

REVIEW

# Genetic and molecular mechanisms leading to eosinophilic esophagitis

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## ABSTRACT

From the epidemiologic studies, to the first genome wide association study in 2010, the understanding of the molecular pathogenesis of EoE has been both inspiring and puzzling. Epidemiologic studies have highlighted the contribution of the genetic in the EoE disease by emphasizing the presence of familial type of EoE, but has also revealed the complexity of its transmission that does not follow a Mendelian inheritance. The molecular pathogenesis advances have helped in the understanding of the mechanisms underlying this esophageal inflammation but has also allow the identification of candidate genes for which single nucleotide polymorphisms (SNP) are associated with the disease. Recently, the genome wide analysis of more than half a million single nucleotide polymorphism has allowed the identification of gene variations associated with the EoE disease and has led to substantial advance in the understanding of the molecular mechanisms leading to EoE. Undeniably, EoE is a complex polygenic disease and we certainly are only at the ground level of its detailed comprehension.

**Key words:** Eosinophilic esophagitis. Pathogenesis. Genetic. Polymorphism. Expression.

## WHAT WE LEARNED FROM EPIDEMIOLOGY STUDIES

Epidemiologic data performed on EoE cohorts have alighted the genetic component of EoE disease (1-3) and more particularly the strong genetic heritability of the disease. In Noel et al. study, 6.8 % of the EoE patients had at least one family member diagnosed with EoE disease (2). This suggests that some genetic factors are transferred by the parents and are responsible for disease susceptibility. Asthma has been shown to have genetic and environmen-

tal factors involved in the heritability of the disease. It is believed that 50 % of the heritability of allergic disease is due to genetics (4). When EoE is compared to another allergic disease like asthma, a sibling of asthmatic child has a recurrence risk ratio of 2, the sibling of a EoE patient has a recurrence risk ratio of 80 (4-6). This suggests that there is a stronger genetic background predisposing for EoE compared to other allergic disease like asthma. This heritability has however a non-Mendelian pattern of transmission and seems to be involving numerous genes (polygenic etiology) like other allergic diseases. However, the presence of EoE patients with unaffected homozygote twins suggest that the environment (and thus the epigenetics factors) may have a fundamental importance on the disease onset. There are also sporadic cases of EoE, that occur in patients that do not have any related family member affected with EoE (6). There is so far no differentiating marker or parameter that can separate the two presentations (sporadic or familial). The eosinophils, molecular changes or clinical presentation seems not significantly different to the exception of familial patients with mucosal furrows which present lower peak eosinophil counts in the distal esophagus compared with sporadic patients (6). However this has not been yet confirmed in another independent study.

It is now well documented that about three quarter of the EoE patients are males (1-3); this gender bias suggests that males are genetically predisposed to develop the disease. We can hypothesize that the sexual chromosomes or hormones may predispose to EoE; the scientific evidence for such a hypothesis is still largely missing. Finally, EoE

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disease seems still to favor Caucasian with white ancestries (1-3). In the study from Spergel 2009, 90 % of the EoE patients were White, 4 % African-American, 3 % Asian and 3 % others (3). Polymorphisms and more particularly the allele frequency of numerous single nucleotide variants (SNVs) differ from population to population. These SNVs may have conferred selective advantage in some populations to adapt to their specific environment, allowing the maintenance of this allele frequency difference between populations. In the Caucasians with white ancestries, some of these genetic polymorphisms either protect the other population or may predispose Whites to develop EoE. Indeed it has been recently shown that patient with inherited connective tissue disorders are at risk (risk multiplied by 8) of developing EoE.

Historically, study of the pathogenesis came before the study of the genetic susceptibility. Thus, in EoE disease, the genetic analysis have first derived from the known molecular pathogenesis of EoE: Candidate gene approach. It is only recently that genetic analysis of SNVs has led to significant molecular insights.

