligibility criteria

Given the focus on biological mechanisms, the PECOS model (Population, Exposure, Comparator Outcome, Study design) was used for eligibility criteria (see Supplementary Table S1, Supplementary Digital Content, http://links.lww.com/CTG/B535). The article search was based on the keywords: "eosinophilic esophagitis," "eosinophilic esophagitis," "pathogenesis," "cytokines," "immune response," "IL-5," "IL-13," "TSLP," "tight junction," "epithelial barrier," "fibroblasts," "extracellular matrix," "barrier dysfunction," "extracellular matrix." Articles without molecular outcome measures were not included in the research. The detail description of records search can be found in Supplementary Material (see Supplementary Text S1, Supplementary Digital Content, http://links.lww.com/CTG/B535).

Only observational and experimental research articles were included in this study, while reviews (including systematic reviews), book chapters, meta-analyses, comments, and letters were excluded. Studies from conference proceedings or gray literature were also excluded.

The selected studies provide original data collected through direct observation or experimentation, which is essential for drawing independent conclusions and performing quantitative synthesis.

The focus of this study was on observational and experimental research with direct participation of humans (e.g., cohort studies, case-control studies). Moreover, animal models of EoE, as well as *in vitro* and *in vivo* studies using human and/or animal tissues and/or cells were included in this investigation.

Articles that met the following criteria were analyzed in our review: (i) epidemiological studies investigating at least one of the following EoE indices: membrane or cytoplasm proteins, nuclei acids, immunoglobulins, cytokines, eosinophils, basophils, interleukins, mast cells (MC); (ii) studies evaluating animal models of EoE; (iii) studies analyzing cell cultures deriving from humans with EoE and/or animal models of EoE; (iv) computational studies analyzing genetic data of EoE patients and healthy controls; (v) studies reporting quantitative measures of EoE representative biomolecules; (vi) studies written in English.

All selected studies focused on immune, epithelial, genetic, or extracellular matrix (ECM) pathways in EoE. Among observational studies with the participation of humans, only studies quantifying the biomolecules difference between patients with EoE and subjects without EoE were considered. This study focused on the immune, epithelial, genetic, or ECM pathways leading to EoE development and progression. Thus, only articles presenting high-quality data on biomolecules variability as an effect of EoE were selected for this systematic review.

More elucidations on exclusion criteria are presented in the supplementary material (see Supplementary Text S2, Supplementary Digital Content, http://links.lww.com/CTG/B535).

Information sources

A comprehensive search was conducted in PubMed, Web of Science, and Scopus for articles published in English from January 1, 2014, to June 1, 2025. All study designs were eligible, including human, animal, and *in vitro* experimental studies.

The literature search was restricted to studies published from January 2014 onward. This starting point was chosen because the years 2014–2015 marked the emergence of several landmark mechanistic discoveries—such as the identification of calpain 14 (CAPN14), the characterization of IL-13–driven epithelial

remodeling, and the first genome-wide risk loci for EoE—that fundamentally reshaped the conceptual and molecular framework of the disease. These studies introduced modern approaches to understanding epithelial barrier dysfunction, Th2-mediated inflammation, and genetic susceptibility, and collectively established the basis for contemporary mechanistic research in EoE. Earlier publications generally lacked multiomics methodologies and did not consistently report biomolecular endpoints relevant to our review. Selecting 2014 as the lower bound therefore ensured that the included literature reflects current experimental standards and mechanistic perspectives.

Search strategy

The search strategy was based on the keywords: "eosinophilic esophagitis," "eosinophilic esophagitis," "pathogenesis," "cytokines," "immune response," "IL-5," "IL-13,' "TSLP," "tight junction," "epithelial barrier," "fibroblasts," "extracellular matrix," "barrier dysfunction," "extracellular matrix." The detail description of records' search is presented in Supplementary Text S1 (see Supplementary Digital Content, http://links.lww.com/CTG/B535).

All selected studies were published in English (language filter) between January 1, 2014, and June 1, 2024 (date range filter).

Study selection

No automation tolls were used in the selection process. Four reviewers (J.L., A.D., S.F., and M.C.F) separately and independently screened the records gathered from the databases, assessing each record's eligibility based on titles, abstracts, and full texts when necessary.

Studies that satisfied the following criteria were included in this review: (i) epidemiological studies investigating at least one of the following EoE indices: membrane or cytoplasm proteins, nuclei acids, immunoglobulins, cytokines, eosinophils, basophils, interleukins, MC; (ii) studies evaluating animal models of EoE; (iii) studies analyzing cell cultures deriving from humans with EoE and/or animal models of EoE; (iv) computational studies analyzing genetic data of EoE patients and healthy controls; (v) studies reporting quantitative measures of EoE representative biomolecules; (vi) studies written in English.

Four reviewers independently screened titles, abstracts, and full texts. Conflicts were resolved via discussion of all 4 reviewers. The PRISMA flow diagram summarizes the selection process.

Data items and data collection process

A standardized extraction sheet was used to collect the following data: (i) author(s), (ii) year, (iii) country, (iv) study design, (v) experimental models, (vi) population size, (vii) molecular targets investigated, (viii) main mechanistic results.

Quality and risk-of-bias assessment

The quality of included studies was independently assessed by 4 reviewers using 3 different tools based on study type: the Newcastle–Ottawa Scale (NOS) for human observational studies, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool for animal experiments, and

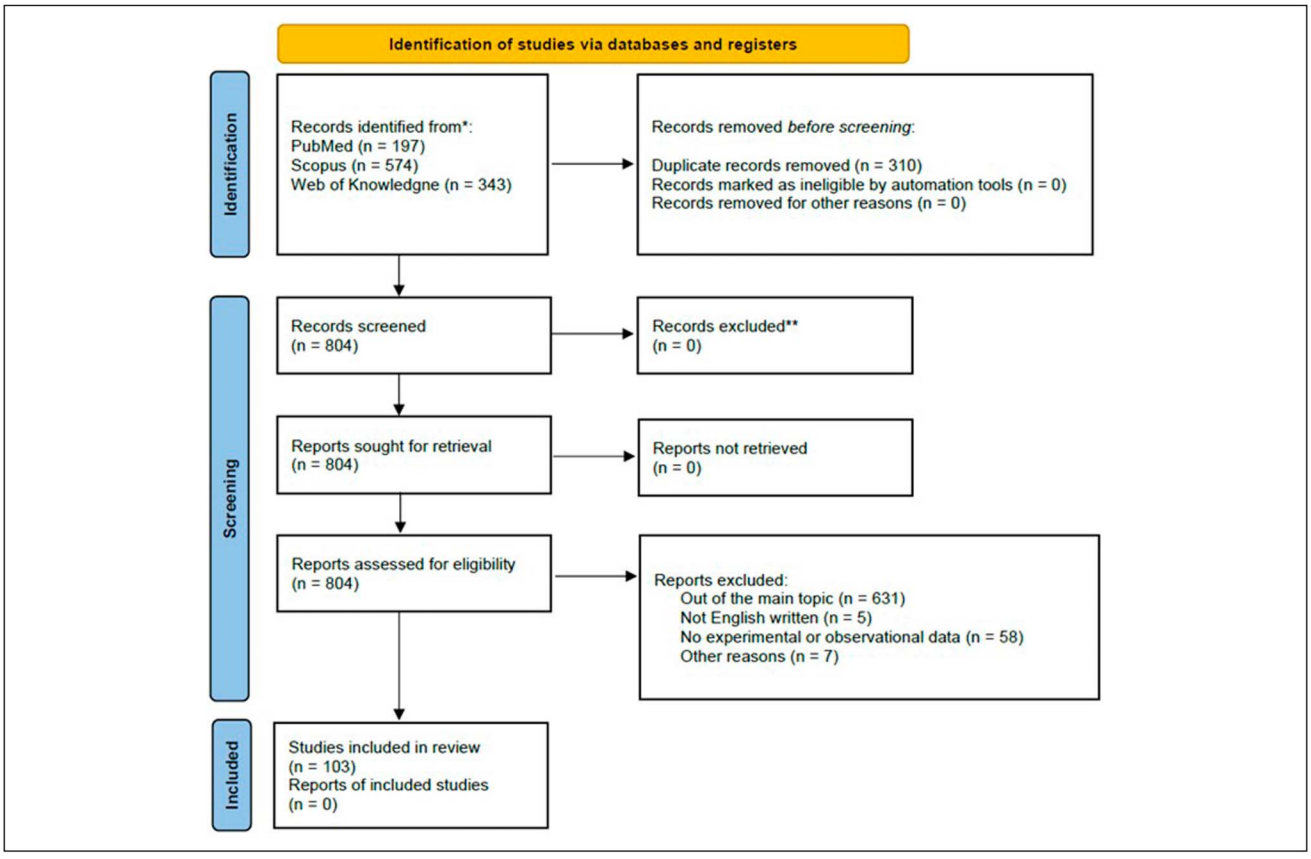


Figure 1. Flowchart of records screening process (17).

Table 1. Integrated synthesis of the principal therapeutic targets emerging from contemporary mechanistic research in EoE

Therapeutic target	Mechanistic role in EoE pathogenesis	Supporting evidence (from included studies)	Development stage
IL-13Rα1	Receptor mediating IL-13–induced epithelial hyperplasia, fibroblast activation, collagen deposition, and type-2 inflammatory amplification	Avlas et al (18) demonstrated that EoE pathology is critically mediated by IL-13 signaling through IL-13Rα1; blocking IL-13 pathways reduced epithelial proliferation and fibrosis	Proven/late-stage clinical (IL-4Rα blockade with dupilumab indirectly blocks IL-13Rα1 signaling)
TSLPR$^+$ Th2A cells	Highly pathogenic memory CD4$^+$ Th2 subset producing IL-4, IL-5, IL-13, and IL-9 through TSLP–STAT5 signaling; strongly correlates with disease severity	Rochman et al (19) identified expansion of TSLPR$^+$ Th2A cells in EoE, demonstrating their central role in amplifying chronic type-2 inflammation	Preclinical → early-phase translational (anti-TSLP therapy validated in asthma; mechanistic data strong for EoE)
LIGHT–HVEM axis (TNFSF14–HVEM)	Drives fibroblast differentiation into an inflammatory-myofibroblast phenotype; enhances collagen production and eosinophil tethering through ICAM-1	Manresa et al (20) showed overproduction of LIGHT in EoE T cells; LIGHT deficiency limited remodeling and inflammation in murine models	Preclinical (no current EoE-specific clinical trials)
CD73 (Ecto-5′-nucleotidase)	Metabolic checkpoint regulating fibroblast phenotype, migration, and ECM deposition; decreased CD73 activity contributes to profibrotic reprogramming	Jumabay et al (21) demonstrated defective CD73 activity in EoE fibroblasts; adenosine supplementation restored normal fibroblast function	Preclinical
KLK5/PAR2 signaling	KLK5 activates PAR2 on epithelial cells, promoting inflammation, barrier breakdown, Th2 cytokine release, and epithelial hyperplasia	Azouz et al (39) showed KLK5/PAR2 activation induces EoE-like inflammation; KLK5 inhibition reduced eosinophilia and barrier loss	Preclinical (potential small-molecule inhibitor target)
MIF–CXCR4 axis	MIF promotes eosinophil recruitment, mast cell accumulation, and subepithelial fibrosis	Souza et al (22) and others demonstrated MIF-dependent eosinophilia and tissue remodeling; CXCR4 signaling required for eosinophil trafficking	Preclinical (CXCR4 antagonists exist but not tested in EoE)
IL-18/NLRP3 pathway	Epithelial alarmin IL-18 activates iNKT cells → IL-5/IL-13 production, mast cell accumulation, fibrosis	Yadavalli et al (23) showed NLRP3/IL-18 activation initiates EoE; pharmacologic inhibition prevented disease development in mice	Preclinical
SCF/c-Kit axis	SCF promotes eosinophil survival, mast cell activation, and IL-4/IL-13 amplification	Ptaschinski et al (24) showed SCF inhibition reduced Th2 inflammation and cellular infiltration in EoE mouse models	Preclinical
Eotaxin-3/CCR3 pathway	Central eosinophil chemoattractant induced by IL-4/IL-13; contributes to eosinophilia and epithelial injury	Multiple included studies confirm elevated CCL26 (eotaxin-3) in EoE; PPI-REE and EoE both show reversible CCL26 upregulation	Proven biologic relevance (but no direct clinical inhibitors approved)
PIR-B (Paired Ig-like receptor B)	Inhibitory receptor restraining IL-13–mediated eosinophil activation and tissue remodeling	Baruch-Morgenstern et al (25) showed PIR-B deficiency exacerbates remodeling; restoring PIR-B activity reduces fibrosis	Preclinical
ATP12A/Ca^{2+}-dependent pathways	Drives intracellular calcium increases leading to eotaxin-3 secretion; contributes to epithelial injury	Odiase et al (26) showed Th2 cytokines activate ATP12A-dependent proton pump pathways increasing Ca^{2+} and eotaxin-3 release	Preclinical (PPI-sensitive)
CD4$^+$ tissue-resident IFN-γ axis	Non-Th2 inflammatory pathway causing epithelial apoptosis, tight-junction disruption, and tissue damage	Lal et al (27) demonstrated IFN-γ disrupts barrier integrity and induces epithelial cell death in EoE biopsies	Preclinical
IgG4-related TFH pathway	TFH IL-10$^+$ cells promote IgG4 deposition and immune activation distinct from IgE-mediated allergy	Lozano-Ojalvo et al (28) identified esophageal IgG4 signatures linked to TFH activation in pediatric EoE	Preclinical

