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Current Opinion in Immunology

Role of genetics, environment, and their interactions in the pathogenesis of eosinophilic esophagitis

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The rise in incidence and prevalence of eosinophilic esophagitis (EoE) since the 1990s has prompted investigations into its pathogenesis, natural history, and management. Identified genetic variants in *FLG, DSG1, CAPN14, SPINK5,* and *SPINK7* link EoE to epithelial barrier dysfunction, whereas variants in *CCL26, POSTN,* and *TSLP* associate EoE with T helper type 2-mediated immunity. Early-life, infectious, and geographic factors have been implicated in promoting esophageal microbial dysbiosis and, subsequently, T helper type 2 immune responses. However, research into environmental factors and their interactions with genetic variants are not as developed as their genetic counterparts. Further research into the subgroups and epigenetics of EoE will likely promote further understanding.

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Current Opinion in Immunology 2019, **60**:46–53

This review comes from a themed issue on **Allergy and hypersensitivity**

Edited by **Pamela A Frischmeyer-Guerrerio** and **Joshua D Milner**

For a complete overview see the Issue and the Editorial

Available online 25th May 2019

https://doi.org/10.1016/j.coi.2019.04.004

Introduction

Eosinophilic esophagitis (EoE) is a chronic, food antigen-driven, T helper type 2 (Th2)-mediated inflammatory disease primarily characterized by a marked esophageal eosinophilic infiltrate (≥15 eosinophils per high-power microscopic field [eos/hpf]) and esophageal dysfunction [1]. Symptoms vary by age and can be nonspecific, including food refusal, nausea/vomiting, poor weight gain or failure to thrive, heartburn, odynophagia, chest pain, abdominal pain, dysphagia, and food impaction [1]. Since its recognition in the 1990s, EoE's incidence and prevalence have risen from a rare, case-reportable disease to one more frequently encountered in clinical practice [2]. EoE has an overall prevalence estimated at 56.3 cases per 100000 persons [2], is costly with an annual health care estimate between $500 million to $1.4 billion [3], and has significant negative impact on a patient's health-related quality of life [4]. Because of these reasons, the pathogenesis of EoE has been an avid area of research, with studies aiming to discover specific therapeutic targets for treatment as patient compliance to and effectiveness of current therapies (food antigen elimination and swallowed corticosteroids) continue to be issues [5–7]. Unfortunately, none of the currently available therapeutics have a universal beneficial effect in all patients, likely due to EoE's polygenic nature and interaction with complex and poorly understood environmental factors. As research continues for specific biologic therapeutics, we will review herein the available evidence concerning genetic variants, environmental factors, and their interactions to advance the discussion of EoE's pathogenesis.