## THE UNDERSTANDING OF THE MOLECULAR PATHOGENESIS

The first study that paved the way for the understanding of the molecular pathogenesis of EoE was published in 2001 by Straumann et al. In this study, the authors identified key molecules overexpressed in the esophagus of the human EoE biopsies that underscored the disease as a Th2 inflammation that support the presence of eosinophils (IL-5, IL-13) (7). Murine models have also highlighted the involvement of Th2 cytokines in disease pathogenesis (such as IL-5, IL-13, IL-4), Th2 cell chemoattractants (such as eotaxins), or cytokines involved in tissue remodeling (TGF- $\beta$ , IL-5) (8).

Microarray analysis of the transcriptomic changes (mRNA expression) of EoE patients compared to non-EoE patients have highlighted numerous genes expression changes in EoE. More than 500 gene expression levels are dysregulated in EoE patient esophageal biopsies (9). These genes are not necessarily all involved in the pathogenesis and some may just be markers of the disease state: Some genes may just be increased because the cells that express the mRNA are infiltrating the tissue in EoE. However, numerous genes are potential candidate for having an important role in the pathogenesis. Eotaxin-3 (*CCL26*) has been identified as the number one gene dysregulated in EoE tissue biopsies. This is central since eotaxin-3 is a chemokine involved in the recruitment of eosinophils. In this study *Ccr3* KO mice, (receptor for the eotaxin family) do not recruit eosinophils in the esophagus (9). This study highlighted that eotaxin-3 is strongly correlated with the eosinophils levels in the esophagus. Interestingly, a SNP (rs2302009,

SNP +2,496 T→G) in the 3'UTR of the eotaxin-3 gene was found to be associated with the disease susceptibility. And this was the only SNP validated so far in a transmission disequilibrium test (9).

The remodeling occurring in the lamina propria and underlying the esophageal epithelial inflammation can be significant. Genetic seems to account for the response of the patients to the steroids. Numerous cytokines are involved in EoE inflammation such as, IL-13 or TGF- $\beta$ . TGF- $\beta$  is involved in numerous processes including a remodeling of underlying tissue. S. Aceves have indeed uncovered that *TGFB1* was highly overexpressed in the esophagus of EoE patients (10). TGF- $\beta$ 1 is responsible for the overexpression of molecules involved in the remodeling process such as collagen, periostin (11). A studied showed that a SNV in the promoter of *TGFB1-509* (C/T) was associated with the numbers of lamina propria TGF- $\beta$ 1 positive cells in patients (10). The patients bearing the allele C on both chromosomes (CC genotype) were more likely to be responders to glucocorticoid treatment. Indeed, the use of steroid is associated by increased expression of other glucocorticoid-specific genes such as esophageal *FKBP51* level that may have predictive value for glucocorticoids response in patients with EoE (12).

In addition to the remodeling in the lamina propria, the histology of the esophageal epithelium of EoE patients sees large modifications (13). Numerous inflammatory cells infiltrate the esophageal epithelium and an extensive epithelial hyperplasia occurs and leads to elongated papillae (13). Indeed, the histopathology is quite nicely fitting with the molecular dysregulation observed. Epithelial changes observed in EoE go together with a downregulation of the epithelial differentiation genes (14,15). Most of these genes are located on a locus of chromosome 1 in the epidermal differentiation complex gene locus that encompasses involucrin, filaggrin, SPRR proteins. Filaggrin is a key element of the skin cornification and is thus involved in skin barrier function. A decrease in filaggrin expression in the skin, due to a downregulation or DNA variants, leads to a disturbed barrier function. A study recently highlighted the fact that 2282del4, a loss-of-function genetic variant in the gene of filaggrin, usually known to be associated to allergic sensitization and atopic dermatitis, is associated with EoE disease (14). In EoE, the Th2 cytokine, IL-13, is highly upregulated and is responsible of numerous dysregulation by epithelial gene (16,17). IL-13 down regulates filaggrin mRNA in EoE patients and may lead to an esophageal epithelial barrier dysfunction in most EoE patients (14). On the other hand, the mutations in filaggrin gene are present in only 4-6 % of the proband. These suggest that the loss of function of filaggrin in EoE is mainly due to the inflammatory context rather than the genetic modification of the gene that is mutated in only a minority of the patients.