The table summarizes molecular, immunologic, epithelial, and stromal pathways that have been repeatedly implicated in disease initiation, amplification, or fibrostenotic progression. For each target, the table outlines its specific mechanistic role within EoE pathophysiology, highlights key supporting evidence from the included studies, and indicates the current stage of therapeutic development, ranging from early preclinical investigation to late-phase clinical evaluation.
ATP12A, ATPase H+/K+ transporting non-gastric alpha-2 subunit; CCL26, C-C motif chemokine ligand 26; CD73, cluster of differentiation 73/ecto-5′-nucleotidase; CXCR4, C-X-C motif chemokine receptor 4; ECM, extracellular matrix; EoE, eosinophilic esophagitis; HVEM, herpesvirus entry mediator; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon γ; IgE, immunoglobulin E; IgG4, immunoglobulin G4; IL, interleukin; IL-13Rα1, interleukin-13 receptor alpha 1; iNKT, invariant natural killer T cell; KLK5, kallikrein-related peptidase 5; MIF, macrophage migration inhibitory factor; NLRP3, NLR family pyrin domain-containing protein 3; PAR2, protease-activated receptor 2; PPI, proton pump inhibitor; SCF, stem cell factor; TFH, T follicular helper; TSLP, thymic stromal lymphopoietin.

a custom checklist for *in vitro* studies. Discrepancies in scoring were resolved through discussion or by involving a fifth reviewer.

For clinical observational studies, we used the NOS, a validated tool widely used to assess the quality of non-randomized studies in meta-analyses (14). This scale evaluates 3 main domains: Selection of study groups, Comparability of groups, and Outcome assessment. The maximum score is 9 stars for cohort and case-control designs and 7 stars for cross-sectional studies. According to established thresholds (15), studies were considered of good quality if they received 3–4 stars in Selection, 1–2 in Comparability, and 2–3 in Outcome; fair quality if they scored 2 in Selection, 1–2 in Comparability, and 2–3 in Outcome; poor quality if they received 0–1 in Selection, 0 in Comparability, or 0–1 in Outcome.

Animal studies were assessed using the SYRCLE's Risk of Bias tool (16), an adaptation of the Cochrane tool for animal research. This tool comprises 10 domains evaluating bias across 6 categories: selection bias (e.g., random sequence generation, allocation concealment), performance bias (e.g., random housing, blinding of personnel), detection bias (e.g., blinding of outcome assessors), attrition bias, reporting bias, and other potential biases. Each domain was judged as "low," "high," or "unclear" risk of bias.

For *in vitro* studies, a structured quality assessment was conducted using a custom checklist developed for this review. The checklist was based on widely accepted standards for robust cell-based experiments and included 6 criteria: Cell line origin and characterization (e.g., source, authentication), experimental design clarity (e.g., defined interventions and timepoints), inclusion of replicates (biological and/or technical), use of appropriate controls (positive/negative), methodological transparency (detailed protocol), statistical rigor (use of appropriate tests and sample size justification). Each study was assessed across all 6 domains, with those fulfilling all criteria considered at low risk of bias.

In several studies included in this review, the number of participants, animals, or experimental replicates was either incompletely reported or not specified. To ensure consistency in our quality evaluation, missing sample size information was handled according to the requirements and scoring structures of the specific risk-of-bias tools applied. For observational human studies assessed with the NOS, sample size is not an explicit scoring domain; however, when the absence of sample size reporting affected the interpretability of selection or outcome adequacy, the corresponding items were conservatively judged as "unclear" rather than "low risk." For animal studies evaluated using the SYRCLE tool, lack of sample size reporting contributed to an "unclear risk" rating in domains related to selection bias, performance bias, or detection bias when the missing information prevented full assessment of allocation, housing conditions, or outcome measurement reliability. *In vitro* studies assessed with our custom methodological checklist were similarly scored as "unclear" in the relevant criteria (e.g., replicates and methodological transparency) when sample size or replicate details were not provided.

Across all study types, missing sample size information therefore did not result in automatic exclusion but was incorporated into the risk-of-bias judgement by reducing confidence in the affected domains. This approach ensured that uncertainty arising from incomplete reporting was explicitly reflected in the overall assessment while maintaining inclusion of mechanistically informative studies central to this review.

Sensitivity analysis

We made the analyses to evaluate the eventual causes of heterogeneity and examined the robustness of the outcome evaluations: (i) We limited our analyses to biomolecules qualitative and quantitative analysis between models with and without EoE. (ii) We independently analyzed data collected in observational studies (adults and pediatrics separately), *in vivo* animal models and *in vitro* cellular studies. (iii) In all data elaborations, we chose the outcome estimate that had the highest significance for each study group and showed a significant difference between samples with and without EoE characteristics.

Effect measures

The effect outcomes presented as quantitative and qualitative analysis of biomolecules, which significantly differs between models with and without EoE were taken from the original manuscript.

Synthesis methods

The synthesis of findings was structured around 4 mechanistic domains relevant to EoE: (i) innate and adaptive immunity, (ii) genetic and epigenetic mechanisms, (iii) ECM remodeling, and (iv) transcription factors and other regulatory proteins. Studies were grouped according to their biological focus and experimental model (human observational, *in vivo* animal, or *in vitro* cellular), allowing for stratified analysis across systems and age groups.

Quantitative and qualitative data on biomolecular markers were extracted and compared between EoE and non-EoE models. The synthesis emphasized molecular targets with consistent differential expression, functional relevance, and therapeutic potential. Where applicable, convergence across multiple studies was highlighted using visual tools (e.g., Venn diagrams) to identify shared gene signatures and pathway-level insights.

This approach enabled a comprehensive mechanistic mapping of EoE pathogenesis, integrating multiomics data and identifying candidate biomarkers and therapeutic targets across diverse experimental contexts.

RESULTS

Study selection

Our literature search in the time range January 2014—June 2025 in 3 databases (PubMed, Scopus, and Web of Science) delivered 1,114 records (Figure 1). After removing 310 duplicates, we analyzed 800 records and selected 103 eligible articles, which were further used for analysis. Table 1 summarizes the main characteristics of 103 records used for the review analysis.

Study characteristics

The pathomechanisms of EoE can be divided into 4 main pathways, for instance, innate and adaptive immunity mechanisms, genetic driven mechanisms, ECM-related pathways, and transcription factors together with other protein-related pathways. Thus, 103 selected records were analyzed in these 4 categories.

Innate and adaptive immunity pathway was analyzed on the base of 69 records, among which 14 records presenting data on epithelial barrier dysfunction, 39 records presenting data on innate immune cells and cytokines, and 16 records describing the recent findings on the role of adaptive immunity in EoE.

Genetic basis of EoE were analyzed based on 21 records relative to genetic risk loci and polymorphism (2 records), epigenetic

alternations (2 records), and multiomics and transcriptomics approaches (17 records).

There were 8 records describing ECM involvement in EoE, and 5 articles presenting role of other proteins and transcription factors in EoE.

The analysis was made on records presenting data collected during observational studies (65 research studies on adults and 11 studies on pediatric patients), *in vivo* models (22 records), and *in vitro* cellular studies using cells derived from human or animal biopsies (50 records).

Heterogeneity and sensitivity analyses

Given the methodological diversity of the included studies—spanning human observational cohorts, *in vivo* models, and *in vitro* mechanistic experiments—addressing heterogeneity was essential to ensure interpretability and robustness of the findings. To systematically manage variability across study types, we performed a series of planned sensitivity and subgroup analyses that stratified the evidence according to key biological and methodological dimensions.

First, observational clinical studies were analyzed separately for adult and pediatric populations. This distinction reduced age-related heterogeneity in epithelial, immunologic, and transcriptomic profiles and enabled clearer identification of convergent mechanistic pathways shared across life stages. Second, *in vivo* animal models were synthesized independently from clinical studies to avoid cross-species variability influencing overall mechanistic interpretations. Third, *in vitro* cellular studies were evaluated as a distinct evidence layer, recognizing the controlled but highly reductionist nature of cell-based systems and allowing their molecular insights (e.g., cytokine signaling, epithelial remodeling, and fibroblast activation) to be interpreted without conflation with whole-organism findings. Fourth, within preclinical work, we further distinguished between human-derived and animal-derived primary tissues, allowing us to assess the consistency of epithelial and stromal responses across species and experimental platforms.

These structured subgroup analyses helped reduce methodological heterogeneity by ensuring that mechanistic signals were evaluated within biologically coherent categories. This approach also revealed strong convergence across systems: epithelial barrier disruption, IL-13–driven inflammation, fibroblast reprogramming, and ECM alterations emerged consistently across adult and pediatric cohorts, across *in vivo* and *in vitro* models, and across human-derived and animal-derived tissues. Such cross-model alignment strengthened confidence in the robustness and generalizability of the mechanistic pathways identified.

Despite these efforts, formal meta-analytic subgroup analysis was not feasible. The mechanistic nature of the included evidence—characterized by diverse biomolecular endpoints, variable assay platforms, distinct experimental designs, and heterogeneous reporting formats—precluded the identification of shared quantitative effect measures necessary for meta-analytic pooling. Because studies frequently reported fold-changes, qualitative molecular patterns, or pathway-level results rather than standardized numerical outcomes, statistical aggregation would have been inappropriate and potentially misleading.

Nonetheless, our stratified narrative synthesis allowed us to address heterogeneity as systematically as possible. By isolating sources of biological and methodological variation and synthesizing evidence within comparable subgroups, we were able to identify coherent mechanistic themes while preserving the integrity of the diverse data types included in this review. Future mechanistic research would benefit from standardized reporting of quantitative molecular outcomes, which could enable more formal comparative analyses and quantitative integration across studies.

Risk of bias assessment analysis

The risk of bias across the included studies was assessed using domain-specific tools: the NOS for observational clinical studies, the SYRCLE Risk of Bias tool for animal models, and a customized checklist tailored to *in vitro* experiments. Overall, the methodological quality was acceptable, although certain limitations—particularly in preclinical studies—warrant attention.