Genetic components

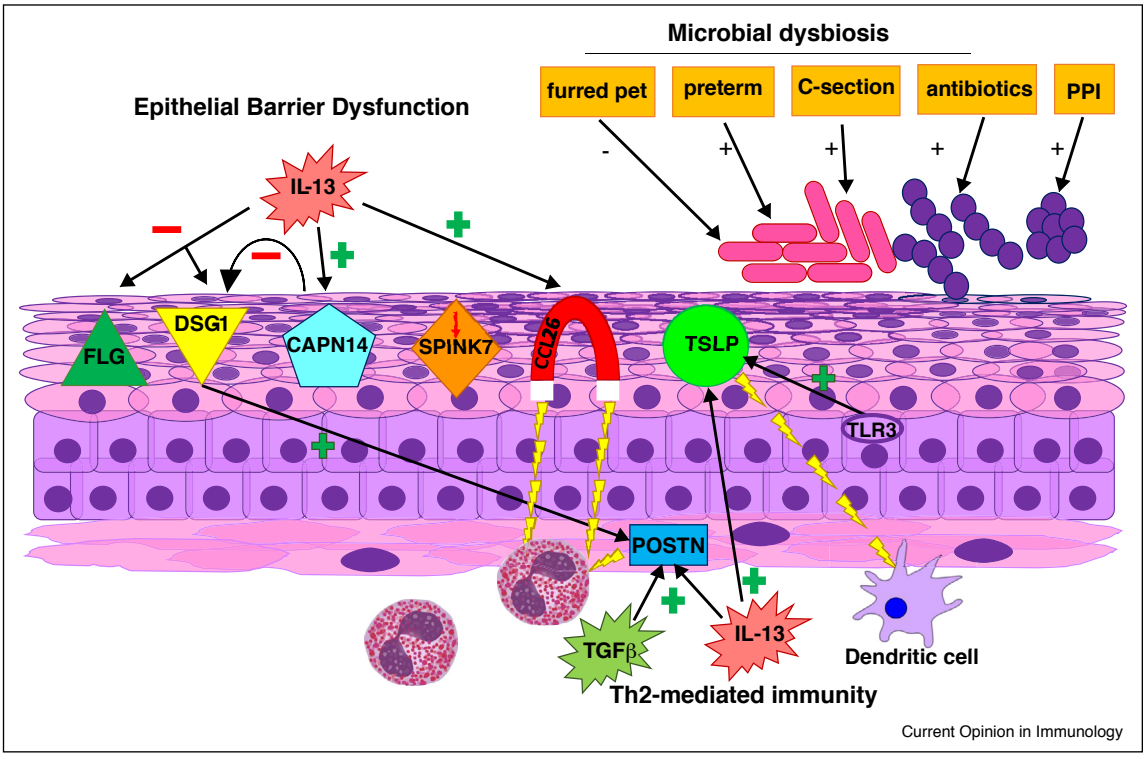
EoE frequency is increased among first-degree family members, suggesting a genetic etiology, at least in part [8,9]. Alexander *et al.* [8] aimed to estimate both the genetic and environmental contributions to EoE, demonstrating that first-degree male relatives of patients with EoE had 64-fold (brothers) and 43-fold (fathers) increased risk of EoE, whereas monozygotic and dizygotic twins had a 41% and 22% frequency of EoE, respectively. Likewise, a large population-based study identified an increased risk of EoE in first-degree relatives of pediatric patients with EoE (OR, 16.3; 95% CI, 9.4–28.3); however, this study was conducted within a single state (Utah) and could reflect a less heterogenous population overall [9]. Familial patterning of EoE does not follow autosomal dominant, recessive, or X-linked patterns, arguing for a complex mode of inheritance [8]. The genetic components of EoE identified to date via candidate-gene studies, genome-wide association studies (GWAS), and analysis of Mendelian disorders associated with EoE most often affect either epithelial barrier function or Th2-mediated immune responses [10•,11••].

Epithelial barrier function

In 2006, Blanchard *et al.* [12] described the EoE transcriptome, a set of 574 differentially expressed genes in EoE that is conserved across age, sex, and atopic status. Of note, it contains a 'hot spot' of dysregulation at the

