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Genetic dissection of eosinophilic esophagitis provides insight into disease pathogenesis and treatment strategies

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Activity Objectives

1. To identify the epidemiology of eosinophilic esophagitis (EoE).
2. To distinguish EoE from gastroesophageal reflux disease (GERD).
3. To apply knowledge of susceptibility to clinical cases.
4. To apply genetic knowledge to emerging diagnostics and therapeutics.
5. To integrate results from genetic susceptibility loci studies with gene expression changes in patients with EoE.

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Eosinophilic esophagitis (EoE) is a chronic inflammatory disorder of the esophagus that is compounded by both genetic predisposition and aberrant responses to environmental antigens, particularly those that are food derived. Data have indicated a unique transcriptional response *in vivo* that defines EoE and that appears to be partially attributable to the T_H2 cytokine IL-13. Moreover, a number of genetic risk variants in proinflammatory and epithelial cell genes associate with EoE susceptibility, demonstrating novel heritable mechanisms that contribute to disease risk. Here we discuss recent advances in our understanding of the intrinsic (genetic) and extrinsic (environmental) components that illustrate the complex nature of EoE. (J Allergy Clin Immunol 2011;128:23-32.)

Key words: *Eosinophilic esophagitis, genetics, candidate gene, genome-wide association, polymorphism*

The phenomenon of esophageal eosinophilia can be traced through the published literature as far back as 1962.[1] Since these early reports, much progress has been made at the molecular and clinical levels to tease apart the intricacies that distinguish eosinophilic esophagitis (EoE) from other inflammatory disorders, including gastroesophageal reflux disease (GERD). As the prevalence of these diseases has increased since the late 1990s, the need for improved diagnoses, both from a therapeutic and research standpoint, has arisen. Moreover, the high degree of overlap in presenting symptoms in EoE and GERD have been problematic clinically and have necessitated the establishment of diagnostic criteria to differentiate the 2 diseases.

In 2007, the First International Gastrointestinal Research Symposium published initial guidelines for the clinical diagnosis of EoE based on the symptomatology and histology of the disease.[2] More recently, updated consensus recommendations from a panel of allergists, pathologists, and gastroenterologists have been stated (see Liacouras et al[3] in this issue of the *Journal*). These recommendations emphasize that EoE is a chronic,

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Terms in boldface and italics are defined in the glossary on page 24.

Abbreviations used
AD: Atopic dermatitis
CRLF2: TSLP receptor singe nucleotide polymorphism
DSG1: Desmoglein-1 gene
EDC: Epidermal differentiation complex
EoE: Eosinophilic esophagitis
FLG: Filaggrin gene
GERD: Gastroesophageal reflux disease
GWAS: Genome-wide association study
hpf: High-powered field
POSTN: Periostin gene
SNP: Single nucleotide polymorphism
TSLP: Thymic stromal lymphopoietin
WDR36: WD repeat domain 36 gene

antigen-driven clinicohistopathological disorder that is causatively and epidemiologically distinct from GERD. Clinically, EoE is characterized by a spectrum of symptoms indicative of esophageal dysfunction. Pathologically, 1 or more esophageal mucosal biopsy specimens show eosinophil-predominant inflammation in excess of 15 intraepithelial eosinophils per high-powered field (hpf). The disease is isolated to the esophagus, and other causes of esophageal eosinophilia should be excluded. The peak age of EoE diagnosis occurs within the first 3 years of life,[4] most likely resulting from antigen hypersensitivity as solid foods are introduced, although diagnosis in adults is also common. Disease remission typically occurs with treatment, which might include dietary exclusion, topical corticosteroids, or both.[3] The symptomatology of EoE, if untreated, follows a trend according to patient age. In pediatric patients, these symptoms begin as difficulty feeding and vomiting and can result in failure to thrive.[5,6] In adolescent and adult patients, abdominal pain, dysphagia, and food impaction are the chief presentations of the disease.[6,7] Endoscopic examination has identified common esophageal abnormalities associated with EoE, such as ***linear furrowing*** with loss of vascularity, ring-like structures, and the presence of ***white exudate*** on the esophageal epithelium.[2,8] Histologically, the esophageal epithelium exhibits extensive ***basal zone hyperplasia*** with ***papillary elongation*** and fibrosis within the lamina propria and accumulation of eosinophils, B lymphocytes,[9] $CD4^+$ and $CD8^+$ T lymphocytes,[10] regulatory T cells,[11] and mast cells.[12-14]

In addition to being resistant to acid neutralization therapy, another distinguishing feature of EoE is the high rate of concurrent atopy. Studies have indicated a prominent role for food allergies in patients with EoE, with published frequencies ranging from 46% to 79% within the EoE population.[4,5,15,16] In comparison with the estimated 22% of patients with peanut allergy who have tolerance later in life,[17] only a very small percentage (<10%) of patients with EoE have tolerance to food antigens, as defined by sustained disease remission,[15] demonstrating the

GLOSSARY

BASAL ZONE HYPERPLASIA, PAPILLARY ELONGATION: Histologic findings in patients with EoE that are caused by active basal cell proliferation and increased extension of the vascular papillae and subepithelial lamina propria into the epithelial space. Other typical histologic features of EoE include dilated intercellular spaces and lamina propria fibrosis.

CCR3, EOTAXINS: CCR3 binds eotaxins. Although eotaxin-1 and eotaxin-2 target eosinophils to the lung and lower gastrointestinal tract, eotaxin-3 is present only in human subjects and functions as a chemoattractant for esophageal eosinophils.