Clinical studies. The observational clinical studies showed consistently high methodological quality according to the NOS, with total scores ranging from 7 to 9 out of a maximum of 9 stars. All studies were judged to be at low risk of bias. The strongest domains included the representativeness of the exposed cohort, the adequacy of the control group, and the ascertainment of exposure. The most common source of uncertainty was related to follow-up duration and adequacy, which was not always clearly described.

Animal studies. The quality of the animal studies, assessed using the SYRCLE Risk of Bias tool, was generally moderate, with all studies categorized as having a moderate risk of bias. A particularly consistent and concerning issue was the lack of information on random sequence generation, allocation concealment, and blinding procedures—all of which were systematically either unreported or explicitly absent. These limitations introduce substantial risks of selection, performance, and detection bias. This issue is not isolated: According to a large-scale analysis of preclinical studies, up to 87% failed to implement random allocation and 86% did not use blinding, despite SYRCLE's explicit inclusion of these domains as critical quality criteria (29). While outcome data were generally complete and selective reporting was low, the lack of methodological safeguards significantly limits the internal validity and translational potential of the findings.

In vitro studies. *In vitro* experiments were evaluated with a custom risk of bias checklist developed for this review. These studies were uniformly judged to be at low risk of bias. All fulfilled the 6 methodological criteria, including detailed cell line characterization, clear experimental design, inclusion of replicates, appropriate use of controls, transparent methodology, and rigorous statistical analysis. The clarity and reproducibility of the protocols substantially increase the reliability of these findings.

Taken together, the studies included in this review present a methodologically robust foundation for our analysis. However, the systematic absence of randomization and blinding in animal studies represents a notable limitation that could affect the internal validity of preclinical findings. Improved reporting and implementation of these methodological safeguards in future studies will be essential to enhance the translational value of animal models in gastrointestinal research.

Results of individual studies

Innate and adaptive immunity in eosinophilic esophagitis.

Epithelial barrier dysfunction Among the studies analyzed, 8 were experimental translational investigations using *in vitro* and/

or *in vivo* models, 6 of which also included patient-derived samples, while 6 were purely clinical case-control studies based on esophageal biopsies (see Supplementary Table S2a, Supplementary Digital Content, http://links.lww.com/CTG/B535). Despite these differences, a consistent theme emerges: Epithelial barrier dysfunction plays a pivotal role in EoE pathogenesis.

Rather than being a consequence of inflammation, barrier disruption actively contributes to disease onset. Structural junctional proteins are among the most affected. Desmoglein-1 (DSG1) is consistently reduced in EoE, compromising epithelial cohesion and promoting periostin deposition (30,31). IL-13–induced CAPN14 specifically degrades DSG1, exacerbating barrier loss (32). Tight junction proteins are similarly affected: Claudin-7 and filaggrin (FLG) are reduced by transforming growth factor beta 1 (TGF-β1) and IL-13, respectively, increasing permeability (33,34). Involucrin downregulation further suggests impaired epithelial maturation (35). Reduced E-cadherin and zonula occludens 1 expression, together with nuclear β-catenin and Twist1 activation, indicate epithelial–mesenchymal transition (EMT), contributing to remodeling and fibrosis (36).

Differentiation and cytoskeletal regulators further link cytokine signaling to epithelial remodeling. Synaptopodin (SYNPO), upregulated by IL-13, impairs epithelial motility and barrier integrity (19). Resistin-like molecule alpha (RELM-α) promotes epithelial hyperplasia and basal layer thickening, driving $CD4^+$ T cell and eosinophil accumulation (37), while bone morphogenetic protein-driven nuclear factor erythroid 2-related factor 2 signaling regulates epithelial differentiation; its inhibition by follistatin induces basal cell hyperplasia (38).

Protease and inflammatory pathways also contribute to epithelial injury. Kallikrein-related peptidase 5 (KLK5) activates protease-activated receptor 2, promoting inflammation and barrier breakdown, whereas its inhibition reduces eosinophilia (39). Krüppel-like factor 4-driven Ras homolog family member F (RHOF) and nuclear factor kappa B activation in keratinocytes induces epithelial inflammation and remodeling (40). Moreover, IL-4 stimulates the H^+,K^+-ATPase in epithelial cells, leading to calcium-dependent eotaxin-3 secretion and enhanced eosinophil recruitment, an effect reversed by PPIs or calcium-channel blockade (26).

Metabolic regulation plays an additional role. Loss of hypoxia-inducible factor 1-alpha signaling shifts epithelial metabolism toward oxidative phosphorylation and impairs differentiation, whereas its stabilization restores glycolysis and barrier integrity (41).

Collectively, these findings support a model where early structural and metabolic dysregulation of epithelial cells facilitates antigen penetration and chronic inflammation. Targeting epithelial differentiation programs, protease activity, and metabolic reprogramming may represent promising therapeutic approaches to restore mucosal integrity and prevent disease progression in EoE.

Innate immune cells and cytokines Of the studies examined, 22 were experimental translational investigations using *in vitro* and/or *in vivo* models, 16 of which also incorporated patient-derived samples, whereas 17 were purely clinical case-control or retrospective studies based on human biopsies or blood specimens (see Supplementary Table S2b, Supplementary Digital Content, http://links.lww.com/CTG/B535). Models ranged from human biopsies, blood, and serum to genetically modified mice and epithelial cell lines, covering pediatric and adult cohorts and comparisons with gastroesophageal reflux disease (GERD) and celiac disease.

Epithelial-derived alarmins, IL-33, IL-18, and TSLP, initiate type 2 inflammation. IL-33 promotes eosinophilia through IL-5 and IL-13, while IL-18 activates invariant natural killer T (iNKT) cells to produce IL-5/IL-13, driving mast cell accumulation and fibrosis (42–44). Peripheral iNKT cells show reduced frequency and increased low-affinity immunoglobulin E receptor II expression, suggesting tissue homing and biomarker potential (45). Dysregulation of noncanonical nuclear factor kappa B signaling also amplifies epithelial inflammation (46). TSLP bridges innate and adaptive immunity by activating $TSLPR^+$ $CD4^+$ T cells through STAT5 signaling, enhancing IL-5 and IL-13 production (47).

MC and eosinophils act as central effector cells. MC persist during remission, releasing IL-13 and oncostatin M that weaken epithelial junctions and perpetuate inflammation (48–50). Histamine receptor signaling (H1R, H2R, H4R) enhances epithelial cytokine release (51), while vasoactive intestinal peptide–chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells signaling couples mast cell and eosinophil activation, contributing to dysmotility (23).

Eosinophils are sustained by IL-5, IL-33, and macrophage migration inhibitory factor (MIF)–C-X-C motif chemokine receptor 4 signaling (22,43) and regulated by inhibitory mechanisms such as paired immunoglobulin-like receptor B (PIR-B) (25). They mediate cytotoxicity (52).

Macrophage-derived colony-stimulating factor 1 promotes fibrosis, epithelial proliferation, and ECM remodeling (53), while stem cell factor enhances eosinophilia and Th2 cytokine production (24). IL-13 and its receptor interleukin-13 receptor alpha 1 (IL-13Rα1) drive epithelial proliferation and fibrotic remodeling (18), and high IL-5/IL-13 levels distinguish EoE from celiac disease (54). Long-term control of eosinophilia reduces fibrostenotic risk (55).

Beyond Th2 circuits, interferon γ (IFN-γ) from $CD8^+$ resident memory T cells disrupts epithelial integrity (27). Forkhead box P3 Tregs expand during active disease but decline in remission, indicating dynamic immune adaptation. Recent transcriptomic data revealed downregulation of C-X-C motif chemokine receptor 2 and an inverse correlation with Treg markers, suggesting a dysregulated neutrophil–Treg axis sustaining chronic inflammation (56).

In summary, epithelial alarmins orchestrate innate and adaptive immune responses, driving eosinophilic inflammation and tissue remodeling. Key therapeutic targets include IL-13Rα1, TSLP–STAT5, interleukin-18/NLR family pyrin domain-containing protein 3, MIF–CXCR4, and stem cell factor. Supplementary Figure S1 (see Supplementary Digital Content, http://links.lww.com/CTG/B535) presents a color-coded diagram of these immunological pathways.

Adaptive immunity in eosinophilic esophagitis To explore the contribution of adaptive immunity to EoE, 16 studies were reviewed (see Supplementary Table S2c, Supplementary Digital Content, http://links.lww.com/CTG/B535). Nine adopted an experimental translational design integrating *in vitro* and/or *in vivo* models, 6 of which also analyzed patient-derived samples, while 7 were purely clinical case-control or retrospective investigations based on esophageal biopsies or blood specimens. Together, these studies delineate how T cell–driven cytokine polarization, fibroblast activation, and impaired tolerance sustain chronic inflammation and remodeling in EoE.

Adaptive immunity drives chronicity in EoE through antigen-specific T-cell responses and type 2 cytokine polarization. IL-13 is a central mediator, activating STAT3/STAT6, inducing secreted frizzled-related protein 1, and promoting epithelial hyperplasia (57). It also regulates leucine-rich repeat-containing protein 31, which maintains barrier integrity; its loss worsens dysfunction and predicts poor treatment response (58). IL-4 enhances eotaxin-3 secretion through calcium and proton-pump pathways, amplifying eosinophil recruitment (26).

Wright et al (59) demonstrated increased GATA-binding protein 3 and T-bet expression in esophageal $CD4^+$ T cells from patients with EoE and proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) compared with GERD and controls, with both markers decreasing after treatment, indicating shared T-helper type 1/T-helper type 2 activation patterns between EoE and PPI-REE.

Th2 cytokines also promote stromal remodeling: IL-9 and granulocyte–macrophage colony-stimulating factor activate MC and myeloid cells (60), while suppression of tumorigenicity 2 Th2 cells produce amphiregulin in response to IL-33, stimulating fibroblasts and collagen deposition (61). $TSLPR^+$ memory $CD4^+$ Th2A cells, activated through TSLP–STAT5 signaling, produce IL-4, IL-5, IL-13, and IL-9 in a feed-forward loop (47). Their frequency correlates with eosinophilia and disease severity, making them a biomarker and therapeutic target.

Beyond Th2 pathways, IFN-γ, secreted by $CD8^+$ tissue-resident memory T cells, disrupts epithelial proliferation and tight junctions, inducing apoptosis (27). Plasmacytoid dendritic cells may amplify this through type I interferons. These findings reveal a non-Th2 axis contributing to epithelial damage and chronic inflammation (62).

Forkhead box P3 regulatory T cells (Tregs) normally suppress excessive immune responses and maintain tolerance. In EoE, Tregs are increased in the esophageal mucosa but show impaired function. Their numbers decline after histologic remission, indicating dynamic immune remodeling during disease progression and treatment (63). Compared with GERD, patients with EoE show reduced Treg induction, suggesting a failure of tolerance mechanisms that favors Th2-driven inflammation (64). IL-33 signaling may further suppress Treg activity, tipping the balance toward inflammation.

T follicular helper (TFH) cells contribute to humoral responses in EoE, which diverge from classical immunoglobulin E (IgE)-mediated food allergy. TFH $IL\text{-}10^+$ cells promote immunoglobulin G4 (IgG4) production, leading to local IgG4 deposition in esophageal tissue (28). This contrasts with TFH13-driven IgE responses seen in milk allergy. Esophageal $CD4^+$ T cells produce IL-4, IL-5, IL-13, IL-10, and IL-21, sustaining both inflammation and IgG4-mediated tissue changes.