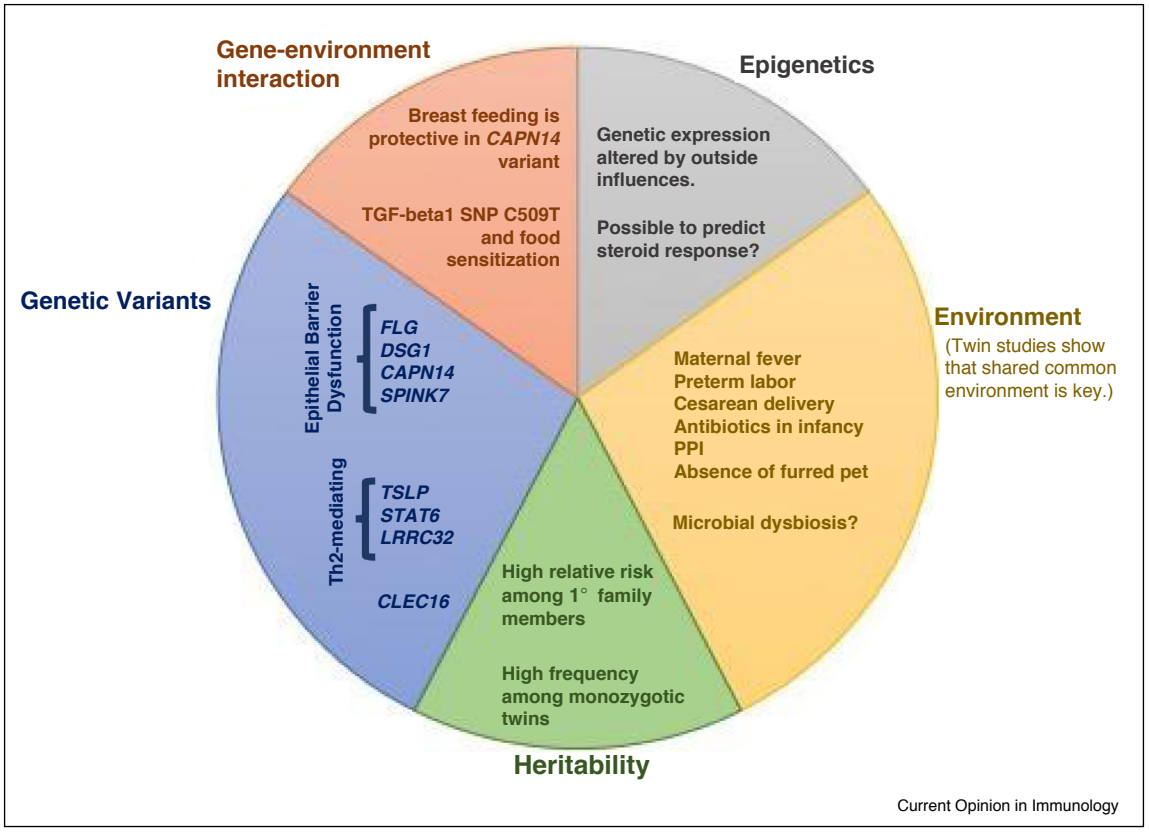
epidermal differentiation complex (1q21) [12]. Several genes involved in epithelial cell differentiation reside in this area, including filaggrin *(FLG)*, which is downregulated in EoE, leading to impaired epithelial barrier function [12]. Likewise, desmoglein 1 *(DSG1)* is downregulated in EoE by interleukin 13 (IL-13), and the absence of DSG1 is sufficient to induce epithelial cell barrier dysfunction in EoE even in the absence of dysregulation of tight junction genes (*OCLN*, *CLDN*, *TJP*) in EoE [13]. SAM (severe atopic dermatitis, multiple allergies, and metabolic wasting) syndrome is caused by homozygous loss-of-function mutations in either *DSG1* or desmoplankin, further demonstrating *DSG1*'s importance to epithelial barrier function [10•,11••]. The strongest GWAS association with EoE to date is found at 2p23, which encodes calpain 14 *(CAPN14)* whose product CAPN14 is an esophagus-specific proteolytic enzyme induced by IL-13 [14]. Increases in CAPN14, such as that observed in EoE, lead to loss of *DSG1* expression and impaired epithelial barrier function [14,15]. However, absence of *CAPN14* also impairs the epithelium's ability for repair following IL-13-mediated damage, leading Litosh *et al.* [16•] to hypothesize that CAPN14 is as an intermediary in IL-13-mediated esophageal epithelial homeostasis. Lastly, Netherton syndrome is caused by loss-of-function mutations in serine peptidase inhibitor, kazal type 5 (*SPINK5*), leading to epithelial barrier dysfunction via uncontrolled serine protease activity [10•,11••]. Though both *SPINK5* and *SPINK7* are downregulated in EoE, *SPINK7* has a higher degree of dysregulation and is sufficient for epithelial barrier dysfunction independent of *SPINK5* dysregulation [17••]. The above evidence supports the independent action of these genetic variants on the esophageal epithelium in EoE; however, *in vivo* they are likely dysregulated in concert. Therefore, multiple pathways allow for dysregulation with a potentially additive effect on esophageal epithelial integrity. These circumstances allow us to hypothesize and study downstream effects on tissue permeability and antigen uptake, which lead to