CHROMATIN IMMUNOPRECIPITATION (CHIP): ChIP technology uses antibodies to precipitate a protein bound to DNA. The bound DNA sequence can be analyzed to look for target sequences for transcription factors or histone-associated regions of DNA.

EPIGENETICS: The study of changes in DNA configuration that allow for changes in gene expression independent of sequence changes. For example, DNA methylation can cause DNA closing, making specific genetic regions inaccessible to RNA polymerase and transcription factors, thus silencing gene expression. Histone acetylation can allow DNA to open and can increase transcription. CREB-binding protein (CBP, p300) is a histone acetylase implicated in EoE pathogenesis.

FILAGGRIN, INVOLUCRIN: Involucrin is present in the cytoplasm of keratinocytes and is cross linked to cell membrane proteins through transglutaminase. This allows the formation of a strong epithelial barrier and decreases skin invasion by microorganisms. The usual function of filaggrin is to function as a natural moisturizing factor. Loss of function in the filaggrin gene causes ichthyosis vulgaris and predisposes to eczema, asthma, and EoE.

GENOME-WIDE ASSOCIATION STUDY (GWAS): GWASs use gene chip technology and bioinformatics to analyze the human genome for SNPs in diseased and nondiseased states. Haplotypes (in blocks) that vary between diseased and nondiseased subjects are considered to be associated with the disease state.

IL-13: IL-13 is a T_H2 cell–derived interleukin capable of inducing multiple aspects of eosinophil-associated tissue remodeling. IL-13 overexpression in target organs of transgenic animals is associated with pulmonary, esophageal, and cutaneous fibrosis, as well as angiogenesis.

LAMBDA (λ): The familial relative risk is defined by the statistic λ, which assesses the risk of disease in a subject with a diseased first-degree biological relative to the risk in the population at large. The larger the λ value, the stronger the genetic effect is on the disease.

LINEAR FURROWING, WHITE EXUDATES: Typical endoscopic findings in patients with EoE include esophageal lichenification, linear furrowing, pallor, and white exudates/plaques (histologically comprised of eosinophils), strictures, and concentric rings caused by motility or fibrosis.

SINGLE NUCLEOTIDE POLYMORPHISM (SNP): Genetic variant in a single nucleotide that might be normally present in the population and associated with risk for certain complex polygenic diseases.

THYMUS AND ACTIVATION-REGULATED CHEMOKINE (TARC): TARC, also known as CCL17, induces migration of $CCR4^+$ T cells to the skin in patients with eczema, and its expression can be increased in the periphery of some patients with EoE.

TRANSCRIPTOMES, PROTEOMES: Gene expression microarray defines transcriptional differences among subjects with and without a disease state. Gene chip technology and bioinformatics are used to analyze the level of gene expression (referred to as the "transcriptome") across the genome in diseased versus control populations. Proteomes are the protein expression pattern of an organism. Proteomic analysis can use techniques, such as 2-dimensional gel analysis and peptide sequencing or antibody arrays, to find both previously known and unknown proteins associated with a particular disease state.

TGF-β: TGF-β is produced by epithelial cells and inflammatory cells, including eosinophils, and mast cells and has profibrotic effects. TGF-β1, 2, and 3 reside on distinct chromosomes but use the same signaling pathway and receptors.

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chronic nature of EoE. The most effective therapies currently used to manage EoE are food antigen avoidance or swallowed glucocorticoid treatment.[18] Although these treatments can reduce or eliminate disease symptoms, relapse commonly occurs after reintroduction of allergens or discontinuation of treatment, suggesting that food antigen hypersensitivity is a fundamental feature of EoE. A number of empiric and test-based food elimination trials have further implicated food hypersensitivity in the population with EoE; however, the high variability and low predictive value of skin prick tests and serum IgE measurements as demonstrated in these studies suggest that the clinical utility for standardized assessment for food-specific reactivity in patients with EoE remains to be determined (see Chehade and Aceves[16] for a thorough review of clinical food trials in patients with EoE). Other atopic diseases, such as atopic dermatitis (AD), asthma, and allergic rhinitis, are also common in the population with EoE.[5] Although these diseases are largely mediated by enhanced sensitivity to aeroallergens, the exacerbated T_H2 inflammation and tissue remodeling that occurs within the affected tissues indicate shared mechanisms of disease with EoE.

EPIDEMIOLOGY

With the expansion of EoE cases reported worldwide, multiple studies have aimed to establish a baseline prevalence of EoE and determine whether disease incidence has increased. From 2000-2003, the estimated prevalence of EoE in a pediatric population was approximately 4 in 10,000, with an incidence rate of 0.9 to 1.3 in 10,000 new cases per year.[5] A similar prevalence (approximately 2 cases per 10,000) and incidence rate (1.4 per 100,000) during a 16-year period were observed in an adult Swiss cohort.[19] A study of 1000 esophageal biopsy specimens from a randomized Swedish cohort showed a disease prevalence of 0.4%, as defined by using an esophageal eosinophil level of greater than 20/hpf.[20] However, in a retrospective study examining esophageal biopsy specimens from 666 pediatric patients given diagnoses of esophagitis from 1982-1999, data suggest that although the prevalence of EoE was increasing, the incidence of disease was relatively stable, despite the marked increase in esophagogastroduodenoscopies during that time period.[21] By using the current recommended eosinophil threshold for EoE diagnosis (>15/hpf), 198 of these patients had sufficient eosinophils levels, with many having histologic indications of basal layer expansion and lamina propria fibrosis to indicate a retrospective diagnosis of EoE.[21] These findings suggest that enhanced disease recognition, rather than a true increase in disease incidence, underlies the emergence of EoE within the last decade. Notably, dysphagia was significantly associated with retrospective EoE, and the ancestry and sex of these cases were similar to those currently reported, with the majority being white (81%) and male (72%).[21]