## THE MOLECULAR PATHOGENESIS DERIVED FROM GENOME WIDE ANALYSIS

A genome wide analysis was carried out on 351 EoE patients and 3104 unrelated controls. This type of analysis allows the identification of SNVs associated with diseases, without any previous evidence or rationale for a function in the disease. This approach is very different from the one described above, in which the polymorphism has been found in a candidate gene approach as their role in the pathogenesis was already understood before the SNVs in the candidate genes were analyzed. The involvement of

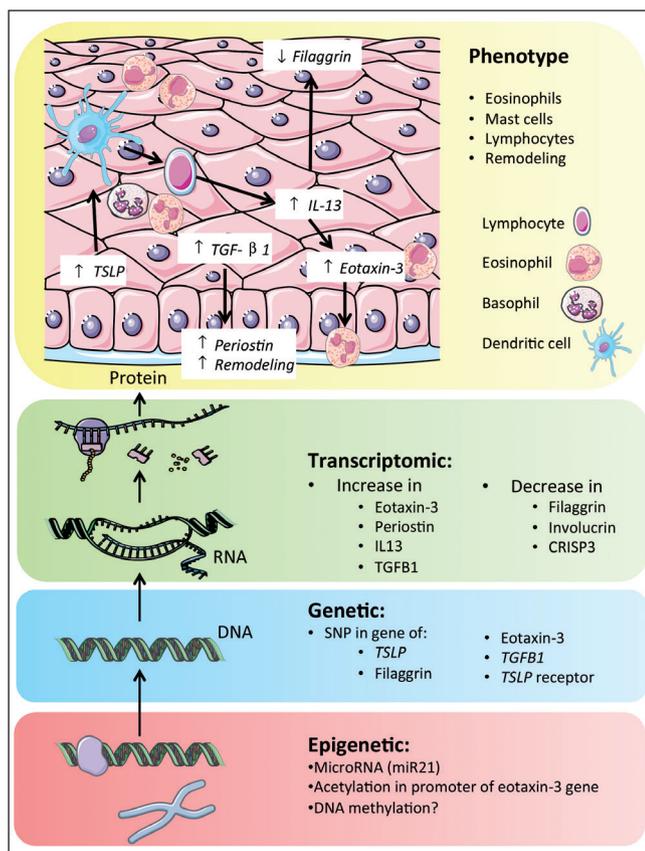


Fig. 1. Genetic variants and pathogenesis associated with EoE disease. EoE histopathology is characterized by an infiltration of eosinophils, mast cells and lymphocytes, an hyperplasia of the epithelial cells and an elongation of papillae. When the lamina propria is present in the biopsies, an intensive remodeling can be observed. The transcriptomic analysis has facilitated the identification of genes such as eotaxin-3 and periostin that are keys for the recruitment of eosinophils in the tissue. Filaggrin, known to be involved in skin barrier function is decrease in EoE. The Th2 cytokine IL-13, is involved in the dysregulation of these genes in EoE. TGF-beta is overexpressed and is involved in tissue remodeling characteristic of the lamina propria of EoE. Single nucleotide polymorphisms in the TSPL, TSLP receptor, Eotaxin-3 and filaggrin genes, have been shown to be associated with EoE disease. The understanding of epigenetic modifications in EoE is at its groundwork. So far, acetylation in the promoter of eotaxin-3 and microRNA expression changes have been observed. Inherited methylation changes in EoE have not been yet described. Figure was produced using Servier Medical Art, www.servier.com.