Figure 1



Our current understanding of the pathogenesis of EoE involves multiple pathways for epithelial barrier dysfunction and Th2-mediated immune responses. IL-13 downregulates *DSG1* and *FLG* while upregulating *CAPN14*, which subsequently downregulates *DSG1*, and *SPINK7* dysregulation has been found to be sufficient for epithelial barrier dysfunction as well. *DSG1* downregulation subsequently induces *POSTN*. IL-13 also induces *CCL26* (eosinophil chemotaxis), *POSTN* (eosinophil adherence), and *TSLP* (dendritic cell chemotaxis). TGF-beta also induces POSTN. Multiple environmental factors (absence of furred pet, preterm labor, cesarean delivery (C-section), antibiotics in infancy) are hypothesized to lead to microbial dysbiosis and Th2-mediated immunity; however, temporal associations are difficult to prove, and it remains unclear if the observed microbial dysbiosis in the esophagi of patients with EoE is causing or due to the disease. Proton pump inhibitors (PPI) are strongly associated with EoE; however, this association suffers from protopathic bias.

Figure 2



Twin studies have shown that the heritability of EoE is a complex issue raising evidence for both genetic and environmental influence. As few studies have examined the interaction between identified genetic variants and environmental factors, further studies are needed. Likewise, epigenetics has the potential to explain gene–environment interactions, identify further risk factors, and advance development of novel therapeutics; however, studies to date are scarce.

an inappropriate Th2-mediated immune response (Figures 1 and 2).

Th2-mediated immune response

EoE is a Th2-associated inflammatory disease involving several cytokines, such as IL-4, IL-5, and IL-13. IL-13 has been shown to induce esophageal eosinophilia in experimental models and regulates several identified genes associated with EoE [10•]. Early work by Blanchard *et al.* [12] identified eotaxin-3 *(CCL26)*, the most highly upregulated (53-fold) gene in the EoE transcriptome, which when induced by IL-13 causes eosinophil chemotaxis via the CCL26 receptor, CCR3 [10•]. Later work demonstrated that when induced by IL-13 and TGF-beta, periostin (*POSTN*) increases eosinophil adhesion to fibronectin [18]. Thus, eotaxin-3 attracts eosinophils, and POSTN increases their adhesion. Interestingly, absence of *DSG1* causes increased *POSTN* expression leading to increased epithelial production of thymic stromal lymphopoietin (*TSLP*) [13], a pro-allergic cytokine. This highlights the interaction between epithelial barrier dysfunction and the Th2-mediated immune response characteristic of EoE. In the first published GWAS, Rothenberg *et al.* [19] described how activated epithelial cells in EoE release TSLP, which is encoded for by the cheif EoE susceptibility locus (5q22) and regulates dendritic cell-mediated Th2 responses, following IL-13 stimulation. TSLP has also been implicated in eosinophil survival [20], mast cell activation [21], and basophil responses [22] within esophageal epithelium. Therefore, TSLP is a central component of the Th2-mediated inflammatory cascade leading to the development of EoE. Moreover, the association between EoE and *TSLP* was later found to be independent of its expression in other atopic disorders [11••,23]. More recently, Martin *et al.* [24•] described the first gene–gene interaction in EoE by demonstrating that the association between EoE and *TSLP* strengthened when accompanied by an *IL4* variant, indicating that this interaction may act as a positive feedback loop to augment allergic inflammation within EoE. Further work identified a previously unrecognized susceptibility locus in toll-like receptor 3 (*TLR3*) through which esophageal epithelium express *TSLP* [25].

STAT6 (12q13) has also been shown to independently associate with EoE, is activated by IL-4 and IL-13, and encodes for a transcription factor that activates many genes identified within EoE [10•,11••]. Furthermore, though *LRCC32's* role in EoE is still being investigated, it independently associates with EoE and is highly expressed by FOXP3+ T regulatory cells and plays a role in TGF-beta surface expression [10•]. Therefore, EoE is likely a disease of immune dysregulation in addition to increased Th2-mediated responses. Of note, *c11orf30* (also linked to *EMSY*) and *ANKRD27* both independently associate with EoE; however, while they are linked to tissue growth and wound healing, respectively, their role in the pathogenesis of EoE has yet to be established [14].