An interesting questionnaire-based study on the geographic distribution of EoE across the United States has indicated higher disease prevalence in urbanized areas, with a higher concentration of EoE observed in the northeastern states. Here the estimated prevalence of EoE in the entire United States was 52 per 100,000.[22] A similar trend of a higher EoE prevalence in urban areas was shown to be independent of race, indicating that environment has an equally important contribution as genetics to EoE risk.[23] Certainly the high incidence of asthma among urban populations, as demonstrated by multiple groups, has garnered significant attention[24] and given credence to the general hypothesis that increased exposure to aeroallergens is a predisposing factor. The findings by Spergel et al[22] and Franciosi et al,[23] which define these EoE "hot zones" within urban settings, implicate a similar effect of socioeconomic factors in EoE susceptibility.

GENETIC HERITABILITY

An underlying genetic predisposition to EoE has been proposed by multiple groups that show a disproportionate prevalence of disease in white subjects and male subjects and within families of affected subjects.[15,23] For instance, data over a 14-year period demonstrated that 90% of the patients with EoE were white and 75% were male.[15] Reports of a familial occurrence of EoE and esophageal dilatation in 6.8% and 9.7% of patients with EoE, respectively, suggest that the prevalence of EoE and associated esophageal dysfunction is high among related subjects.[5] Furthermore, the increased risk of EoE among siblings is dramatic when compared with other disorders. For instance, the estimated sibling recurrence risk among siblings of patients with EoE ($\lambda_s = \sim 80$) is markedly higher compared with that of siblings with other atopic diseases with familial inheritance patterns, such as asthma ($\lambda_s = \sim 2$).[25] However, despite this strong familial inheritance, comparison of familial with sporadic cases of EoE showed no difference in esophageal pathology (with the exception of linear furrowing) and gene expression profiles.[26] Nonetheless, genetic predisposition and family history likely have a significant role in EoE susceptibility, and thus detailed family histories are paramount when encountering these patients.

TRANSCRIPTOME ANALYSIS

A major step toward the molecular mapping of EoE was achieved when gene expression profiling of patients' esophageal biopsy specimens showed a remarkable transcript signature that distinguishes patients with EoE from healthy control subjects and patients with chronic esophagitis.[27] Altered expression of approximately 574 genes comprises this EoE "***transcriptome***," which exhibits a high level of conservation among patients' sex, age, and atopic history and strongly correlates with esophageal eosinophil levels. The most highly induced gene in the esophagi of patients with EoE is the eosinophil chemoattractant ***eotaxin***-3 *(CCL26)*, which was overexpressed 53-fold in esophageal biopsy specimens from patients with EoE compared with normal esophageal biopsy specimens.[27] Eotaxin-3 belongs to the eotaxin family (eotaxin-1 to eotaxin-3) of CC chemokines and, through its receptor, ***CCR3***, activates downstream G protein signaling to drive eosinophil chemotaxis and activation. Of the eotaxins, only *CCL26* is upregulated in patients with EoE, and its expression correlates with eosinophil (and mast cell) levels within esophageal biopsy specimens, indicating a specific contribution in the disease.[27] Notably, levels of *CCL26* transcript in a single biopsy specimen are highly sensitive (89%) in distinguishing EoE from control populations[28] despite the histological "patchiness" of EoE across multiple biopsy specimens. In fact, histological examination of at least 3 biopsy specimens is required to achieve similar diagnostic sensitivity.[2,3] Immunofluorescence and *in situ* hybridization studies on esophageal biopsy specimens identify the esophageal epithelium as the main source of eotaxin-3 production.[27] *In vivo* models of EoE further illustrate the crucial role of eotaxin-3 in disease because mice deficient in

the eotaxin receptor *Ccr3* are protected from esophageal eosinophilia after allergen challenge.[27] Steroid therapy, in particular swallowed glucocorticoids, effectively normalizes as much as 98% of the EoE transcriptome,[29] including *CCL26*, indicating the dynamic nature and reversibility of the genetic dysregulation.

In addition to eotaxin-3, a number of immune cell–specific genes exhibit differential expression levels in patients with EoE. For instance, expression of immunoglobulin genes and genes involved in antibody class switching is increased, reflecting the increase in the esophageal B-cell population in patients with EoE.[9] Mast cell–specific genes, specifically carboxypeptidase 3A *(CPA3)*, high-affinity IgE receptor *(FCERI)*, and tryptase-α *(TPSAB1)*, are abundantly represented in the EoE transcriptome, and mast cells are indeed a prominent inflammatory cell in the esophagi of patients with EoE when specifically examined with anti-tryptase staining.[12,27] Based on mast cell levels, a specific esophageal transcriptome is also identified in patients with EoE, which only partially overlaps with the transcriptome defined by eosinophil levels alone,[12] indicating that mast cells and eosinophils are likely independently involved, at least in part. Significant increases in mast cell degranulation and mastocytosis within the epithelium, lamina propria, and smooth muscle layer,[12,13] which can be ameliorated with steroid therapy,[12] further implicate these cells in the local inflammatory milieu within the esophagus.