Lymphotoxin-like, exhibits inducible expression, competes with herpes simplex virus glycoprotein D for herpesvirus entry mediator, a receptor expressed by T lymphocytes (LIGHT) or TNFSF14 (tumor necrosis factor superfamily member 14), produced by activated T cells, drives stromal remodeling in EoE. It induces an inflammatory phenotype in fibroblasts through intercellular adhesion molecule 1 (ICAM-1), promotes eosinophil adhesion, and synergizes with TGF-β1 to stimulate myofibroblast differentiation and collagen deposition (20,65). These findings link adaptive immune signals directly to fibrosis.

Adaptive immunity in EoE is dominated by IL-13–driven Th2 responses but also involves pathogenic memory Th2A cells, impaired Treg tolerance, non-Th2 interferon pathways, and TFH–B-cell circuits. This multifaceted immune landscape sustains chronic inflammation and fibrostenotic remodeling. Therapeutic strategies may target IL-13, TSLP, LIGHT–fibroblast interactions, and interferon-driven epithelial injury.

Supplementary Figure S2 (see Supplementary Digital Content, http://links.lww.com/CTG/B535) graphically summarizes the role of adaptive immunity in EoE.

The genetic basis of eosinophilic esophagitis. Twenty-one studies (see Supplementary Table S3, Supplementary Digital Content, http://links.lww.com/CTG/B535) were analyzed to assess the genetic basis of EoE. These include case-control designs, prospective trials, cohort studies, and computational analyses using large datasets. Sample types range from esophageal biopsies and blood leukocytes to extracellular vesicles and cell lines, with both pediatric and adult cohorts represented.

A wide array of molecular techniques was used, including RNA sequencing (RNA-seq), single-cell RNA-seq, microRNA (miRNA) profiling, deoxyribonucleic acid (DNA) methylation, genome-wide association study, proteomics, and pathway analysis. Sample sizes varied from small cohorts (n = 18) to large-scale studies (n = 1,214), although most genome-wide association study cohorts were predominantly of European ancestry.

Key findings include altered expression of IL-13, periostin (POSTN), and C-C motif chemokine ligand 26 (CCL26) (eotaxin-3), epigenetic changes (e.g., CpG methylation), and

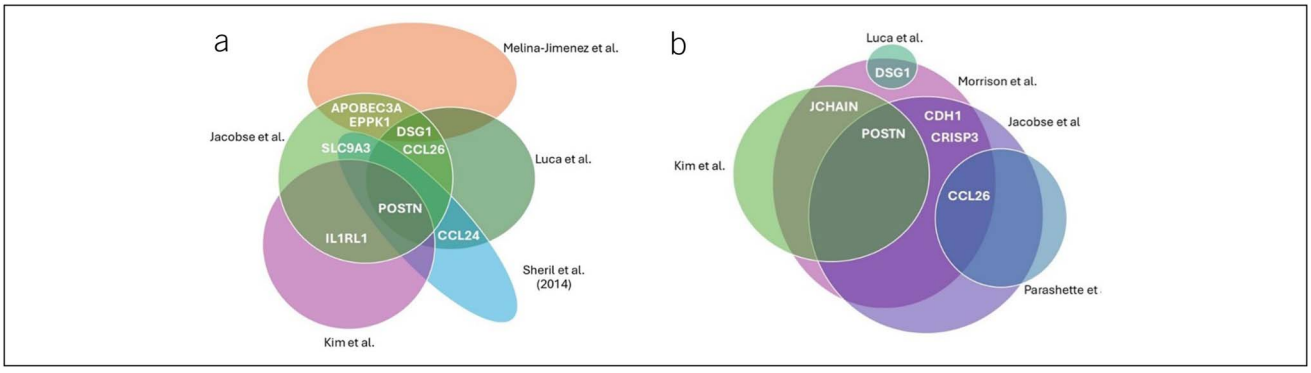


Figure 2. Venn diagram of (**a**) DE genes in selected studies on adult EoE biopsies and (**b**) DE genes in selected studies on pediatric and adult EoE biopsies. EoE, eosinophilic esophagitis.

dysregulated miRNAs (e.g., miR-155 and miR-375). Studies also revealed shifts in immune cell composition and microbiome alterations (e.g., *Fusobacterium* enrichment). Some focused on treatment response, while others explored genetic susceptibility and biomarker discovery.

Collectively, these studies highlight the role of genetic susceptibility in EoE, implicating pathways involved in epithelial barrier integrity, immune regulation, and allergic inflammation. The integration of transcriptomic, epigenomic, and proteomic data provides a foundation for personalized approaches to diagnosis and therapy.

Genetic risk loci and polymorphisms Genetic studies have identified key EoE risk loci, notably at 16p13, involving C-type lectin domain family 16 member A, dexamethasone-induced protein, and class II major histocompatibility complex transactivator, with immune-cell-specific expression patterns (66). Variants in TSLP and toll-like receptor 3 suggest a pathogenic epithelial signaling axis (67). Rare damaging mutations in dehydrogenase E1 and transketolase domain-containing protein 1, oxoglutarate dehydrogenase-like protein, dedicator of cytokinesis 8 (DOCK8), and myeloid differentiation primary response protein 88 impair mitochondrial and immune functions, reinforcing the immune-genetic basis of EoE (68). These findings highlight the role of genetic susceptibility in epithelial barrier dysfunction, Th2 inflammation, and immune dysregulation, offering insights into disease mechanisms and potential therapeutic targets.

Epigenetic alterations Epigenetic mechanisms such as DNA methylation also seem to modulate EoE pathogenesis. Methylation profiles of esophageal biopsies from patients with EoE—especially children—clearly separate them from healthy controls, even after first-line therapy, suggesting a lasting epigenetic imprint associated with disease (69). Another study confirmed that methylation differences are associated with treatment responsiveness, highlighting pathways related to cell adhesion, epithelial barrier integrity, and cellular aging (70).

Multiomics and transcriptomic approaches Differential gene expression in EoE has been explored using diverse methods, including single-cell RNA-seq (49,71–73), bulk RNA-seq (19,68,74), polymerase chain reaction (PCR) (71,75), microarrays (76), meta-transcriptomics (77), and miRNA profiling (78–80). These approaches reveal consistent upregulation of genes linked to inflammation and epithelial remodeling, such as POSTN, arachidonate 15-lipoxygenase, eosinophil peroxidase, and collagen type I alpha-2 chain.

Wen et al (81) further demonstrated that PPI-REE shares an EoE-like Th2 transcriptomic profile characterized by elevated CCL26, POSTN, carboxypeptidase A3, and DSG1 expression; PPI monotherapy normalized this signature and identified differential clusters (notably potassium inwardly rectifying channel subfamily J member 2) distinguishing PPI-REE from EoE.

Single-cell RNA-seq identified cell-type–specific expression of IL13, joining chain of multimeric immunoglobulins, C-X-C motif chemokine ligand 8, and fibroblast-associated signatures correlating with fibrosis (72). Rochman et al (47) found increased TSLP-responsive memory $CD4^+$ T cells, supporting anti-TSLP therapy as a precision approach.

Keever-Keigher et al (73) profiled peripheral blood mononuclear cells across eosinophilic and inflammatory bowel disease conditions, identifying EGR1 as a key differentially expressed gene (DEG) in Th2 cells, upregulated by zinc. This links zinc dysregulation to eosinophilia and epithelial stress.

miRNA studies revealed dysregulation of miR-203, miR-375, miR-21, and miR-223, with limited correlation to circulating levels (78). miRNAs in plasma-derived extracellular vesicles (e.g., miR-10b-5p and miR-125a-5p) were elevated in active EoE (79). miR-155 was shown to impair barrier integrity by targeting claudin 7 (80).

Comparative analysis of 8 adult EoE studies identified shared DEGs (Figure 2a): apolipoprotein B mRNA editing enzyme catalytic subunit 3A, epiplakin 1, DSG1, CCL26, solute carrier family 9 member A3, POSTN, IL1RL1, and C-C motif chemokine ligand 24. Cross-age comparisons (Figure 2b) revealed 6 common DEGs in pediatric and adult EoE: joining chain of multimeric immunoglobulins, DSG1, POSTN, cadherin 1, cysteine-rich secretory protein 3, and CCL26.

These findings support a multiomics approach to understanding EoE and highlight potential biomarkers and therapeutic targets.

The main characteristics of DE genes presented in Figure 2 are described in Supplementary Table S6 (see Supplementary Digital Content, http://links.lww.com/CTG/B535).

In line with current international consensus, PPI-REE is no longer regarded as a separate diagnostic entity but rather as part of the EoE continuum. This position, endorsed by the A Working Group on Proton Pump Inhibitor-Responsive Esophageal Eosinophilia (AGREE) conference and subsequent international guidelines, reflects accumulating evidence that PPI responsiveness represents a therapeutic phenotype rather than a distinct disease mechanism. Our findings further support this perspective: transcriptomic, immunologic, and molecular signatures reported in studies of PPI-responsive patients closely mirror those observed in classical EoE, including shared Th2-associated pathways, epithelial barrier alterations, and overlapping gene-expression profiles. These convergent mechanistic features reinforce the view that PPI-REE constitutes a phenotypic variant within the broader EoE spectrum, underscoring the importance of evaluating PPI response as a treatment attribute rather than a basis for differential diagnosis.

Extracellular matrix in esophagitis eosinophils. Over the past decade, 8 studies have investigated ECM remodeling in EoE, including 5 *in vitro* studies using fibroblasts from esophageal biopsies, 1 *in vivo* mouse model, and 2 pediatric biopsy-based studies (see Supplementary Table S5). These studies vary in design, sample type, and molecular focus, offering a broad view of ECM involvement in EoE pathogenesis.

Zdanowicz et al (82) examined matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinases 1 (TIMP-1) expression in pediatric EoE biopsies. TIMP-1 was absent in EoE but present in EoE and controls, suggesting a proteolytic imbalance favoring ECM degradation. Strong MMP-1 expression correlated with poor response to standard therapies, indicating its potential as a prognostic biomarker.

Clevenger et al (83) used single-cell RNA-seq to show that basal cell hyperplasia in EoE is driven by suprabasal epithelial cells retaining progenitor-like transcriptional programs. These cells overexpress SRY-box transcription factor 2 and Krüppel-like factor 5, fail to differentiate, and exhibit a wound-repair–like phenotype, distinct from GERD. This suggests chronic inflammation disrupts epithelial homeostasis through altered differentiation.

