

ORIGINAL CONTRIBUTION

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Food allergen triggers are increased in children with the TSLP risk allele and eosinophilic esophagitis

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Abstract

Objectives: TSLP has been shown to be associated with eosinophilic esophagitis (EoE). Specifically, children with EoE often have the nucleotides AA or AG instead of GG at the single nucleotide polymorphism position RS3806932. Presently, the phenotypic characteristics in EoE children with the TSLP EoE risk allele are unknown.

Methods: A retrospective analysis was performed of all children with EoE who had TSLP genotyping at The Children's Hospital of Philadelphia from 2008–2014. EoE food allergen triggers, presence of atopic features, IgE mediated food allergy and skin prick testing results were reviewed. The number and type of EoE food allergen triggers were compared with genotype using chi-square analysis. Primary cell cultures from EoE patients with or without the risk allele were stimulated with ovalbumin and TSLP secretion was measured by ELISA.

Results: Fifty three of 309 patients were found to have no copies of the TSLP risk allele, whereas 256 patients were found to have one or more copies of the risk allele. There was an increase in the number of patients with three or more EoE food allergens among those who were either homozygous or heterozygous for the risk allele compared to those without the risk allele ($P < 0.0001$). This was independent of their atopic background. Primary cultures from patients homozygous for the risk allele had greater TSLP secretion than those isolated from heterozygous patients.

Conclusions: The TSLP risk allele is associated with having multiple EoE food allergen triggers. This novel EoE genotypic-phenotypic correlation may guide future treatment for those with the TSLP risk allele.

Introduction

Eosinophilic esophagitis (EoE) is an atopic disease characterized by an infiltration of eosinophils limited to the esophagus. Food allergens are the antigens that most

commonly trigger the immunologic response in patients with EoE^{1–7}. Multiple studies suggest that food allergens elicit T helper lymphocyte Type 2 (Th2) inflammation characterized by the presence of the typical Th2 cytokines (i.e., interleukin (IL)-4, IL-13, IL-5), chemokines (chemokine (C–C motif) ligand (CCL) 26, CCL5), and other inflammatory cells (i.e., eosinophils, mast cells, Th2 lymphocytes, basophils) independently from an Immunoglobulin (Ig)-E mediated allergic reaction⁸. It is believed that in genetically predisposed individuals, a dysfunctional epithelium facilitates a delayed cellular mediated allergic response^{7,8}.

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The foods that most often cause pediatric and adult EoE are milk, wheat, egg, and soy^{7,9,10}. Most individuals respond to a single food elimination diet, whereas a subgroup of people require elimination of multiple foods from the diet in order to control their symptoms^{7,9,11}. Currently, patients are initially either placed on a 6 food elimination diet or on a milk free diet. Following this, a series of dietary changes are made based on the histopathological and clinical response to the diet¹². Establishing the correct diet is often difficult and requires multiple upper endoscopies in order to identify the trigger foods. In addition, there is no indication as to which of the two initial dietary approaches will be most beneficial for a particular patient¹². Therefore, there is a need to find a way to predict who will respond better to the single food vs. the multiple food elimination diet.

From a genetic standpoint, our institution previously described the association between thymic stromal lymphopoietin (TSLP)/WDR36 locus and EoE¹. In a collaborative effort, Rothenberg et al. showed for the first time the presence of the single-nucleotide polymorphism (SNP) RS3806932 in the promoter region of the TSLP gene on 5q22. The protective minor allele (G) is present in a higher percentage of control patients (45.8%) compared to EoE patients (31.2%). Individuals homozygous for the TSLP risk allele (A)(AA or AG) have increased TSLP expression and basophil infiltration in the esophageal epithelium compared to those carrying homozygous (GG) protective minor alleles^{1,2}. This locus has been confirmed in two subsequent independent studies as a significant risk factor for EoE^{13,14}.

It has also been previously shown that chicken egg ovalbumin (OVA) induces differentiated esophageal epithelial cells to secrete TSLP via unknown mechanisms, an effect abolished by budesonide and NF- κ B inhibition^{15,16}. Therefore, similar to other atopic allergic diseases such as asthma, OVA can be used as a model of an antigen-induced atopic inflammatory process in EoE (i.e., production of TSLP from differentiated epithelial cells)¹⁵.