A significant portion of the gene transcriptional changes associated with EoE occurs within the esophageal epithelium. These structural cells can influence multiple aspects of the disease phenotype, including inflammatory cell recruitment, tissue remodeling, and hyperproliferation. The human esophageal epithelium is composed of nonkeratinized, stratified squamous epithelia with a proliferating basal layer of 1 to 3 cells in depth and a differentiating suprabasal layer migrating toward the esophageal lumen.[30] Many of the histopathological features of the esophagus that are associated with EoE indicate gross defects in cell adherence, as indicated by dilated intercellular spaces, expansion of the basal cell layer, and extracellular matrix deposition within the lamina propria. Studies have highlighted ***IL-13*** as a critical signaling molecule capable of altering global gene expression of the esophageal epithelium. *Ex vivo* microarray analysis showed that treatment of biopsy-derived primary esophageal epithelial cells with IL-13, which is upregulated at the mRNA level in patients with EoE, can largely recapitulate the EoE transcriptome.[29] This study also confirmed epithelial cells as the primary source of *CCL26* in patients with EoE, which was upregulated by an astounding 279-fold after IL-13 stimulation *ex vivo*.[29] Notably, esophageal epithelial cells derived from patients with EoE and control subjects respond similarly to IL-13, as assessed based on *CCL26* production.[31]

Animal models have provided demonstrative data highlighting the robust proinflammatory action of IL-13 in an *in vivo* setting. Lung-specific overexpression of *Il13* in mice induces an asthma-like phenotype in the absence of antigen challenge that is characterized by marked inflammatory cell infiltration into the lungs and enhanced airway mucus production.[32] However, this model also promotes inflammation within the esophagus, such as esophageal eosinophilia and tissue remodeling, including fibrosis, angiogenesis, and epithelial hyperplasia.[33] The esophageal remodeling in this model occurs independently of eosinophilia and is inhibited by the type 2 IL-13 receptor (IL-13Rα2).[33] In summary, these findings implicate the esophageal epithelium as the pathogenic target of IL-13 signaling in patients with EoE, as demonstrated by the induction of pronounced histologic and molecular changes that occur in the presence of this potent T_H2 cytokine.

The epidermal differentiation complex (EDC) on human chromosome 1q21 is a cluster of genes that regulates terminal differentiation and formation of the cornified envelope of the epithelium.[34] Despite the lack of a cornified layer in the esophagus, the EDC locus contains the highest density of dysregulated genes in the EoE transcriptome compared with all other loci in the genome.[31] Loss-of-function mutations in several EDC genes, including ***filaggrin*** *(FLG)*, have been reported for various cutaneous disorders.[35-39] *FLG*, ***involucrin*** *(IVL)*, and several small proline-rich repeat (*SPRR*) family members (2C, 2D, and 3) are expressed in esophageal epithelial cells but are downregulated in response to IL-13 *ex vivo*,[31] implicating a homeostatic role for the EDC in the esophageal epithelium. Loss of *FLG* expression and subsequent defects in epidermal barrier function have been demonstrated in patients with AD,[40,41] which frequently co-occurs with EoE. However, no significant difference in *FLG* expression is observed between atopic and nonatopic patients with EoE,[31] suggesting an alternative function for filaggrin in regulating the epithelial structure within the human esophagus.

It is important to note that 2% of the EoE transcriptome is not reversible after disease remission induced by swallowed glucocorticoids.[29] Interestingly, these transcripts include genes that are involved in regulating homeostatic and pathogenic responses in the epithelium, such as cadherin-like 26 *(CDH26)*, uroplakin 1B *(UPK1B)*, periostin *(POSTN)*, and desmoglein-1 *(DSG1)*.[29] Desmoglein-1 is a transmembrane desmosomal cadherin component of desmosomes and facilitates the calcium-dependent homotypic interactions between adjacent cells that impart both structure and mechanical strength to the epithelia. Expression of *DSG1* is decreased in both glucocorticoid-treated and untreated patients with EoE (77% and 87%, respectively) compared with that seen in healthy control subjects. Desmoglein-1 is of particular importance because it is the target of multiple inherited and acquired cutaneous disorders. Pemphigus foliaceus and pemphigus vulgaris are autoimmune diseases in which autoantibodies targeting desmoglein-1 decrease cellular adhesion, resulting in epidermal blistering.[42] Notably, epithelial microabcesses exhibiting pronounced eosinophilic inflammation that can be associated with pemphigoid disorders have also been demonstrated within the esophagus, such as in pemphigus vegetans.[43] Furthermore, multiple heterozygous mutations in the extracellular domain coding region of *DSG1* have been linked with striate palmoplantar keratoderma, a disease characterized by epidermal thickening on the palms and soles.[44] Collectively, these findings substantiate the significance of alterations in desmoglein-1 in a spectrum of human diseases; it is tempting to speculate that tissue-specific decreases in desmoglein-1 might be pathogenic and partially responsible for the tissue-specific inflammation in patients with EoE.

POSTN is the gene of another key molecule that demonstrates steroid resistance in patients with EoE. Periostin, which functions as a cell adhesion molecule that regulates extracellular matrix deposition,[45,46] is dramatically upregulated in patients with EoE by approximately 52-fold; although glucocorticoid therapy can reduce a significant portion of this overexpression, *POSTN* expression remains increased in glucocorticoid-treated patients (approximately 2-fold).[47] Periostin is expressed in the basal epithelium and papillae[47] of the esophagus, suggesting a contributing role for the increased lamina propria fibrosis. Indeed, ***TGF-β***, a profibrotic stimulus that is expressed by eosinophils

and mast cells in biopsy specimens from patients with EoE,[13,48] can induce a dramatic upregulation of *POSTN* expression in primary esophageal fibroblasts, supporting this potential mechanism for the tissue fibrosis observed in patients with EoE.[47,49] Moreover, periostin can enhance eosinophil adhesion *in vitro*, and *Postn*-deficient mice are protected from allergen-induced eosinophilia in the lung and esophagus.[47] Interestingly, periostin upregulation in bronchial epithelial cells enhances TGF-β–induced collagen synthesis.[50] Because periostin also enhances cross-linking of collagen fibrils through upregulating the cleavage of mature active lysyl oxidase,[51] whose gene expression is also increased in the EoE transcriptome, these cumulative data suggest a positive feedback loop in which periostin has a central role in promoting the fibrotic responses in multiple inflammatory conditions.