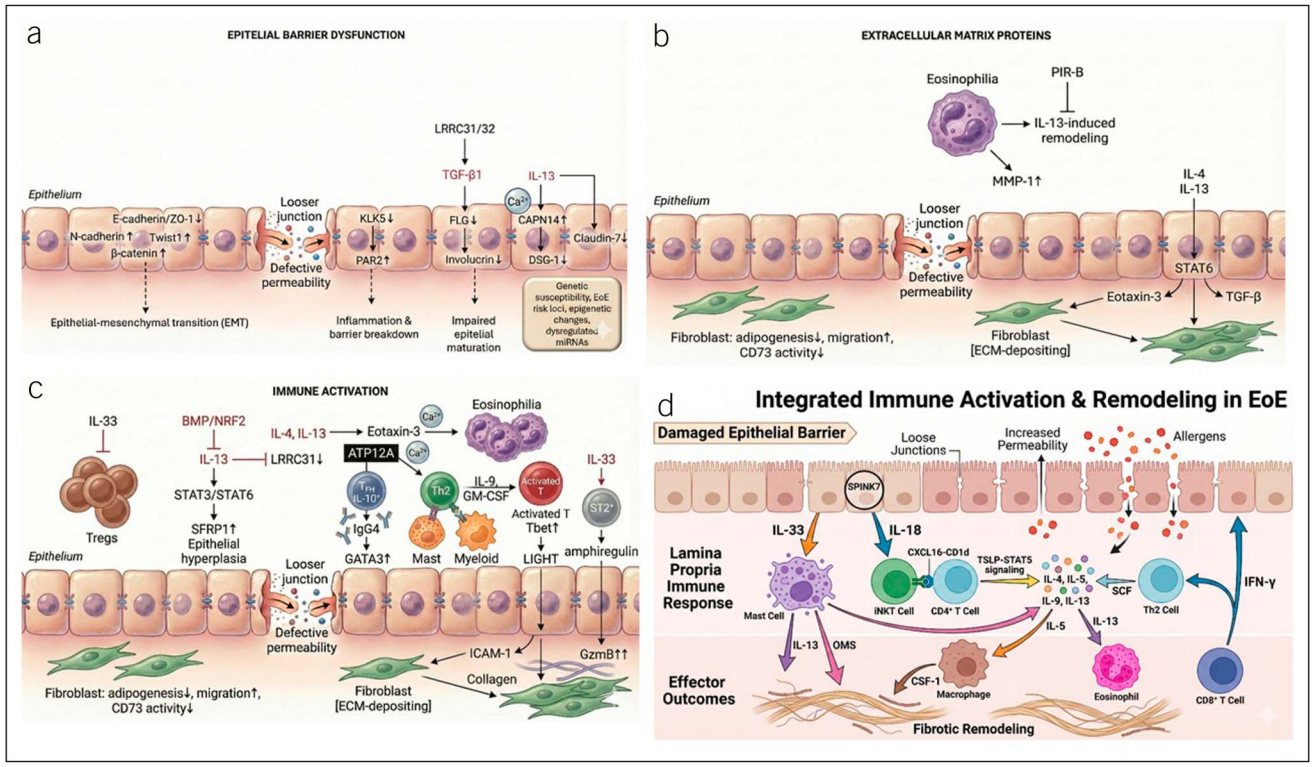


Figure 3. Integrated pathophysiological mechanisms of EoE. (**a**) Loosening of epithelial junctions reflects increased permeability and compromised barrier integrity. Epithelial-mesenchymal transition is indicated by upregulation of N-cadherin, β-catenin, and twist 1, alongside downregulation of E-cadherin and ZO-1, contributing to loss of epithelial cohesion. Inflammation and barrier breakdown are mediated by KLK5 and PAR2 signaling. Impaired epithelial maturation is shown through IL-13–induced upregulation of CAPN14 and downregulation of filaggrin and involucrin, with DSG-1 reduction linked to disrupted calcium signaling. Genetic susceptibility factors—including EoE risk loci, epigenetic modifications, and dysregulated microRNAs—are summarized in the orange box. (**b**) IL-13–induced remodeling leads to inhibition of PIR-B, contributing to eosinophilia. IL-4 and IL-13 activate STAT6 signaling, promoting the Eotaxin-3, a key eosinophil chemoattractant. TGF-β is also involved in epithelial-fibroblast crosstalk. Fibroblasts exhibit altered behavior, including reduced adipogenesis, increased migratory activity, and decreased CD73 expression. Upregulation of MMP-1 further contributes to tissue remodeling and fibrosis. (**c**) Epithelial hyperplasia is driven by increased expression of SFRP1 and influenced by BMP/NRF2 and IL-13 signaling, with IL-13 acting through STAT3/STAT6 pathways. Immune activation involves a diverse array of cells, including regulatory T cells, mast cells, myeloid cells, Th2 cells, eosinophils, and IL-10^+ T follicular helper cells. Key mediators include IL-33, IL-9, GM-CSF, LIGHT, ICAM-1, amphiregulin, and GATA-binding protein 3, with increased granzyme B (GzmB) expression. IgG4 production is also noted. Eotaxin-3 enhances intracellular Ca^{2+} through ATP12A channels, contributing to epithelial dysfunction. Fibroblast interactions with collagen in the extracellular matrix further support tissue remodeling. **(d)** Allergens initiate the release of IL-33 and IL-18, which activate mast cells and invariant natural killer T cells, respectively. Mast cells, IL-13, and OMS, promote fibrosis. iNKT cells produce CXCL16-CD1d and interact with $CD4^+$ T cells, leading to TSLP-STAT5 signaling and the production of IL-4, IL-5, IL-13, and IL-9. Th2 cells, influenced by stem cell factor, further amplify cytokine production. Macrophages release CSF-1, contributing to fibrotic remodeling, while $CD8^+$ T cells produce IFN-γ, inducing epithelial injury. Eosinophilia is driven by IL-5 and IL-13. The cumulative effect of these immune interactions results in epithelial barrier dysfunction, characterized by loose junctions and increased permeability. SPINK7 is noted within the epithelial layer, potentially reflecting a protective or regulatory role. ATP12A, ATPase H+/K+ transporting non-gastric alpha-2 subunit; BMP, bone morphogenetic protein; CAPN14, calpain 14; CSF-1, colony-stimulating factor 1; EoE, eosinophilic esophagitis; GM-CSF, granulocyte–macrophage colony-stimulating factor; GzmB, granzyme B; ink T, invariant natural killer T; KLK5, kallikrein-related peptidase 5; NRF2, nuclear factor erythroid 2-related factor 2; SPINK7, serine peptidase inhibitor Kazal type 7; STAT, signal transducer and activator of transcription; ZO-1, zonula occludens 1.

Baruch-Morgenstern et al (43) demonstrated that PIR-B, an inhibitory receptor on eosinophils, suppresses IL-13–induced remodeling in a mouse model. PIR-B deficiency led to increased eosinophil activation, epithelial hyperplasia, collagen deposition, and angiogenesis. Eosinophil depletion reversed these effects, confirming PIR-B's role in limiting tissue damage.

Fibroblasts have emerged as key ECM regulators in EoE. Studies show their functional plasticity and reprogramming under inflammatory conditions, contributing to fibrosis and structural remodeling. Together, these findings highlight ECM dysregulation as a central feature of EoE, linking immune activation to tissue remodeling and offering new therapeutic targets such as MMP-1, PIR-B, and epithelial differentiation pathways.

Five *in vitro* studies have explored fibroblast behavior in EoE using cells derived from human esophageal biopsies. Cheng et al (84) showed that IL-4/IL-13 stimulate eotaxin-3 via STAT6 in both epithelial cells and fibroblasts, contributing to inflammation and fibrosis. Janus kinase-STAT6 inhibitors block this effect, while PPIs act only on epithelial cells, suggesting limited impact on subepithelial fibrosis.

Muir et al (85) demonstrated that matrix stiffness and TGF-β synergistically drive fibroblast activation and myofibroblast differentiation, perpetuating fibrosis even in inactive disease. Li et al (86) identified 2 fibroblast subtypes: F_A (contractile) and F_B (ECM-depositing), with F_B dominant in active/remission EoE and enriched in EMT pathways.

Jumabay et al (21) found EoE fibroblasts undergo pathogenic reprogramming, showing impaired adipogenesis, enhanced migration, and reduced cluster of differentiation 73/ecto-5′-nucleotidase (CD73) activity. Adenosine supplementation restored fibroblast function, suggesting a metabolic checkpoint.

Manresa et al (65) identified LIGHT (TNFSF14) as a T cell–derived cytokine that drives fibroblast differentiation into an inflammatory–myofibroblast hybrid. LIGHT upregulates ICAM-1, promoting eosinophil tethering and localized inflammation.

Together, these studies reveal fibroblast heterogeneity and their central role in fibrosis, inflammation, and epithelial remodeling, highlighting therapeutic targets such as STAT6, CD73, and LIGHT.

Nonproteins and metabolic mediators. The role of nonprotein molecules and metabolites in EoE pathomechanisms has been explored in several studies (see Supplementary Table S6, Supplementary Digital Content, http://links.lww.com/CTG/B535). These investigations employ a variety of designs, from prospective clinical cohorts to *in vitro* epithelial models and analytical chemistry techniques. The sample types span esophageal mucosa (through impedance-pH), serum, and plasma, allowing assessment of mucosal integrity, systemic metabolic markers, and eosinophil activation signatures.

One of the most robust contributions comes from Frazzoni et al (87), who used impedance-pH monitoring in 60 patients with EoE (40 PPI-responsive, 20 PPI-refractory), 60 GERD, and 60 control subjects. They found that low mean nocturnal baseline impedance gradient between mid and distal esophagus predicts PPI refractoriness, and that postreflux swallow–induced peristaltic wave index distinguishes PPI-responder from nonresponder cases. These findings suggest that gastroesophageal reflux and impaired mucosal integrity may actively contribute to EoE pathogenesis and that part of PPI effectiveness may derive from improving chemical clearance and barrier properties.

At the epithelial and cellular level, Wheeler et al (88) explored the protective role of 17β-estradiol (E2) against IL-13–induced injury. In esophageal epithelial cell line 2 ALI esophageal epithelial cells, E2 pretreatment mitigated the loss of barrier function and remodeling effects by suppressing tyrosine kinase 2/STAT6 phosphorylation and restoring DSG1 expression, in an ESR2-dependent manner. This suggests a modulatory influence of sex hormones on EoE susceptibility and barrier integrity.

In the translational domain, Brusilovsky et al (89) examined vitamin D ($25(OH)D_3$) in pediatric EoE: They present evidence that vitamin D antagonizes IL-13–induced epithelial disruption and hyperproliferation, supporting barrier restoration. Cameron et al (90) then assessed serum $25(OH)D_3$ in 40 EoE vs 40 non-EoE controls, finding lower mean levels in patients with EoE and an association between lower vitamin D and lamina propria fibrosis, although deficiency prevalence was similar.

Finally, Thomas et al (91) focused on 3-bromotyrosine (a marker of eosinophil activation) and L-tyrosine in plasma. They developed a sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method and applied it to 4 patients with EoE, finding no clear correlation between plasma 3-bromotyrosine levels and eosinophil counts, although possible associations with esophageal structural changes were noted. This opens the possibility of noninvasive metabolic biomarkers for EoE activity.

Collectively, these studies hint that impedance metrics, hormonal modulation, vitamin D status, and eosinophil-derived metabolic byproducts may modulate or reflect EoE pathophysiology. However, most findings remain preliminary and require validation in larger cohorts and mechanistic models. Future research integrating multiomic biomarker panels (impedance, metabolomics, and hormonal assays) with histologic and clinical endpoints may reveal novel diagnostic or therapeutic pathways beyond the proteome.

DISCUSSION

This systematic review builds on the foundational framework proposed by O'Shea et al (92), reinforcing the concept of EoE as a chronic, antigen-driven, type 2 immune-mediated disease marked by epithelial barrier dysfunction, eosinophilic inflammation, and progressive tissue remodeling. While O'Shea emphasized IL-13–induced CAPN14 expression and desmosomal protein degradation (e.g., DSG1 and FLG), our review identifies additional contributors to barrier disruption, including KLK5, SYNPO, and RELM-α, and highlights EMT and transcriptional reprogramming in suprabasal cells. Figure 3 summarizes the integrated pathophysiological mechanisms of EoE, including epithelial barrier dysfunction, immune activation, fibroblast reprogramming, and genetic susceptibility.

Recent findings support barrier dysfunction as a primary pathogenic event. McGowan et al (93) linked reduced DSG1 expression to altered immune gene profiles (e.g., IL13), while Akdis (94) proposed the "epithelial barrier hypothesis," implicating environmental exposures in barrier damage and immune dysregulation.

Our review confirms the central role of type 2 inflammation, with IL-4, IL-5, and IL-13 driving eosinophil, mast cell, and Th2 activation. We expand on O'Shea's (92) findings by identifying Th2A memory cells, group 2 innate lymphoid cells, and basophils as key cytokine producers and describe novel signaling axes [thymic stromal lymphopoietin–signal transducer and activator of transcription 5 (TSLP–STAT5), suppression of tumorigenicity 2/interleukin-33 receptor, MIF–CXCR4] that amplify inflammation.