TSLP is a pro-inflammatory cytokine in the interleukin 7 family that is secreted by epithelial cells in response to stressors such as infection, damage or allergens, and specifically promotes a Th2 immune response¹⁷. Recent studies showed that TSLP may promote Th2 inflammation in EoE through basophil infiltration^{2,5}. TSLP is activated not only in EoE but also in several allergic diseases such as atopic dermatitis and asthma¹⁵.

There are no large scale studies to date which correlate the TSLP genotype with phenotype in pediatric patients with EoE. The aims of this study were to determine whether the presence of one or more copies of the TSLP risk allele is correlated with having multiple EoE food allergen triggers and to assess the relationship between TSLP genotype and patient phenotype in a carefully characterized population.

We hypothesized that the presence of the TSLP risk allele would correlate with a more atopic phenotype and having multiple EoE food allergen triggers.

Furthermore, we sought to investigate possible correlations between the TSLP risk allele polymorphism and epithelial responses to food antigens in vitro.

Methods

Study population

This study was approved by the Institutional Review Board at The Children's Hospital of Philadelphia (CHOP) and written informed consent was obtained from the legal guardians. All confirmed pediatric EoE patients ages 0–18 years old who were seen in the outpatient clinic at CHOP between 2008 and 2014 were invited to participate in this genetic study. A total of 347 patients consented to the study, of which 14 patients were excluded from the study because they did not meet the strict definition of EoE due to either having another gastrointestinal diagnosis, or because they did not have an 8 week trial of high-dose proton pump inhibitor medication before endoscopy. Three patients were excluded due to very limited medical records, and 21 patients were excluded because they did not undergo multiple endoscopies in an attempt to identify their food allergen triggers. Data from three hundred and nine patients were analyzed. A further sub-analysis was then done excluding an additional 23 patients who did not have any food allergen triggers identified despite multiple endoscopies. EoE diagnosis was confirmed by upper endoscopy with biopsy showing isolated esophageal eosinophils of greater than or equal to 15 eosinophils per high powered field after gastroesophageal reflux had been ruled out by a proton pump inhibitor trial. The maximum histologic eosinophil count was used to determine potential response to treatment.

Genotyping

Genotyping was completed at the Center for Applied Genomics at CHOP, as previously described by Sleiman et al.¹⁴. The samples were genotyped on the Illumina HumanHap550 or HH610. Standard quality control parameters were applied. Samples with chip-wide genotyping failure rate <5% were excluded from the study, as were SNPs with minor allele frequencies of <1%, genotyping failure rates of greater than 2% and Hardy-Weinberg P -values < 1×10^{-6} . Smartpca, a part of the EIGENSTRAT package, was used on 100,000 random autosomal SNPs in linkage equilibrium to compute principal components on the dataset, thereby determining genetic ancestry. K means clustering was used to cluster the patient samples into four continental ancestry group clusters using the kmeans package in R. These ancestry groups included Caucasian, African (including African American), Asian, and Native American/Hispanic. A risk

allele was defined as a genetic variation in the gene that increased the probability of having EoE¹⁴. RS3806932 was the TSLP SNP that was previously identified as being associated with EoE by Rothenberg et al. and was the TSLP risk allele that was assessed in this study¹.

Identification of clinical characteristics

After genotyping was complete, a retrospective chart review was performed of all of these pediatric patients. Clinical data that was reviewed included atopy, IgE mediated food allergy, skin prick allergy testing, confirmation of histologic diagnosis of EoE, identification of EoE food allergen triggers with serial endoscopies, and gender. Atopy was defined as having one or more of the following: asthma, allergic rhinitis or atopic dermatitis. The data were also evaluated in a sub-analysis including IgE mediated allergy as a fourth possible atopic characteristic. The number of patients with at least one of these atopic features was compared with the number of patients with no atopic features.