In summary, esophageal transcript profiling has defined an EoE-specific transcript signature that is composed of dysregulated gene networks involved in T_H2 inflammation and epithelial cell responses. These studies demonstrate that IL-13 is a central mediator and link between the immunologic and histologic changes that are germane to EoE, largely through its effects on the esophageal epithelium. Given the well-documented role of IL-13 in other atopic diseases, such as asthma and AD, it is reasonable to speculate that IL-13 production in response to inhaled or absorbed antigens can also predispose subjects to other T_H2 comorbidities, such as EoE.

GENETIC VARIANTS AND DISEASE SUSCEPTIBILITY

The number of studies investigating genetic variants associated with EoE are few compared with those for other more common and more widely recognized atopic diseases, such as AD and asthma. Regardless, there have been significant strides in uncovering EoE risk variants in a relatively short period of time[14,27,52,53] due in part to the technological advances in genotyping ***single nucleotide polymorphisms*** (SNPs) in large case-control cohorts. In all, there have been 4 candidate gene studies that tested for polymorphisms in genes with a published (or suspected) functional role in EoE and one ***genome-wide association study*** (GWAS) that was used to identify EoE risk variants across the entire genome in an unbiased fashion (Table I).[14,27,31,52,53]

Candidate gene studies

Blanchard et al[27] identified the first EoE risk variant in a likely candidate, *CCL26*. The *CCL26* SNP (rs2302009) was shown to be highly associated with disease risk ($P = .001$), with an odds ratio of 4.55 in a case-control cohort. Transmission disequilibrium testing, which measures the transmission of a disease allele from unaffected heterozygous parents to an affected offspring, confirmed that the association of rs2302009 with EoE was not due to ancestral differences in the case-control analysis.[27] Additional studies have also linked this SNP to increased serum IgE levels and asthma susceptibility.[54] However, the observed association between rs2302009 and EoE was independent of atopic status, indicating a direct link with EoE susceptibility. Although rs2302009 is located within the 3′ untranslated region of the *CCL26* transcript and could potentially affect mRNA stability, a functional effect of this SNP in either asthma or EoE has yet to be described.

TGF-β, an eosinophil- and mast cell–derived mediator of fibrotic tissue responses, has been implicated in the same pathogenic process in patients with EoE.[48] Moreover, TGF-β1 has recently been shown to stimulate esophageal smooth muscle contractility and potentially contribute to esophageal dysmotility in patients with EoE.[13] An SNP within the *TGFB1* promoter (C-509T) that associated with asthma susceptibility[55,56] was shown to create a binding site for the transcription factor YY1 that subsequently enhanced promoter activity.[56] In a small cohort of 20 patients with EoE, homozygotes for the minor T allele of C-509T exhibited increased TGF-$\beta1^+$ lamina propria cell numbers.[14] Conversely, the major C allele of C-509T was a positive prognostic indicator for therapeutic responses in patients with EoE.[14] Determining the association of this and other *TGFB1* SNPs in a larger disease cohort will be vital to assess the full genetic contribution of *TGFB1* in patients with EoE.

Polymorphisms in epithelium-specific genes have also been associated with EoE susceptibility. First, a loss-of-function SNP in *FLG* (2282del4) that was previously linked with AD susceptibility[38] also associates with EoE risk; similar to the *CCL26* SNP, this association is specific to EoE because atopy was found not to be a confounding factor.[31] A second and larger candidate gene study examined 736 SNPs in 52 genes known to be involved in epithelial cell structure or inflammatory responses. Here, an EoE cohort of 170 patients was genotyped by using a custom SNP chip and compared with similarly genotyped control subjects with various atopic histories.[53] Importantly, SNPs in the gene for thymic stromal lymphopoietin *(TSLP)*, a cytokine recently described as a "master regulator" of T_H2 responses,[57] were shown to associate with EoE independent of the patient's atopic status. TSLP is derived primarily from epithelial cells in response to cytokines,[58] noxious substances,[59] and mechanical stress[60] and exerts its effects on nearly every cell type involved in T_H2 inflammation, including eosinophils[61] and mast cells.[62] For instance, TSLP activates dendritic cells to adopt a T_H2 priming phenotype through the secretion of the chemokines ***thymus and activation-regulated chemokine (TARC)***, macrophage-derived chemokine, and eotaxin-2 and OX40 ligand expression, which activates T_H2 cytokine production by naive $CD4^+$ T cells.[63-65] Thus, it is remarkable that TSLP critically regulates the exact processes involved in allergen sensitization that underscore the EoE phenotype. This study also identified an association between male patients with EoE and a nonsynonymous SNP in the TSLP receptor *(CRLF2)*,[53] which, given the male predilection for EoE, presents an intriguing scenario because *CRLF2* is encoded on pseudoautosomal region 1 of the X and Y chromosomes.[66]