the genes described below has thus been explained posteriorly to their identification with this technique. This analysis tested more than half a million common SNVs (18). From this study, the locus 5q22 encompassing *TSLP* and *WDR36*, appeared to be highly associated with the disease. *TSLP* is the most likely gene associated to EoE in this region as its expression is upregulated in EoE patients biopsies compared to control biopsies with a normal histology. TSLP is an epithelial cell-derived cytokine known to regulate allergic inflammation and to activate multiple immune cells (19-22); such as dendritic cells that induce Th2 cytokine production in naïve T cells (23). The association of the SNV rs3806932 with the EoE disease was found to be highly significant in a discovery cohort and in a replication cohort. While *TSLP* mRNA expression seems to be increased in most EoE patients and while, the genotype influence the level of *TSLP* expression in the disease, the minor allele frequency was 31-37% in the EoE population and 45-46 % in the controls (18). This is emphasizing that while associated to the disease susceptibility, this SNV presence, in the majority of the cases does not preclude for the disease to occur nor prevent an individual from being healthy. Allergic diseases are comorbidities of EoE patients in the pediatric and adults EoE populations (24). Since variants in this gene locus are also associated with other allergic diseases (25), it is important to separate allergic from non-allergic EoE and to compare EoE patients to allergic controls. When some SNVs in 53 genes were analyzed in EoE patients compared to atopic controls the association of *TSLP* with EoE was maintained while other association in Th2 gene SNVs such as *IL4* were lost (26). In addition to its role in activating dendritic cells to prime a Th2 response, TSLP has recently been found in a murine model of EoE to elicit basophil response and to be involved in EoE pathogenesis (27).

It is interesting to note that cytokine receptor-like factor 2 (*CRLF2*) gene, that codes for the receptor of TSLP, is located on the pseudoautosomal region of the sexual chromosomes (conserved region to both X and Y chromosomes). A SNV (rs36133495) in *CRLF2* gene, that modifies the protein was found to be associated to EoE in males and is thus the first molecular mechanism that associate EoE to its male predominance (26).

It is important to keep in mind that these associations point to an increased risk of developing the disease but a significant portion of the normal population have these mutations and will never develop EoE.

## ONE WORD ON EPIGENETICS

Epigenetics changes are the modifications, heritable or non-heritable, that are not directly linked to the DNA sequence. Epigenetic are responsible of the changes and differences between identical homozygotes twins for examples. Epigenetics mechanisms are numerous such

as DNA methylation, histone modification, acetylation, microRNA. In EoE, histone 3 acetylation of the eotaxin-3 promoter occurs in the esophageal epithelial cells, in the promoter of eotaxin-3 gene during the disease (28). Another epigenetic mechanism that occurs in EoE is the dysregulation of MicroRNA. MicroRNAs are non-coding RNA sequence, that pair with a complementary messenger coding RNA, and thus leads to the mRNA degradation or translation repression. Numerous microRNAs are differentially regulated in EoE esophageal biopsies (29-31). These microRNAs seem to be highly dependent on the inflammatory state of the esophagus since most microRNAs expression levels are normalized by a successful corticosteroid therapy. Interestingly, some of these microRNA where found to be targeting genes involved in the Th2 differentiation such as miR21 that may modulated the Th1/Th2 response (30). Other MicroRNAs might be used as noninvasive biomarkers as found dysregulated in the plasma of EoE patients. Other studies are required to confirm the sensitivity and or specificity of such biomarkers (30).

## CONCLUSION

A single nucleotide variation in a gene can have huge consequence on disease onset when only one gene drives the disease. Here, since EoE is a disease with a polygenic etiology, several genes are certainly responsible for the disease susceptibility. Moreover, the environment certainly influences the onset of the disease in genetically predisposed individuals. It is suspected that multiple SNPs association will increase the odd of developing the disease. In EoE, analyses done so far have led to 2 key findings: a) The molecular changes in the diseased esophagus is highly conserved in most of the EoE individuals; and b) on the other side, the SNVs associated with EoE are only present with a minorities of the EoE population, and so far cannot accurately predict the disease. The missing heritability seems to be particularly important and highlights the fact that some variations with very small allele frequency might play an important role. It is most likely that a subset of several SNVs will have a higher predictability than the independent SNVs found so far. It is important to keep in mind that the environmental factors seems to influence greatly EoE and it is thus key to understand what these factors might be. Epigenetic studies in the next years will certainly help unraveling these influencing factors.

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