Other Mendelian disorders associated with EoE include connective tissue disorders such as Loeys-Dietz syndrome and Ehlers-Danlos syndrome (hypermobility type). These are due to increased TGF-beta production and/or signaling, which promote smooth muscle contractility and tissue remodeling [10•]. Ultimately, it is apparent that the genetic components of EoE's pathogenesis are complex and affect multiple levels within esophageal epithelial function and Th2-mediated immune responses. As research continues within the genetic basis of EoE, advances are likely to derive from continued investigation of known variants to discover root causes with potential for therapeutic intervention (Figures 1 and 2).

It is worth mentioning that while the main focus of this review is the pathophysiology of EoE, many of these genetic loci contribute to the pathophysiology of other atopic conditions such as atopic dermatitis (*c11orf30*, *FLG*), asthma (*TSLP*, *c11orf30*, *CCL26*), allergic sensitization (*TSLP*, *c11orf30*, *STAT6*), allergic rhinitis (*TSLP*, *c11orf30*), and total serum IgE levels (*STAT6*) via epithelial barrier dysfunction and Th2-mediated immune responses [14,19,26].

Environmental components

Though our understanding of EoE's genetic components continue to evolve, the complexity uncovered thus far underscores the challenges ahead, especially when considering the potential myriad of interactions with environmental factors. Despite dizygotic twins and non-twin siblings both sharing ~50% genetic material, EoE frequency in dizygotic twins (22%) is much higher than non-twin siblings (1.3–3.5%), suggesting that a shared common environment, even in utero, plays a significant role in the development of EoE [8]. In addition, heritability among twins decreased from 99.5% to 14.5% ± 4% when a common environment (81% ± 4%) was accounted for [8]. These data and the rising incidence and prevalence of EoE suggest that a strong component of EoE's pathogenesis is the environment. Likewise, while the exact mechanisms of allergic sensitization in EoE are not known, it appears to be multifactorial but heavily influenced by the environment as indicated by the success of dietary elimination in addition to several murine models in which epicutaneous [22], ingested [27], and inhaled allergens [28] have been shown to induce experimental EoE.

Research into early-life, infectious, and geographic factors have been explored and reported. In the largest and most recent case-control study to date, Jensen *et al.* [29••] found positive associations between maternal fever, preterm labor, cesarean delivery, antibiotic use in infancy, and proton pump inhibitor (PPI) use and the development of EoE, whereas the presence of furred pets in infancy demonstrated an inverse association and breast feeding had no association with EoE. It has been hypothesized that most of these factors lead to esophageal microbial dysbiosis, potentiating a shift to a Th2-mediated immune response and EoE in certain susceptible individuals [29••,30••]; however, it is also plausible that this observed dysbiosis is a result and not a cause of EoE. For instance, differences in esophageal microbiome have been described in active EoE compared to controls; however, there were no genus differences observed between inactive EoE and controls, suggesting that microbial dysbiosis in these subjects may not be a primary event [31,32]. Unfortunately, both of these studies were underpowered to detect differences in the esophageal microbiome of EoE based on treatment [31,32]. The association between PPI use and EoE likely suffers from protopathic bias; put more simply, because the symptomatology of EoE and reflux are very similar in early life, treatment of presumed reflux symptoms may in fact be unrecognized EoE [30••].

Infectious factors, such as *Helicobacter pylori* and herpes simplex virus (HSV) esophagitis, have been shown to have inverse and possible associations with EoE, respectively [10•,30••]. *H. pylori* is thought to polarize toward Th1 immunity [33]; however, this has not been experimentally proven. There are case reports of HSV esophagitis preceding development of EoE; however, temporal association and causality have not been established [30].

Initial data on the association of rurality and EoE are mixed, with various studies demonstrating associations between EoE and suburban areas [30••] or rural areas [34] or no difference between urban and rural areas [35]; however, if the hygiene and microbial dysbiosis hypotheses are correct – or at least along the right track – then one would expect a higher incidence of EoE within urban settings, as they generally have higher incidences of air pollutants and allergens that could affect esophageal epithelial development and barrier function to a greater degree than do rural and suburban settings. On the other hand, living in a rural environment or certain regions may place individuals at higher risk of developing EoE due to increased aeroallergen exposure as demonstrated in prior murine models of EoE [28]; however, a recent review argues against inhalant aeroallergens as the cause or

worsening of EoE in humans but did not rule out the possibility of pollen-food allergen cross reactivity in EoE [36]. Overall, geographic association with EoE requires more rigorous study. Though there are increased odds of EoE in cold climates and seasonal variation of EoE diagnosis within temporal and cold climates, both require further study in order to replicate these findings and establish temporal association [30••] (Figures 1 and 2).