Racca et al (95) outlined genetic predisposition (e.g., TSLP, FLG, CAPN14, and STAT6) and environmental triggers. Our review adds mechanistic depth, detailing how epithelial-derived cytokines (IL-33, IL-18) activate iNKT cells and group 2 innate lymphoid cells, and how PIR-B and vitamin D modulate IL-13–driven responses.

We also highlight adaptive immunity's complexity, identifying thymic stromal lymphopoietin receptor ($TSLPR^+$) Th2A cells as biomarkers and therapeutic targets, and describing a non-Th2 IFN-γ axis involving $CD8^+$ tissue-resident memory T cells. TFH cells promote IgG4 rather than IgE, distinguishing EoE from classical food allergy.

Fibrosis is driven by TGF-β1, periostin, and LIGHT, with our review detailing LIGHT–ICAM-1 interactions and CSF-1–dependent macrophage activation. These findings support precision medicine strategies targeting epithelial–immune–stromal crosstalk.

In summary, our review enriches the mechanistic understanding of EoE, integrating recent discoveries in immune regulation, epithelial biology, and fibrosis and identifying novel therapeutic targets for personalized intervention.

Building on the review of O'Shea et al (92), which identified CAPN14 and TSLP as key genetic risk loci, our synthesis integrates multiomics data to expand the genetic landscape of EoE. Additional risk genes such as leucine-rich repeat-containing protein 32, DOCK8, and class II major histocompatibility

complex transactivator, along with persistent epigenetic changes (e.g., DNA methylation and miRNA dysregulation), suggest mechanisms of disease memory and treatment resistance.

Recent literature (96) emphasizes ECM remodeling as central to EoE pathogenesis. Fibroblasts from patients with EoE secrete a distinct ECM proteome, with thrombospondin-1 emerging as a key driver of collagen I production and fibrosis. Thrombospondin-1 is upregulated by TGF-β1, forming a feed-forward loop that perpetuates tissue rigidity.

Studies reveal heterogeneous fibroblast subpopulations (92), including $ACTA2^+$ myofibroblasts and ECM-depositing fibroblasts, shaped by cytokine signaling (e.g., IL-13 and LIGHT), and metabolic checkpoints (e.g., CD73 and adenosine). Mechanotransduction through matrix stiffness further drives fibroblast activation and differentiation.

Serum biomarkers such as N-terminal propeptide of type III collagen, C-terminal propeptide of type VI collagen, and MMP-derived fragments (matrix metalloproteinase-generated fragment of type III collagen and matrix metalloproteinase-generated fragment of type IV collagen) correlate with fibrosis and symptom resolution, offering noninvasive tools for disease monitoring. Notably, TIMP-1 absence in pediatric biopsies suggests early ECM degradation preceding fibrosis.

Our review complements prior findings by identifying epithelial-specific targets (e.g., ATPase H+/K+ transporting non-gastric alpha-2 subunit and calcium channels), transcriptional regulators (Krüppel-like factor 4 and RHOF), and immune biomarkers [major basic protein (MBP), $TSLPR^+$ Th2A cells, and IgG4]. These insights support a shift toward precision medicine, with stratified approaches based on molecular phenotype.

In summary, ECM remodeling, immune activation, and epithelial dysfunction are tightly interconnected in EoE. Integrating genetic, epigenetic, and proteomic data enhances our understanding of disease mechanisms and highlights novel therapeutic and diagnostic strategies. Future research should validate ECM biomarkers across diverse populations and explore their role in personalized treatment algorithms.

Both Muir et al (97) and our systematic review highlight the central role of Th2 cytokines—particularly IL-13, IL-4, and IL-5—in driving epithelial barrier dysfunction, fibroblast activation, and ECM deposition in EoE. IL-13 promotes eotaxin-3 secretion, fibroblast migration, and collagen production, supported by upregulation of POSTN, arachidonate 15-lipoxygenase, and secreted frizzled-related protein 1, and downregulation of DSG1 and FLG.

TGF-β is a master regulator of fibrosis, inducing α-SMA, fibronectin, and periostin, and activating suppressor of mothers against decapentaplegic signaling Muir et al (97). Our review adds downstream targets like plasminogen activator inhibitor 1, lysyl oxidase, and connective tissue growth factor, which contribute to ECM crosslinking and stiffness.

EMT markers (Twist1, β-catenin) and loss of junctional proteins (claudin-7, E-cadherin) promote fibroblast-like behavior and collagen secretion. Fibroblasts respond to matrix stiffness by upregulating contractile markers, perpetuating fibrosis even in remission.

Immune cells—MC, basophils, iNKT cells, and macrophages—release profibrotic mediators (IL-18, LIGHT, amphiregulin). PIR-B acts as a brake on IL-13–driven eosinophil activation.

Oxidative stress and impaired nuclear factor erythroid 2-related factor 2 signaling exacerbate epithelial hyperplasia (97). Clinically, fibrosis assessment is challenging; endoluminal functional lumen imaging probe and serum biomarkers (MBP, CD73, IL-13, and LIGHT) offer promising noninvasive tools for disease monitoring and stratified therapy.

EoE is defined by epithelial barrier disruption, immune activation, fibroblast reprogramming, and mechanical stress. Future research should validate noninvasive biomarkers, refine endotype classification, and develop targeted therapies. Diagnostic criteria may evolve to include molecular profiling, with PPI responsiveness removed. Equitable access to biologics and transcriptomic tools is essential, especially for pediatric care. Early screening in at-risk children may prevent fibrostenotic progression. Long-term studies should assess fibrosis reversibility and treatment durability. Emerging targets—CD73, LIGHT–HVEM, IFN-γ, IgG4, and microbiome–immune interactions—offer promising directions for precision medicine and anti-remodeling strategies.

The expanding understanding of EoE at the molecular, immunologic, and epithelial levels has created an important opportunity to reimagine how the disease may be treated in the years ahead. Traditional therapies—dietary elimination, PPIs, and topical corticosteroids—have played a central role in management, yet they predominantly address downstream inflammation or symptomatic control rather than the upstream processes that initiate and perpetuate the disease. The approval of dupilumab, which inhibits IL-4 and IL-13 signaling through IL-4Rα, marked a pivotal shift toward mechanistically targeted therapy. However, the complex interplay of epithelial barrier dysfunction, type-2 cytokine networks, fibroblast activation, and stromal remodeling revealed by recent studies indicates that additional therapeutic avenues are necessary to achieve deeper and more durable disease modification.

Within this context, the therapeutic targets synthesized in Table 1 represent a diverse but interconnected set of molecular pathways that define the pathobiology of EoE. The IL-13 pathway stands out as a particularly compelling axis, not only because of the established role of IL-13 in epithelial remodeling and fibroblast activation but also because of accumulating clinical trial data involving agents such as cendakimab. These investigations have demonstrated that selective IL-13 blockade can normalize key components of the EoE transcriptome and improve both histologic and symptomatic disease activity. The strength of these data suggests that IL-13 signaling contributes centrally to disease progression and may serve as a biological anchor for future therapeutic strategies.

At the same time, other pathways identified in the literature expand the therapeutic landscape far beyond conventional type-2 inflammation. The discovery of TSLPR-expressing Th2A memory cells as potent amplifiers of cytokine production points to the possibility of intervening at the level of adaptive immune memory and chronic inflammation. Similarly, the LIGHT–HVEM axis offers insight into the mechanisms by which T-cell–derived signals orchestrate fibroblast activation and ECM deposition, processes fundamental to the development of fibrostenosis. Emerging evidence highlighting metabolic checkpoints—such as the impaired CD73-adenosine pathway in EoE fibroblasts—suggests that tissue remodeling may also be influenced by previously unrecognized metabolic dysregulation. The identification of epithelial proteases, including KLK5 acting through protease-activated receptor 2, introduces an additional layer of therapeutic potential by linking epithelial injury directly to inflammatory propagation. Finally, chemotactic and innate immune regulators such as the MIF–CXCR4 axis reveal how eosinophil recruitment and mast cell activation respond to signals beyond classical cytokine pathways.

Together, these findings underscore a more intricate and multifaceted view of EoE pathogenesis than previously appreciated. They demonstrate that EoE is not simply a disease of eosinophilic infiltration, but rather a dynamic disorder in which epithelial barrier biology, innate and adaptive immunity, stromal activation, and metabolic signaling collectively shape disease severity and chronicity. As a result, the therapeutic targets assembled in this review do more than highlight potential drug candidates; they offer a roadmap for a more precise and individualized approach to treatment.

The importance of pursuing these emerging targets in future research cannot be overstated. Each pathway represents an opportunity to intervene earlier in disease progression, to modulate specific components of the inflammatory cascade, and to prevent or reverse fibrostenotic transformation. A deeper exploration of these mechanisms will be essential for defining endotype-specific vulnerabilities, identifying biomarkers capable of predicting treatment response, and refining the classification of EoE beyond current clinical and histologic criteria. Moreover, because new therapies progress through preclinical and clinical development, there will be increasing need to understand how multiple pathogenic pathways converge within individual patients, and whether combination or sequential interventions may offer the most effective means of achieving sustained remission.

In summary, the therapeutic targets presented here provide a comprehensive and forward-looking foundation for future innovation in EoE research. They reflect a field rapidly evolving toward mechanistic precision, and they highlight the potential for transformative advances in disease management as upstream, epithelial, stromal, and metabolic pathways become integrated into the therapeutic armamentarium.

This systematic review synthesizes immunological, genetic, epithelial, and ECM mechanisms in EoE, confirming it as a multifactorial, antigen-driven disease. Epithelial barrier dysfunction, type 2 inflammation, and fibroblast reprogramming drive chronicity and fibrosis. Key contributors include IL-13, TSLP, RELM-α, LIGHT, and metabolic checkpoints like CD73. Genetic and epigenetic alterations (e.g., CAPN14, DOCK8, and miRNAs) underlie disease persistence. Noninvasive biomarkers and molecular profiling may refine diagnosis and guide therapy. Precision medicine approaches, early screening, and pediatric-focused research are essential. Future studies should explore IFN-γ, IgG4, Tregs, and microbiome–immune interactions to address treatment variability and uncover new therapeutic targets.

Strengths and limitations

This systematic review offers a mechanistic synthesis of EoE, integrating findings across immunological, epithelial, genetic, and ECM pathways. It includes 103 original studies—spanning human, animal, and *in vitro* models—selected using Population Exposure Comparator Outcome and Study design (PECOS) criteria and PRISMA 2020 guidelines. Risk of bias was assessed using validated tools (Newcastle–Ottawa Scale, SYRCLE, and a custom *in vitro* checklist), ensuring methodological rigor.

Multiomics data (transcriptomic, proteomic, epigenomic, single-cell RNA sequencing [scRNA-seq]) enabled high-resolution insights into disease heterogeneity. Emerging targets include TSLPR$^+$ Th2A cells, LIGHT–HVEM, CD73, and IL-13Rα1, with biomarkers like MBP, periostin, and IgG4-producing TFH cells. Inclusion of pediatric and adult cohorts supports age-specific insights and early intervention strategies.

However, limitations persist. Methodological diversity complicates cross-study comparisons. Most studies are short-term or case-control, lacking longitudinal data on fibrosis progression and treatment durability. Non-Th2 pathways (e.g., IFN-γ, IgG4, and Tregs) remain underexplored. Biomarker validation is incomplete, and standardization is lacking.

Microbiome–immune interactions and early-life exposures (e.g., antibiotics and C-section) are recognized but poorly understood. Functional consequences of genetic variants and epigenetic regulation (e.g., DNA methylation and miRNAs) require further mapping.