Identification of EoE food allergen triggers

A food was identified as an EoE food allergen trigger if a patient was histologically diagnosed with EoE while eating the specific food, that food was eliminated from the diet, and the number of esophageal eosinophils normalized once the food was removed. A food was also considered to cause EoE if reintroduction of a particular food led to recurrence of esophageal eosinophilia¹¹. We excluded a food as a potential allergen trigger if a patient was started on a swallowed corticosteroid prior to an endoscopy. If a patient was started on a swallowed corticosteroid after EoE food allergen triggers were identified, then they were still included in the study. All subjects included in this analysis underwent identification of food triggers.

Association of genotype with phenotype

The presence of at least one copy of the TSLP risk allele was compared with the absence of the TSLP risk allele. The data was statistically analyzed using chi-square and logistic analysis (STATA). Results were considered statistically significant if the *P* value was less than 0.05.

Isolation and stimulation of primary esophageal epithelial cells

After obtaining Institutional Review Board approval at CHOP and following written informed consent, 2–4 additional pinch biopsies were obtained from the distal esophagus during routine upper endoscopy. Primary epithelial cells (EPCs) were cultivated from these esophageal biopsies using previously published methods⁴. Briefly, biopsies were placed in Hanks BSS buffer, transferred to dispase (0.6 µl/mL in PBS), then trypsinized (trypsin-EDTA) for 20 min at 37°C. Trypsin was

inactivated using soybean trypsin inhibitor (Sigma, St. Louis MO), and biopsies were agitated to release epithelial cells. Cells were pelleted, resuspended, and seeded. Cells used for these experiments were used in passages 2–4. OVA (Sigma) was used at concentrations of 1 mg/mL, as previously described to stimulate cell cultures from patients with established genotypes¹⁵. These 12 patients were the only individuals in this study that also consented to have biopsy specimens obtained and cell lines created. All available specimens with genotyping were included.

ELISA

TSLP secretion was quantified in cell supernatants using a TSLP ELISA kit (eBiosciences, San Diego, CA) according to manufacturer's instructions. TSLP concentrations were calculated based upon a standard curve generated by human recombinant TSLP provided by the company. Results were expressed as the mean ± standard error of the mean (SEM) in pg/mL.

Results

We describe a population of 309 pediatric patients with biopsy-confirmed EoE and known food allergen triggers whose TSLP gene was genotyped. Eighty two percent were males (*N* = 253). Ninety one percent (*N* = 280) were Caucasian. Eighty three percent of the patients had a TSLP risk allele: 37% were found to be homozygous for the risk TSLP allele, 17% had no copies of the TSLP risk allele and, 45% were heterozygotes. By ethnicity, the TSLP risk allele frequency in this study population was 0.87 Caucasian, 0.06 African American, 0.04 Hispanic, 0.02 Asian (Supplemental Table 1).

Correlation between genotype and atopy

First, we examined if the TSLP SNP identified as a risk factor for EoE was a risk factor for atopy in patients with EoE⁷. Interestingly, there was not a statistically significant difference between prevalence of the individual diagnoses of asthma, allergic rhinitis (AR), atopic dermatitis (AD), IgE mediated food allergy (IgE-FA) or a positive skin prick test between the two genotype subgroups, even though having AR was slightly more prevalent in children with the TSLP EoE risk allele. The gender distributions between the various genotypes were equal (Tables 1–2).

TSLP risk allele is correlated with the presence of multiple EoE food allergen triggers

There was a statistically significant increase in the number of patients with two or more EoE food allergen triggers and three or more food allergen triggers among those who had one or more copies of the TSLP risk allele compared to those without the risk allele (*P* < 0.0001) (Table 2). This correlation also held true when analyzing the data after those patients with no identified food

Table 1 Clinical Characteristics of EoE Patients Based on TSLP EoE Risk Genotype

| | Male Gender <i>n</i> (%) | Atopy <i>n</i> (%) | Asthma <i>n</i> (%) | AR <i>n</i> (%) | AD <i>n</i> (%) | IgE-FA <i>n</i> (%) | EoE with ≥3 food allergen triggers <i>n</i> (%) |
|---|-----------------------------|-----------------------|------------------------|-----------------|-----------------|------------------------|--|
| TSLP homozygous risk allele (<i>n</i> = 117) | 93 (79%) | 106 (91%) | 74 (63%) | 95 (81%) | 33 (28%) | 56 (48%) | 77 (66%) |
| TSLP heterozygous risk allele (<i>n</i> = 139) | 116 (83%) | 134 (96%) | 89 (64%) | 120 (86%) | 34 (24%) | 54 (39%) | 97 (70%) |
| No TSLP risk allele (<i>n</i> = 53) | 44 (83%) | 47 (89%) | 31 (58%) | 40 (75%) | 17 (32%) | 26 (49%) | 16 (30%) |