Future research should investigate factors that have had associations with other atopic disorders and increased use within the recent past. Exposure to bisphenol A (BPA) and phthalate, plasticizing agents found in many food and beverage containers, is ubiquitous among humans, even newborns [37]. Both upregulate Th2 immunity *in vitro* [37]. Following perinatal exposure to BPA, murine models demonstrate higher levels of airway eosinophils, increased airway reactivity, and increased levels of serum IgE in addition to altered oral tolerance with decreased levels of immunoregulatory FOXP3+ T cells. Likewise, following dermal exposure to dibutyl phthalate, murine models demonstrate increased levels of TSLP and IL-13 [33]. No clear links have been established between BPA or phthalate exposure and atopic conditions, including EoE; however, their experimental effects on the developing immune system are striking.

Smith *et al.* [38] proposed the false alarm hypothesis stating that recent increases in food allergy may be due to increased levels of advanced glycation end-products (AGEs) within Western diets via increased consumption of fructose and foods cooked by superhigh heating (microwaves, frying, and barbequing). AGEs bind to receptors of advanced glycation end-products (RAGE), which activate mast cells, T cells, and dendritic cells while also inducing a stronger Th2 cytokine response in murine models [38]. This mechanism could be a valuable environmental trigger for EoE in genetically susceptible patients and is worthy of further exploration.

Nickel, which is found in our diet and cooking utensils, has been associated with the provocation and persistence of chronic, allergic-like dermatitis symptoms; however, the mechanism remains unclear [39]. Prior murine models have elicited experimental EoE via cutaneous sensitization with allergens [28,40,41], and a high percentage of patients with EoE have atopic dermatitis [2,42], suggesting links between esophageal and epidermal epithelial dysfunction. Martin *et al.* [24•] hypothesized that dietary nickel could play a role in EoE's pathogenesis; however, this has not been validated. Despite lack of associations between these environmental components with EoE, it is important to pursue these leads and others like them that have arisen within the recent past in order to further our understanding of the heterogeneous and complex environmental milieu contributing to or potentiating EoE (Figure 2).

Interactive and epigenetic components

Few studies directly examine gene–environment interactions. Rawson *et al.* [43] described the first potential gene–environment interaction in EoE between the *TGFB1* SNP C-509T and food sensitization, with subjects demonstrating significantly more TGF-beta1- and tryptase (mast cell)- positive cells. Thus, food could induce mast cell degranulation and TGF-beta release leading to profibrotic gene expression and smooth muscle contraction. They conclude that though this SNP does not independently associate with EoE, it could represent a disease modifying allele with consequences for therapeutic selection and severity of fibrosis. Later, Jensen *et al.* [44••] continued their work in EoE's pathogenesis by examining interactions between 5 gene variants (rs6736278 within *CAPN14*, rs230009 within *CCL26*, rs3806932 within *TSLP*, rs17815905 within the *LOC283710* and *KLF13* region, and rs1800469 within *TGFB*) and six early-life factors previously associated with EoE (cesarean delivery, preterm delivery, neonatal intensive care unit [NICU] admission, breast-feeding, antibiotics in infancy, and absence of a furred pet in infancy). They observed statistically significant interactions between rs6736278 within *CAPN14* and breast-feeding and rs17815905 within the *LOC283710* and *KLF13* region and NICU admission (P = 0.02) and reported that breast-feeding offered a protective effect in the presence of this *CAPN14* variant [44••]. Their data also suggested trends toward significance for absence of furred pets in infancy and NICU admission when interacting with the variant rs3806932 within *TSLP* [44••]. Though this is initially encouraging for patients with the variant rs6736278 within *CAPN14* as their risk of developing EoE is reduced by breast-feeding, it is important to note that the potential of breast-feeding as a modifiable factor is low given that the vast majority of diagnoses are made past six months of life, when breast-feeding would no longer be the sole source of nutrition for these patients. These data could influence a family's feeding choice for subsequent children and strengthens the American Academy of Pediatrics (AAP) advocacy of breast-feeding, when safe and feasible. Ultimately, we must question why and how breast milk interacts with this variant in a protective manner. As Jensen *et al.* [44••] recognize, further investigation into these gene–environment interactions and others is necessary for advancing our knowledge on modifiable risk factors; however, we should likely focus on environmental factors that continue past the neonatal period (Figure 2).