A notable limitation of this review concerns the use of a custom-designed quality assessment checklist for *in vitro* mechanistic studies. While this tool was constructed based on widely accepted experimental standards—such as transparent reporting of cell line origin, authentication procedures, inclusion of biological and technical replicates, appropriate controls, methodological clarity, and statistical rigor—it has not undergone formal external validation. Consequently, the absence of standardized benchmarks may introduce an element of subjectivity during scoring. This subjectivity may affect the consistency with which methodological strengths and weaknesses are interpreted across studies, despite efforts to mitigate bias through independent scoring by multiple reviewers and consensus discussion.

Furthermore, the use of a nonvalidated tool limits comparability with other systematic reviews that evaluate *in vitro* evidence using different or partially overlapping criteria. Because no universally endorsed methodological quality assessment tool exists for *in vitro* experiments, current reviews often rely on heterogeneous or internally developed instruments, complicating cross-study synthesis and the establishment of consistent quality thresholds. This variability underscores a broader methodological challenge in the field: although *in vitro* studies provide indispensable mechanistic insights—particularly in molecular, epithelial, and immunologic research—their appraisal lacks the structured frameworks available for clinical and animal research.

This limitation highlights the need for future initiatives aimed at standardizing and validating quality assessment tools specifically tailored to *in vitro* mechanistic research. The development of a consensus-based, externally validated checklist would enhance methodological rigor, improve reproducibility, and facilitate meaningful comparisons across systematic reviews. Such efforts would also support the integration of *in vitro* evidence into translational frameworks with greater confidence, ultimately strengthening mechanistic inference in fields such as EoE.

Review limitations include language bias (English-only), potential publication bias, and manual study selection without automation tools. The custom *in vitro* checklist lacks external validation. Restricting the search to 2014–2025 may exclude foundational studies. Exclusion of qualitative research may limit contextual understanding.

Despite these limitations, the review supports a paradigm shift toward precision medicine in EoE, emphasizing biomarker-guided diagnosis, stratified therapy, and early screening. Future research should prioritize longitudinal studies, pediatric cohorts, and integrative multiomics to refine disease modeling and improve outcomes.

CONFLICTS OF INTEREST

Guarantor of the article: Massimo Claudio Fantini, MD, PhD.
Specific author contributions: J.L.: planning and conducting the study, collecting and interpreting data, and drafting the manuscript.

A.D.: planning and conducting the study, collecting and interpreting data, and drafting the manuscript. S.F.: planning and conducting the study, collecting and interpreting data. A.F.: manuscript revisions. N.L.: drafting the manuscript. S.O.: planning the study, manuscript revisions. M.C.F.: planning the study, manuscript revisions, supervision.

Financial support: None to report.

Potential competing interests: None to report.

REFERENCES

1. Lutzu N, Favale A, Demurtas M, et al. Eosinophilic esophagitis in the "atopic march": dupilumab as an "umbrella" strategy for multiple coexisting atopic diseases. Front Med (Lausanne). 2024;11:1513417.
2. de Bortoli N, Visaggi P, Penagini R, et al. The 1st EoETALY consensus on the diagnosis and management of eosinophilic esophagitis—definition, clinical presentation and diagnosis. Dig Liver Dis 2024;56(6):951–63.
3. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated international consensus diagnostic criteria for eosinophilic esophagitis: Proceedings of the AGREE conference. In: Gastroenterology. W.B. Saunders, 2018, pp 1022–33.e10.
4. Khokhar D, Marella S, Idelman G, et al. Eosinophilic esophagitis: Immune mechanisms and therapeutic targets. Clin Exp Allergy 2022;52(10):1142–56.
5. Sato S, Nagakura K, Yanagida N, et al. Current perspective on allergen immunotherapy for food allergies. Allergol Int 2024;73(4):501–14.
6. Warners MJ, van Rhijn BD, Verheij J, et al. Disease activity in eosinophilic esophagitis is associated with impaired esophageal barrier integrity. Am J Physiol 2017;313(3):G230–G238.
7. Barchi A, Mandarino FV, Yacoub MR, et al. From pathogenesis to treatment: Targeting type-2 inflammation in eosinophilic esophagitis. Biomolecules 2024;14(9):1080.
8. Dellon ES, Hirano I. Epidemiology and natural history of eosinophilic esophagitis. Gastroenterology 2018;154(2):319–32.e3.
9. Spergel JM, Rothenberg ME, Collins MH, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: Results of a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 2012; 129(2):456–63.e3.
10. Dellon ES, Peterson KA, Mitlyng BL, et al. Mepolizumab for treatment of adolescents and adults with eosinophilic oesophagitis: A multicentre, randomised, double-blind, placebo-controlled clinical trial. Gut 2023; 72(10):1828–37.
11. Rothenberg ME, Dellon ES, Collins MH, et al. Eosinophil depletion with benralizumab for eosinophilic esophagitis. New Engl J Med 2024;390(24): 2252–63.
12. Le Floc'h A, Allinne J, Nagashima K, et al. Dual blockade of IL-4 and IL-13 with dupilumab, an IL-4Rα antibody, is required to broadly inhibit type 2 inflammation. Allergy 2020;75:1188–204.
13. Rethlefsen ML, Kirtley S, Waffenschmidt S, et al. PRISMA-S: An extension to the PRISMA statement for reporting literature searches in systematic reviews. Syst Rev 2021;10(1):39.
14. Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-analyses. Ottawa Hospital Research Institute, Ottawa, 2011, Vol 2, pp 1–12.
15. Ma LL, Wang YY, Yang ZH, et al. Methodological quality (risk of bias) assessment tools for primary and secondary medical studies: What are they and which is better?. Mil Med Res 2020;7(1):7.
16. Hooijmans CR, Rovers MM, De Vries RBM, et al. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14(1):43.
17. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
18. Avlas S, Shany G, Rhone N, et al. Eosinophilic esophagitis is critically mediated by IL-13 signaling via IL13Ra1. J Allergy Clin Immunol 2022; 149(2):AB52.
19. Rochman M, Travers J, Abonia JP, et al. Synaptopodin is upregulated by IL-13 in eosinophilic esophagitis and regulates esophageal epithelial cell motility and barrier integrity. JCI Insight 2017;2(20):e96789.
20. Manresa MC, Miki H, Miller J, et al. A deficiency in the cytokine TNFSF14/LIGHT limits inflammation and remodeling in murine eosinophilic esophagitis. J Immunol 2022;209(12):2341–51.
21. Jumabay M, Abud EM, Okamoto K, et al. Eosinophilic esophagitis drives tissue fibroblast regenerative programs toward pathologic dysfunction. J Allergy Clin Immunol 2025;155(4):1333–45.
22. De Souza HS, Tortori CA, Lintomen L, et al. Macrophage migration inhibitory factor promotes eosinophil accumulation and tissue remodeling in eosinophilic esophagitis. Mucosal Immunol. 2015;8(5):1154–65.
23. Yadavalli CS, Upparahalli Venkateshaiah S, Kumar S, et al. Allergen-induced NLRP3/caspase1/IL-18 signaling initiate eosinophilic esophagitis and respective inhibitors protect disease pathogenesis. Commun Biol 2023;6(1):763.
24. Ptaschinski C, Zhu D, Fonseca W, et al. Stem cell factor inhibition reduces Th2 inflammation and cellular infiltration in a mouse model of eosinophilic esophagitis. Mucosal Immunol 2023;16(5):727–39.
25. Ben Baruch-Morgenstern N, Mingler MK, Stucke E, et al. Paired Ig-like receptor B inhibits IL-13–driven eosinophil accumulation and activation in the esophagus. J Immunol 2016;197(3):707–14.
26. Odiase E, Zhang X, Chang Y, et al. In esophageal squamous cells from eosinophilic esophagitis patients, Th2 cytokines increase eotaxin-3 secretion through effects on intracellular calcium and a non-gastric proton pump. Gastroenterology 2021;160(6):2072–88.e6.
27. Lal M, Burk CM, Gautam R, et al. Interferon-γ signaling in eosinophilic esophagitis has implications for epithelial barrier function and programmed cell death [Internet]; 2024. Accessed February 1, 2026. http://biorxiv.org/lookup/doi/10.1101/2024.01.26.577407
28. Lozano-Ojalvo D, Chen X, Kazmi W, et al. Differential T follicular helper cell phenotypes distinguish IgE-mediated milk allergy from eosinophilic esophagitis in children. J Allergy Clin Immunol 2025;155(3):909–22.
29. Zhang W, Jiang Y, Shang Z, et al. The methodological quality of animal studies: A cross-sectional study based on the SYRCLE's risk of bias tool [Internet], 2019. Accessed February 2, 2026. http://biorxiv.org/lookup/doi/10.1101/701110
30. Sherrill JD, Kc K, Wu D, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. Mucosal Immunol 2014;7(3):718–29.
31. Hoelz H, Faro T, Frank ML, et al. Persistent desmoglein-1 downregulation and periostin accumulation in histologic remission of eosinophilic esophagitis. J Allergy Clin Immunol 2024;155(2):505–19.
32. Davis BP, Stucke EM, Khorki ME, et al. Eosinophilic esophagitis-linked calpain 14 is an IL-13-induced protease that mediates esophageal epithelial barrier impairment. JCI Insight 2016;1(4):e86355.
33. Nguyen N, Fernando SD, Biette KA, et al. TGF-β1 alters esophageal epithelial barrier function by attenuation of claudin-7 in eosinophilic esophagitis. Mucosal Immunol 2018;11(2):415–26.
34. Wu L, Oshima T, Li M, et al. Filaggrin and tight junction proteins are crucial for IL-13-mediated esophageal barrier dysfunction. Am J Physiol 2018;315(3):G341–50.
35. Oshima T, Li M, Tomita T, et al. Filaggrin and tight junction proteins are crucial for IL-13-mediated esophageal barrier dysfunction. Am J Physiol Gastrointest Liver Physiol 2018;315(3):G341–50.
36. Garcia E, Ladak Z, Landry T, et al. Epithelial-mesenchymal transition, regulated by β-catenin and twist, leads to esophageal wall remodeling in pediatric eosinophilic esophagitis. PLoS One 2022; 17(3):e0264622.
37. Mavi P, Niranjan R, Dutt P, et al. Allergen-induced resistin-like molecule-α promotes esophageal epithelial cell hyperplasia in eosinophilic esophagitis. Am J Physiol 2014;307(5):G499–G507.
38. Jiang M, Ku W-Y, Zhou Z, et al. BMP-driven NRF2 activation in esophageal basal cell differentiation and eosinophilic esophagitis. J Clin Invest 2015;125(4):1557–68.
39. Azouz NP, Klingler AM, Pathre P, et al. Functional role of kallikrein 5 and proteinase-activated receptor 2 in eosinophilic esophagitis. Sci Transl Med 2020;12(545):eaaz7773.
40. Shaverdashvili K, Padlo J, Weinblatt D, et al. KLF4 activates NFκB signaling and esophageal epithelial inflammation via the rho-related GTP-binding protein RHOF. PLoS One 2019;14(4):e0215746.
41. Ryan S, Crowe L, Almeida Cruz SN, et al. Metabolic dysfunction mediated by HIF-1α contributes to epithelial differentiation defects in eosinophilic esophagitis. J Allergy Clin Immunol 2024;154(6):1472–88.
42. Judd LM, Heine RG, Menheniott TR, et al. Elevated IL-33 expression is associated with pediatric eosinophilic esophagitis, and exogenous IL-33 promotes eosinophilic esophagitis development in mice. Am J Physiol 2016;310(1):G13–G25.
43. Johnston LK, Bryce PJ. Understanding interleukin 33 and its roles in eosinophil development. Front Med (Lausanne) 2017;4:51.
44. Pyon GC, Masuda MY, Putikova A, et al. Tissue-specific inducible IL-33 expression elicits features of eosinophilic esophagitis. J Allergy Clin Immunol 2024;154(6):1545–53.e2.