Table 2 TSLP EoE SNP Risk Allele is Associated with EoE Triggered by Multiple Food Allergens

| | TSLP risk allele (homozygous/heterozygous), % | No TSLP risk allele (%) | <i>P</i> value |
|---|--|----------------------------|----------------|
| Male Gender | 82 | 83 | 0.813 |
| Any atopic disease | 94 | 89 | 0.191 |
| Asthma | 64 | 58 | 0.478 |
| AR | 84 | 75 | 0.137 |
| AD | 26 | 32 | 0.379 |
| IgE-FA | 43 | 49 | 0.416 |
| 2 or more EoE food allergen triggers (analyzing entire population of <i>N</i> = 309) | 85 | 56 | <0.0001 |
| 3 or more EoE food allergen triggers (analyzing entire population of <i>N</i> = 309) | 68 | 30 | <0.0001 |
| 2 or more EoE food allergen triggers ^a (analyzing sub-population of <i>N</i> = 286) | 75 | 10 | <0.0001 |
| 3 or more EoE food allergen triggers ^a (analyzing sub-population of <i>N</i> = 286) | 62 | 6 | <0.0001 |

^aPatients with zero food allergen triggers identified were eliminated from this sub-analysis

triggers were excluded (Table 2). The number of food allergen triggers categorized by TSLP genotype is detailed in Supplemental Table 2.

We performed a multivariate logistic regression to correct for the risk of having two or more EoE food allergen triggers and three or more food allergen triggers and having either asthma, AR, AD, or IgE-FA, and showed that even after correction for the atopic condition, the TSLP EoE risk allele was still significantly associated with EoE due to multiple food allergens. This was the case for the entire population (*N* = 309), as well as for the sub-population after those with no identified food triggers were excluded (*N* = 286) (Table 3 and Supplemental Table 3). Milk was the most common food trigger for patients with at least 1 copy of the TSLP risk allele, with 50 percent of patients in this sub-group having milk as an EoE trigger (Table 4). Following milk, the second most common EoE food allergen trigger for this risk allele group was meat (41%). In addition, there was a statistically significant increase in the number of

patients with a food allergen trigger of either meat, wheat, or soy among those with at least 1 copy of the TSLP risk allele compared to those without the risk allele (Table 4). Foods defined as meats were pork, beef, turkey, and chicken. Vegetables included sweet potato, corn, carrot, broccoli, cauliflower, and spinach. Fruits included cherry, apple, pear, tomato, peaches, mango, and grapes. The three most common food trigger combinations among patients with two more EoE-causative foods were milk and meat, followed by milk and wheat, as well as milk and soy (Table 5). When analyzing all patients with three or more EoE food allergen triggers, regardless of TSLP genotype, milk was also the most common causative food, followed by meat, soy and wheat.

Pediatric EoE patients homozygous for the TSLP risk allele have enhanced responses to ovalbumin *in vitro*

We obtained EPCs from the 12 EoE patients in our cohort that also consented to have biopsies taken and cell

Table 3 Multivariate Logistic Analysis of TSLP EoE Risk Alleles and Presence of Two or More Food Allergens Triggers^a

| TSLP risk allele (homozygous/heterozygous) | Odds Ratio | Standard Error | P value | 95% confidence interval |
|--|------------|----------------|---------|-------------------------|
| ≥ 2 EoE food allergen triggers | 4.795348 | 2.027524 | <0.0001 | 2.093741–10.9829 |
| Asthma | 0.8618146 | 0.3618593 | 0.723 | 0.3784519–1.962533 |
| AR | 1.028315 | 0.5208934 | 0.956 | 0.3810213–2.775254 |
| AD | 0.7086044 | 0.2843965 | 0.391 | 0.3226832–1.556078 |
| IgE-FA | 0.6648271 | 0.249292 | 0.276 | 0.3188096–1.386392 |
| Any Atopic Disease | 0.9422993 | 0.8018175 | 0.944 | 0.177782–4.994475 |
| Male Gender | 0.9475623 | 0.1969328 | 0.796 | 0.6305241–1.42401 |