EoE GWAS

A broader, unbiased GWAS approach was undertaken to identify SNPs associated with EoE susceptibility. Here, 2 relatively large cohorts of patients with EoE and healthy control subjects were genotyped for 550,000 SNPs across the genome.[52] Although only 1 locus on chromosome 5q22 was genome-wide significant after multiple testing correction, this region contains the genes encoding for *TSLP* and WD repeat domain 36 *(WDR36)*. Esophageal expression of *TSLP*, but not *WDR36*, is increased in patients with EoE, and the protective minor allele for the most significantly EoE-associated SNP on 5q22 (rs3806932), which lies upstream of the *TSLP* locus, correlates with decreased *TSLP* expression in the esophagus. Notably,

TABLE I. Genetic risk variants in patients with EoE

Chromosome	SNP*	Alleles†	Gene/gene locus	SNP location‡	Study design	*P* value and OR	Summary	Reference
7q11	rs2302009	T>G	Eotaxin-3 *(CCL26)*	3′ UTR	Candidate gene study (117 cases and 225 control subjects)	$P = .001$, OR = 4.55	Significance in a case-control association was also replicated by using transmission disequilibrium testing in a trio cohort.	Blanchard et al[27]
19q13	−(C-509T)	C>T	TGF-β1 *(TGFB1)*	Promoter	Candidate gene study (20 cases)	$P = .02$ for response status, $P = .01$ for TGF-$\beta1^+$ cells	CC genotype correlated with therapy response. The T allele was associated with increased numbers of TGF-$\beta1^+$ cells in lamina propria.	Aceves et al[14]
1q21	rs61816761 (2282del4)	CAGT>−	Filaggrin *(FLG)*	Exon	Candidate gene study (365 cases and 164 control subjects)	$P = .018$, OR = 4.89	Loss-of-function mutation in *FLG* associated with EoE	Blanchard et al[31]
5q22	rs10062929	C>A	*TSLP*	Intron	Large–scale candidate gene study (257 cases and 342 control subjects)	Meta-$P = 3.16 \times 10^{-6}$, OR = 0.36-0.45	*TSLP* SNPs associated with EoE risk independent of atopy	Sherrill et al[53]
Xp22/Yp11	rs36133495	G>T	TSLP receptor *(CRLF2)*	Exon	Candidate gene study (199 cases and 78 control subjects)	$P = .039$, OR = 2.05	Ala>Val amino acid change in TSLP receptor associated with male patients with EoE	Sherrill et al[53]
5q22	rs3806932 rs7723819	A>G G>A	*TSLP* *WDR36*	Near gene Near gene	GWAS (351 cases and 3104 control subjects)	Meta-$P = 3.19 \times 10^{-9}$, OR = 0.54-0.73 Meta-$P = 7.67 \times 10^{-9}$, OR = 0.55-0.71	Minor (protective) G allele correlated with decreased esophageal *TSLP* expression	Rothenberg et al[52]

OR, Odds ratio; *UTR*, untranslated region.
*dbSNP Build 131 "rs" identifier given when appropriate.
†Major allele > minor allele.
‡SNP location in relation to the gene/gene locus.

rs3806932 is in linkage disequilibrium with rs3806933,[52,67] suggesting that these 2 SNPs are inherited together more often than would be expected by chance. Data recently implicated rs3806933 in altering the binding of the transcription factor activator protein 1 to the *TSLP* promoter with a modest increase in promoter activity.[67] The other genome-wide significant SNP on 5q22 is upstream of the *WDR36* gene, which is located approximately 14 kb away from *TSLP* and lies within the same linkage block as rs3806932.[52] *WDR36* is critically involved in ribosomal RNA processing[68] and is coregulated with *IL2* in activated T lymphocytes.[69] Moreover, SNPs in the region of *WDR36* have been associated with peripheral blood eosinophilia,[70] as well as glaucoma susceptibility.[71] Thus, although *TSLP* appears to be the likely disease candidate on the 5q22 locus, the role of *WDR36* warrants further investigation.

GWASs on other more common gastrointestinal inflammatory diseases, such as Crohn disease,[72] ulcerative colitis,[73] and celiac disease,[74-76] have successfully identified numerous disease risk variants aided in part by the well-developed patient cohorts being historically investigated for these diseases. For example, meta-analyses across large, independent case-control cohorts (often in excess of 10,000 combined patients) along with further refinement of the human genome polymorphism map have yielded sufficient sample sizes to detect significant disease associations with common variants that have relatively low effect sizes. The low sample size of the current EoE GWAS (251 patients with EoE in total) not only emphasizes the magnitude of the 5q22 SNP associations but also suggests that there are likely additional EoE risk variants to be uncovered as further EoE cohorts are subjected to genome-wide genotyping and similar meta-analyses are