Epigenetics is the process by which a subject's phenotype is altered through methylation and hydroxymethylation, expression of non-coding RNAs (ncRNAs), and posttranslational modifications (PTMs) of histone proteins without changes being made to the DNA nucleotide sequence [45]. Fundamentally, epigenetic modification regulates gene transcription by affecting transcription factor binding (e.g. via methylation or non-coding RNAs, such as

microRNAs) and chromatin structure (heterochromatin is densely packed and transcriptionally silent, whereas euchromatin is lightly packed and transcriptionally active) [45]. In their work to expand knowledge on genetic variants, Kottyan *et al.* [26] described the epigenetic responsiveness of *CAPN14* via increased histone 3 acetylation at lysine 27 after IL-13 exposure, thus linking PTM of histone proteins to a main EoE genetic locus. In addition, the expression of IL-13-induced eotaxin-3 (*CCL26*) mRNA in esophageal epithelial cells is under epigenetic regulation, involving histone 3 lysine 27 acetylation [46]. More recently, broader associations between differentially methylated sites and allergic sensitization have been reported [47]; however, the question remains whether these broader associations with allergy will contribute significantly to our understanding of the pathogenesis of EoE. While EoE-specific epigenetic changes are of more importance to explore presently; ultimately, we are likely to find significant epigenetic modifications that span multiple atopic disorders. Epigenetics provide a plausible link between genetics and environment, offering opportunities to advance clinical management by understanding modifiable environmental risk factors and through the development of novel therapeutics targeting this 'epigenetic machinery' [45]. Jensen *et al.* analyzed steroid responder versus non-responder EoE patients and found 18 statistically significant differentially methylated sites that could be applied in a profile to predict steroid non-responders in the future [48••]. Though this is a good start, much more work is needed to fully unpack the potential of epigenetics within the pathogenesis and management of EoE (Figure 2).

Conclusions

The rapidity of EoE's rise within the fields of Allergy/Immunology and Gastroenterology is matched only by our rapid ongoing understanding of its pathogenesis. Since the early 2000s, investigations into both the genetic basis and environmental factors involved in the pathogenesis of EoE have progressed our understanding greatly. Identified genetic variants can be broadly grouped into epithelial barrier dysfunction and Th2-mediated immune responses. Environmental factors suggest that early risk factors leading to microbial dysbiosis and/or Th2-mediated immunity are key to the development of EoE. However, limited investigations have been performed into gene–environment interactions and epigenetics. Future studies into factors such as BPA/phthalate exposure, AGEs, and nickel exposure in association with EoE have the potential to identify post-diagnosis, modifiable factors. Epigenetics has the potential to explain gene–environment interactions in EoE, identify further modifiable environmental risk factors, and advance the development of novel therapeutics. In addition, more recent work has identified subgroups within EoE, called endotypes, that are molecularly distinguishable [49•], warranting further dissection of EoE as a spectrum of disease. For instance, within pediatrics, we have seen an increase in incidence and prevalence of EoE, with diagnosis occurring as early as six months of age in our experience. Few studies have reported diagnosis of EoE as early as one year of age—one study reported 10 patients that were <12 months of age at diagnosis and described their clinical characteristics as a part of the larger group of patients diagnosed with EoE at ≤5 years [50]; however, it did not evaluate the group diagnosed at ≤12 month in isolation. In-depth study of patients at such an early timepoint will likely yield further novel insights into the pathogenesis, natural history, and management of EoE.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported in part by N.I.H.K24 DK100303 (J.L.) and N.I.H. U19 AI070235, N.I.H. R01 AI124355, N.I.H. R37 A1045898, and U54 AI117804; the Campaign Urging Research for Eosinophilic Disease (CURED) Foundation; the Buckeye Foundation; and the Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning (M.E.R.).

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