^aPatients with zero food allergen triggers identified were eliminated from this sub-analysis

lines created, which included 5 patients homozygous for the TSLP risk allele, and 7 heterozygous patients. Demographics and esophageal biopsy eosinophil count for each patient are detailed in Table 6. When stratified with regard to genotype, EPCs from patients homozygous for the TSLP risk allele had significantly enhanced TSLP responses to OVA compared to TSLP risk heterozygous cell lines (Fig. 1).

Discussion

For the first time we identify a clinical correlation between TSLP genotype and number of food allergen triggers for pediatric EoE patients. We show that the presence of the TSLP risk allele (AA or AG at RS3806932 on the TSLP gene) is correlated with patients having multiple EoE food allergen triggers independently of the atopic status of the patient.

Several genome wide and single polymorphism genetic studies have shown that compared to the general population, multiple SNPs at the TSLP genomic locus are associated with increased asthma susceptibility [RS3806933 (−847C>T), RS1837253]^{2,5,17–23} or protection (RS1837253, RS2289276)^{2,5,15,18,19,23}, increase risk of AD TSLP-SNPs (RS1898671, RS11466749, RS10043985 and RS2289276)^{24,25} and atopy in general in different ethnic backgrounds, gender or age^{1,25,26}.

In our EoE patient population we confirmed a very high prevalence of atopic diseases compared to the general population. Indeed we found the prevalence of atopic disease to be over 90% in our study group compared to the estimated 24–42% prevalence in the general pediatric population^{18,19}. Similarly, the prevalence of IgE mediated food allergy, asthma and allergic rhinitis was elevated in our study group compared to the prevalence of those diseases in healthy children (60% vs 8.8%; 80% vs 13%; 45% vs 4.9%)^{18,19}. However, we found that the TSLP EoE risk allele was not associated with atopy in general or risk of having a particular atopic disease, confirming that the

TSLP EoE risk allele may be specifically linked to the immune dysregulation involved in the severity of non-IgE mediated food allergy in EoE. This finding is consistent with recent studies that have suggested a genotype-phenotype correlation between TSLP and other atopic diseases. Significant associations were also observed for one of the most severe manifestations of AD, eczema herpeticum, and two additional TSLP-SNPs (RS1898671 and RS2416259) and four IL7R-SNPs (RS12516866, RS10213865, RS1389832, and RS10058453)²⁷. In addition, in Japan, Iijima et al.²⁸ reported that TSLP genetic polymorphisms may play a role in the development of asthma in adult smokers.

We also observed that genetic background may influence TSLP production from esophageal epithelial cells in response to an antigen such as OVA. It has been previously demonstrated that OVA is able to induce TSLP in children with and without EoE, and specifically both with and without clinical egg induced EoE¹⁵. This is in line with the classical use of OVA to reproduce atopic inflammation. Both acute and chronic sensitization with OVA is known to cause key features of clinical asthma in murine models, even if egg allergy is rarely a trigger of chronic asthma in humans^{15,29}. Similarly, in this study we used OVA as a model of antigen induction of TSLP from differentiated esophageal epithelial cells, as OVA has been the only allergen able to directly induce esophageal epithelial cells to secrete TSLP¹⁶. Although the mechanisms that lead OVA to induce TSLP is unknown, it is inhibited by steroids and this antigen induced production of TSLP from epithelial cells can be a relevant model to study in vitro TSLP production from human esophageal epithelial cells^{15,16}.