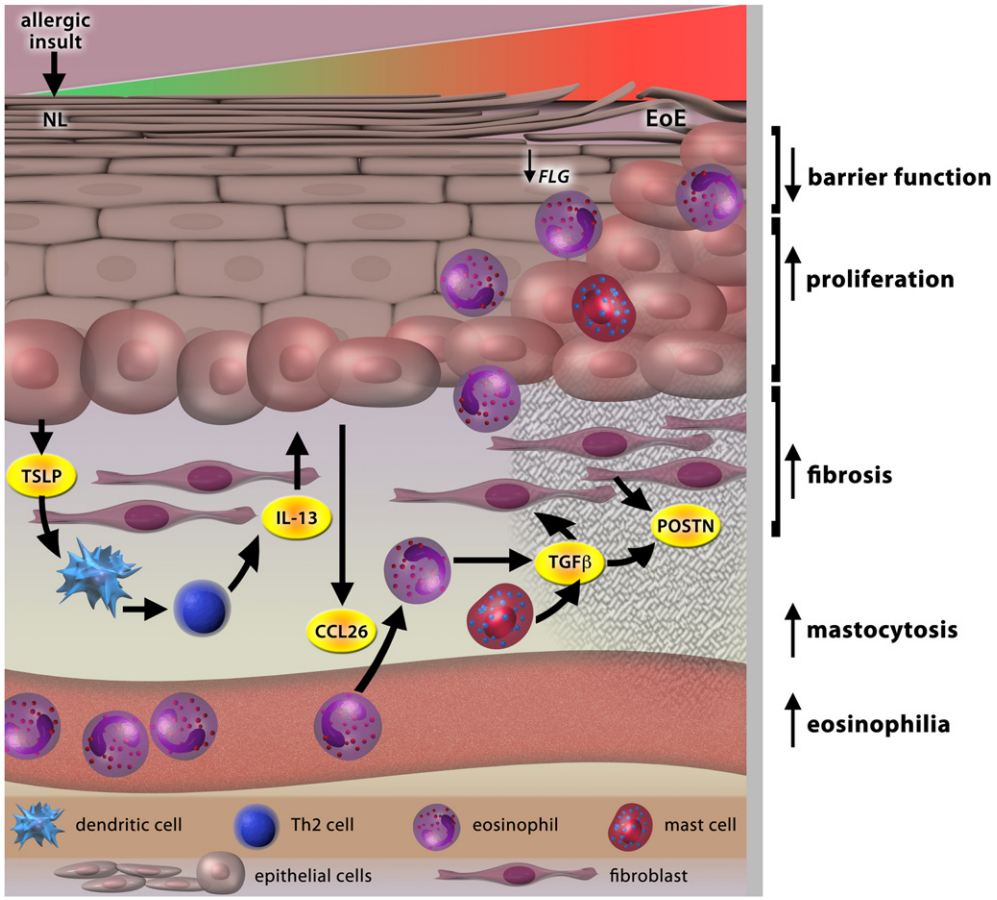

FIG 1. The molecular pathogenesis of EoE. An allergic insult by either food antigens or aeroallergens initiates the transition of the esophagus from a normal *(NL)* to an EoE phenotype through the production of TSLP by the esophageal epithelium. TSLP-activated dendritic cells induce a robust T_H2 response and enhanced IL-13, which in turn mediates marked dysregulation of gene expression (the EoE transcriptome). Enhanced eotaxin-3 (CCL26) secretion by the esophageal epithelium promotes eosinophil migration from the blood into the tissue. Eosinophil- and mast cell–derived TGF-β along with IL-13 act on fibroblasts within the lamina propria to secrete periostin *(POSTN)* and stimulate the fibrotic response. Loss of *FLG* expression, partially because of IL-13 overproduction, genetic variants, or both, might further enhance or even predispose patients with EoE to antigen exposure and exacerbate T_H2 inflammation.

performed (in essence, boosting the statistical power). A hint at what SNPs or gene loci might hold potential significance in these future studies can be gained by means of investigation into those that did not reach the statistical threshold for significance from the previous GWAS.[52] For instance, SNPs in signal transducer and activator of transcription 6 *(STAT6)*, the major signaling molecule downstream of IL-13 signaling, and in the aforementioned *DSG1*, which is largely resistant to steroid-dependent regulation in patients with EoE, are highly associated with EoE but under the genome-wide significance threshold.[52]

CONCLUSIONS AND FUTURE DIRECTIONS

In just over 10 years since the recognition of EoE as a distinct inflammatory disorder, the rapid progress toward characterizing the disease on multiple fronts has underscored its complexity. We now have insight into the natural history of EoE, its strong association with specific ethnicities and sexes, the genetic and environmental factors involved, and the molecular pathogenesis of the disease (Fig 1). Moreover, the burst of data illustrating EoE risk variants in *CCL26*, *TGFB1*, *TSLP* and *CRLF2*, and *FLG* provide insight into the upstream mechanisms that regulate the expression of genes that are operational (and likely synergistic) in multiple aspects of EoE pathogenesis (Fig 2). For instance, perturbations in the TSLP signaling pathway as a result of variants either increasing *TSLP* gene expression or altering receptor function can amplify innate inflammatory responses to food antigens. Moreover, prolonged *CCL26* expression might further enhance eosinophil recruitment and TGF-β1 secretion to exacerbate tissue remodeling. Variants affecting *FLG* expression might disrupt normal esophageal barrier function and result in increased antigen exposure and affect overall tissue integrity. Despite these advances, much work remains in terms of identifying true casual variants and determining their mechanistic function in these pathways. A major initiative currently underway is to expand on the current genome-wide associated polymorphisms by increasing the number of genotyped patients with EoE; this will undoubtedly greatly expand the number of genetic loci linked with EoE risk. Moreover, deep sequencing efforts and extensive fine mapping of the established EoE susceptibility loci, such as *TSLP*, could identify rare variants, casual variants, or both that affect gene transcription.