45. Venkateshaiah SU, Kandikattu HK, Mishra A. Significance of interleukin (IL)-15 in IgE associated eosinophilic esophagitis (EoE). Int J Basic Clin Immunol 2019;2:1–12.
46. Eden K, Rothschild DE, McDaniel DK, et al. Noncanonical NF-κB signaling and the essential kinase NIK modulate crucial features associated with eosinophilic esophagitis pathogenesis. Dis Models Mech 2017;10(12):1517–27.
47. Rochman Y, Kotliar M, Morgenstern NBB, et al. TSLP shapes the pathogenic responses of memory CD4+ T cells in eosinophilic esophagitis. Sci Signal 2023;16(802):eadg6360.
48. Kleuskens MTA, Bek MK, Al HalabiY, et al. Mast cells disrupt the function of the esophageal epithelial barrier. Mucosal Immunol. 2023; 16(5):567–77.
49. Ben-Baruch Morgenstern N, Ballaban AY, Wen T, et al. Single-cell RNA sequencing of mast cells in eosinophilic esophagitis reveals heterogeneity, local proliferation, and activation that persists in remission. J Allergy Clin Immunol 2022;149(6):2062–77.
50. Zhang S, Shoda T, Aceves SS, et al. Mast cell-pain connection in eosinophilic esophagitis. Allergy 2022;77(6):1895–9.
51. Merves J, Chandramouleeswaran PM, Benitez AJ, et al. Altered esophageal histamine receptor expression in eosinophilic esophagitis (EoE): Implications on disease pathogenesis. PLoS One 2015;10(2): e0114831.
52. Ebrahimi S, Khatami S, Mahjoub FE, et al. Detection of eosinophilic cell-free granules based on expression of CCR3 and MBP markers in esophageal biopsy specimens of patients with suspected and confirmed eosinophilic esophagitis. Pediatr Gastroenterol Hepatol Nutr 2025;28(1):19.
53. Benson TM, Markey GE, Hammer JA, et al. CSF1-dependent macrophage support matrisome and epithelial stress-induced keratin remodeling in eosinophilic esophagitis. Mucosal Immunol 2025;18(1):105–20.
54. Pronio A, Covotta F, Pallotta L, et al. Eosinophilic esophagitis: Cytokines expression and fibrotic markers in comparison to celiac disease. Diagnostics 2022;12(9):2092.
55. Strauss Starling A, Ren Y, Li H, et al. Reducing eosinophil counts in eosinophilic esophagitis in children is associated with reduction in later stricture development. Am J Gastroenterol 2024;119(10):2002–9.
56. Wu J, Duan C, Han C, et al. Identification of CXC chemokine receptor 2 (CXCR2) as a novel eosinophils-independent diagnostic biomarker of pediatric eosinophilic esophagitis by integrated bioinformatic and machine-learning analysis. ImmunoTargets Ther 2024;13:55–74.
57. Marella S, Sharma A, Ganesan V, et al. IL-13–induced STAT3-dependent signaling networks regulate esophageal epithelial proliferation in eosinophilic esophagitis. J Allergy Clin Immunol 2023;152(6):1550–68.
58. D'mello RJ, Caldwell JM, Azouz NP, et al. LRRC31 is induced by IL-13 and regulates kallikrein expression and barrier function in the esophageal epithelium. Mucosal Immunol 2016;9(3):744–56.
59. Wright BL, Nguyen N, Shim KP, et al. Increased GATA-3 and T-bet expression in eosinophilic esophagitis versus gastroesophageal reflux disease. J Allergy Clin Immunol 2018;141(5):1919–21.e5.
60. Ding J, Garber JJ, Uchida A, et al. An esophagus cell atlas reveals dynamic rewiring during active eosinophilic esophagitis and remission. Nat Commun 2024;15(1):3344.
61. Kaneko T, Iwamura C, Kiuchi M, et al. Amphiregulin-producing TH2 cells facilitate esophageal fibrosis of eosinophilic esophagitis. J Allergy Clin Immunol Glob. 2024;3(3):100287.
62. Ruffner MA, Hu A, Dilollo J, et al. Conserved IFN signature between adult and pediatric eosinophilic esophagitis. J Immunol 2021;206(6):1361–71.
63. Chen J, Oshima T, Tomita T, et al. Regulatory T cells are increased and correlate with mast cells in eosinophilic esophagitis. J Neurogastroenterol Motil 2024;30(1):29–37.
64. Ayaki M, Manabe N, Nakamura J, et al. A retrospective study of the differences in the induction of regulatory T cells between adult patients with eosinophilic esophagitis and gastroesophageal reflux disease. Dig Dis Sci 2022;67(10):4742–8.
65. Manresa MC, Chiang AWT, Kurten RC, et al. Increased production of LIGHT by T cells in eosinophilic esophagitis promotes differentiation of esophageal fibroblasts toward an inflammatory phenotype. Gastroenterology 2020;159(5):1778–92.e13.
66. Kottyan LC, Maddox A, Braxton JR, et al. Genetic variants at the 16p13 locus confer risk for eosinophilic esophagitis. Genes Immun 2019;20(4): 281–92.
67. Ávila-Castellano R, García-Lozano JR, Cimbollek S, et al. Genetic variations in the TLR3 locus are associated with eosinophilic esophagitis. United Eur Gastroenterol J 2018;6(3):349–57.
68. Sherrill JD, Kiran KC, Wang X, et al. Whole-exome sequencing uncovers oxidoreductases DHTKD1 and OGDHL as linkers between mitochondrial dysfunction and eosinophilic esophagitis. JCI Insight 2018;3(8):e99922.
69. Strisciuglio C, Payne F, Nayak K, et al. Disease-associated DNA methylation signatures in esophageal biopsies of children diagnosed with eosinophilic esophagitis. Clin Epigenetics 2021;13(1):81.
70. Jensen ET, Langefeld CD, Zimmerman KD, et al. Epigenetic methylation in eosinophilic esophagitis: Molecular ageing and novel biomarkers for treatment response. Clin Exp Allergy 2020;50(12):1372–80.
71. Massimino L, Barchi A, Mandarino FV, et al. A multi-omic analysis reveals the esophageal dysbiosis as the predominant trait of eosinophilic esophagitis. J Transl Med 2023;21(1):46.
72. Kim S, Ben-Baruch MN, Osonoi K, et al. Nonepithelial gene expression correlates with symptom severity in adults with eosinophilic esophagitis. J Allergy Clin Immunol 2024;12(12):3346–55.e1.
73. Keever-Keigher MR, Harvey L, Williams V, et al. Genomic insights into pediatric intestinal inflammatory and eosinophilic disorders using single-cell RNA-sequencing. Front Immunol 2024;15:1420208.
74. Jacobse J, Brown R, Revetta F, et al. A synthesis and subgroup analysis of the eosinophilic esophagitis tissue transcriptome. J Allergy Clin Immunol 2024;153(3):759–71.
75. Giannetti M, Schroeder HA, Zalewski A, et al. Dysregulation of the Wnt pathway in adult eosinophilic esophagitis. Dis Esophagus 2015;28(8): 705–10.
76. Morrison HA, Hoyt KJ, Mounzer C, et al. Expression profiling identifies key genes and biological functions associated with eosinophilic esophagitis in human patients. Front Allergy 2023;4:1239273.
77. Hiremath G, Choksi Y, Correa H, et al. Children with eosinophilic esophagitis non-responsive to combination therapy have distinct esophageal transcriptomic and microbiome profile. Allergy 2024;79(10): 2798–811.
78. Zahm AM, Menard-Katcher C, Benitez AJ, et al. Pediatric eosinophilic esophagitis is associated with changes in esophageal microRNAs [Internet]. Am J Physiol 2014;307(8):803–12.
79. Grueso-Navarro E, Rodríguez-Alcolado L, Arias-González L, et al. MicroRNAs in plasma-derived extracellular vesicles as non-invasive biomarkers for eosinophilic esophagitis. Int J Mol Sci 2025;26(2):639.
80. Markey GE, Ryan S, Furuta GT, et al. Hypoxia-inducible microRNA-155 negatively regulates epithelial barrier in eosinophilic esophagitis by suppressing tight junction claudin-7. FASEB J 2024;38(1):e23358.
81. Wen T, Dellon ES, Moawad FJ, et al. Transcriptome analysis of proton pump inhibitor-responsive esophageal eosinophilia reveals proton pump inhibitor-reversible allergic inflammation. J Allergy Clin Immunol 2015; 135(1):187–97.e4.
82. Zdanowicz K, Rycyk A, Reszec J, et al. Evaluation of MMP-1 and TIMP-1 expression in eosinophilic esophagitis and their usefulness in making therapeutic decisions—A preliminary study. Pediatria Polska 2023;98(4):293–9.
83. Clevenger MH, Karami AL, Carlson DA, et al. Suprabasal cells retain progenitor cell identity programs in eosinophilic esophagitis–driven basal cell hyperplasia. JCI insight 2023;8(19):e171765.
84. Cheng E, Zhang X, Wilson KS, et al. JAK-STAT6 pathway inhibitors block eotaxin-3 secretion by epithelial cells and fibroblasts from esophageal eosinophilia patients: Promising agents to improve inflammation and prevent fibrosis in EOE. PLoS One 2016;11(6):e0157376.
85. Muir AB, Dods K, Henry SJ, et al. Eosinophilic esophagitis-associated chemical and mechanical microenvironment shapes esophageal fibroblast behavior. J Pediatr Gastroenterol Nutr 2016;63(2):200–9.
86. Li T, Salomon M, Shao L, et al. Differential contributions of fibroblast subpopulations to intercellular communication in eosinophilic esophagitis. Biology (Basel) 2024;13(7):461.
87. Frazzoni M, Frazzoni L, De Bortoli N, et al. Response of eosinophilic oesophagitis to proton pump inhibitors is associated with impedance-pH parameters implying anti-reflux mechanism of action. Aliment Pharmacol Ther 2021;53(11):1183–9.
88. Wheeler JC, Vanoni S, Zeng C, et al. 17β-Estradiol protects the esophageal epithelium from IL-13–induced barrier dysfunction and remodeling. J Allergy Clin Immunol 2019;143(6):2131–46.
89. Brusilovsky M, Rochman M, Shoda T, et al. Vitamin D receptor and STAT6 interactome governs oesophageal epithelial barrier responses to IL-13 signalling. Gut 2023;72(5):834–45.
90. Cameron BA, Anderson CW, Jensen ET, et al. Vitamin D levels as a potential modifier of eosinophilic esophagitis severity in adults. Dig Dis Sci 2024;69(4):1287–92.

91. Thomas ME, Markowitz JE, Arwood AC, et al. An LC-MS/MS method for the quantification of 3-bromotyrosine in plasma from patients diagnosed with eosinophilic esophagitis. Anal Methods 2024;16(38): 6509–16.
92. O'Shea KM, Aceves SS, Dellon ES, et al. Pathophysiology of eosinophilic esophagitis. Gastroenterology 2018;154(2):333–45.
93. McGowan EC, Singh R, Katzka DA. Barrier dysfunction in eosinophilic esophagitis. Curr Gastroenterol Rep 2023;25(12):380–9.
94. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions?. Nat Rev Immunol 2021;21(11):739–51.
95. Racca F, Pellegatta G, Cataldo G, et al. Type 2 inflammation in eosinophilic esophagitis: From pathophysiology to therapeutic targets. Front Physiol 2022;12:815842.
96. Hsieh LY, Chiang AWT, Duong LD, et al. A unique esophageal extracellular matrix proteome alters normal fibroblast function in severe eosinophilic esophagitis. J Allergy Clin Immunol 2021;148(2): 486–94.
97. Muir AB, Wang JX, Nakagawa H. Epithelial-stromal crosstalk and fibrosis in eosinophilic esophagitis. J Gastroenterol 2019;54(1):10–8.

Open Access This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.