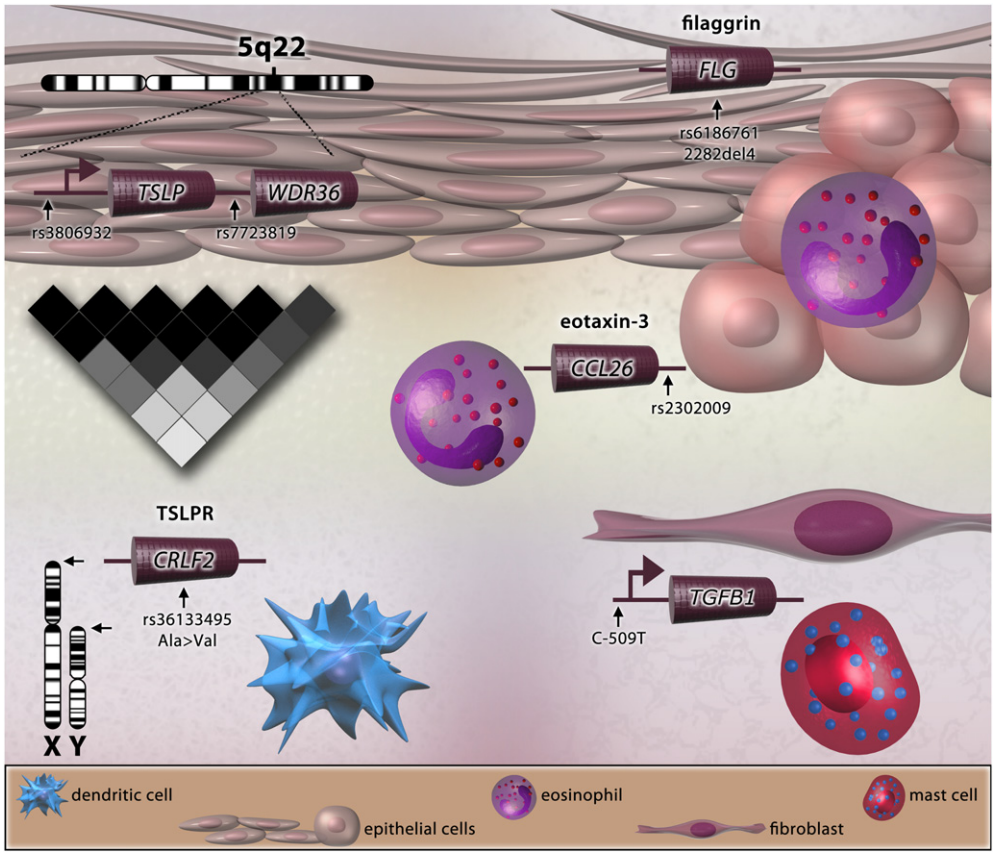

FIG 2. Genetic risk variants in patients with EoE. EoE risk variants near *TSLP* and in the TSLP receptor gene *(CRLF2)* highlight a potential role for the TSLP pathway in EoE. SNPs in other key genes, such as *CCL26*, *TGFB1*, and *FLG*, can affect multiple aspects of EoE pathogenesis, including eosinophil chemotaxis, fibrosis and smooth muscle dysfunction, and decreased esophageal barrier function, respectively.

An additional area to be explored in EoE heritability will be the role of ***epigenetics***, which can be defined as the study of heritable changes in gene expression that are not associated with DNA sequence variations, which can include noncoding RNAs, histone modifications (acetylation and methylation), and DNA methylation.[77] Importantly, because these genomic alterations can be influenced by external stimuli, such as diet and drugs, epigenetics can provide insight into the complex interactions between environmental exposures and disease-associated genes. The profiling of global epigenetic changes in large disease cohorts has already yielded promising results for cancer, cardiovascular disease, obesity,[77] and asthma.[78] Because EoE is also influenced by environmental antigen exposure, the uncovering of an EoE epigenome through microRNA arrays, DNA methylation profiling, and ***chromatin immunoprecipitation*** sequencing technologies will provide a critical link to the global gene transcriptional changes already known to occur in patients with EoE. Recent data have already indicated that IL-13 can elicit acetylation changes to histone H3 at the *CCL26* promoter in esophageal epithelial cells, implicating that epigenetic modifications represent a novel mechanism of gene regulation in patients with EoE.[79]

The pivotal role of the esophageal epithelium in patients with EoE and the transcriptional changes that occur within different stratified layers of the epithelium provide potential opportunities for noninvasive biomarkers for EoE. Laser-capture microscopy allows for the isolation of specific cell types from minute sections of tissues that can subsequently be subjected to microarray or mass spectrometric analysis. Such techniques have already been used to define the transcriptomes[80] and ***proteomes***[81] of the various esophageal layers in patients with Barrett esophagus. Identification of an EoE-specific transcript profile specific to the suprabasal epithelium might yield diagnostic targets from the skin or oral mucosal samples.

In conclusion, it is remarkable how the genetic dissection of EoE susceptibility has uncovered key pathways that are now being considered for treatment strategies. For example, our findings identify new targets for antibody neutralization strategies (eg, anti–IL-13) and specific cell types for directed therapy, such as mast cells and epithelial cells, which also supports the clinical value of topical steroid therapy. Therefore, over the next 10 years, further unraveling of the genetic and environmental factors that compound EoE holds great promise for the future development of novel and highly effective therapies.

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Key concepts

- **EoE is an emerging inflammatory disease that is clinically and causatively distinct from GERD.**
- **Genetic predisposition and food antigen exposure contribute to EoE.**
- **EoE exhibits a familial inheritance pattern and is more common among white subjects and male subjects.**
- **Microarray analysis of the patients' esophageal biopsy specimens has defined an EoE transcriptome that is also highly inducible by IL-13 *ex vivo*.**
- ***CCL26* is the most highly induced gene in the EoE transcriptome.**
- **Candidate gene studies have identified genetic variants in *CCL26*, *TGFB1*, *TSLP*, and *FLG* associated with EoE risk.**
- **A coding SNP in the TSLP receptor gene *CRLF2*, which is encoded on the X and Y chromosomes, is significantly associated with disease risk in male patients with EoE.**
- **A GWAS on 351 patients with EoE identified the 5q21 locus encoding *TSLP* and *WDR36* as an EoE susceptibility locus.**

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