In EoE, it has been demonstrated that esophageal epithelial-derived TSLP may play a central role in inducing resident dendritic cells to orchestrate a Th2-type polarization. A recent study described a mouse model of EoE-like disease esophageal eosinophilic infiltration and

Table 4 EoE Food Allergen Triggers Categorized by TSLP Genotype

| Food allergen | No TSLP risk allele; n,% (n = 53) | At least 1 TSLP risk allele; n,% (n = 256) | P value |
|-------------------------|-----------------------------------|--|---------|
| Milk | 21, 40% | 128, 50% | 0.169 |
| Egg | 7, 13% | 56, 22% | 0.154 |
| Wheat | 8, 15% | 72, 28% | 0.049 |
| Tree Nut | 2, 4% | 33, 13% | 0.057 |
| Peanut | 9, 17% | 51, 20% | 0.622 |
| Shellfish | 1, 2% | 23, 9% | 0.079 |
| Soy | 8, 15% | 72, 28% | 0.049 |
| Meat ^a | 12, 23% | 105, 41% | 0.012 |
| Fruit ^b | 6, 11% | 49, 19% | 0.176 |
| Vegetables ^c | 10, 19% | 77, 30% | 0.099 |
| Legumes ^d | 2, 4% | 13, 5% | 0.688 |
| Rice | 3, 6% | 23, 9% | 0.428 |
| Oat | 4, 8% | 33, 13% | 0.275 |
| Barley | 1, 2% | 20, 8% | 0.119 |
| Seeds | 1, 2% | 5, 2% | 0.975 |
| Other | 0, 0% | 5, 2% | N/A |

^aMeats included pork, beef, turkey and chicken

^bFruits included cherry, apple, pear, tomato, peaches, mango and grapes

^cVegetables included sweet potato, corn, carrot, broccoli, cauliflower and spinach

^dLegumes included peas and green beans

subsequent esophageal food impactions were dependent upon TSLP^{2,5,17,20}. In this paper we demonstrated that antigen dependent TSLP esophageal epithelial production is increased in patients homozygous for the TSLP EoE risk allele, suggesting that the genetic background associated with more severe non IgE mediated food allergy may also be associated with increased antigen induced TSLP expression from differentiated epithelial cells. Future studies will be necessary to study the differences between epithelial cells derived from larger cohorts of EoE patients which specifically include patients that did not have any allelic risk of EoE. Future research may also include stimulation of the cell lines with poly I:C to demonstrate whether the differences in inducible-TSLP secretion was specific to OVA stimulation vs. TLR3 stimulation.

This is the first study reporting clinical relevance of genotypic characterization of pediatric patients with EoE in a large patient cohort. This finding, if reproduced in a larger, prospectively studied population, could be important in helping the physician to determine the best treatment option for the individual patient, in a

Table 5 Most Common Food Allergen Combinations Among Patients with two or more EoE Causative Foods

| Food allergen | Number of patients |
|-------------------------|--------------------|
| Milk, meat | 61 |
| Milk, wheat | 43 |
| Milk, soy | 43 |
| Milk, egg | 42 |
| Meat, vegetable | 41 |
| Milk, vegetable | 33 |
| Wheat, meat | 33 |
| Wheat, soy | 31 |
| Meat, soy | 31 |
| Meat, egg | 30 |
| Wheat, vegetable | 24 |
| Egg, wheat | 22 |
| Egg, soy | 20 |
| Milk, meat, egg | 20 |
| Milk, meat, wheat | 20 |
| Egg, vegetable | 19 |
| Milk, egg, soy | 18 |
| Milk, vegetable, meat | 16 |
| Milk, egg, wheat | 16 |
| Milk, meat, soy | 16 |
| Milk, meat, peanut | 16 |
| Milk, vegetable, egg | 14 |
| Milk, egg, peanut | 13 |
| Milk, vegetable, peanut | 13 |
| Milk, vegetable, soy | 12 |
| Milk, vegetable, wheat | 12 |

disease in which the phenotype of the patient is initially very similar and does not help to direct the treatment choice.

There is currently no consensus standard of care algorithm for pediatric EoE. Nutritional therapy options include hypoallergenic or elemental diets, 6 food elimination diets, elimination diets guided by IgE mediated allergy testing, and targeted food elimination diets with serial endoscopies to identify specific EoE food allergen triggers^{11,12}. Pharmacologic options include swallowed corticosteroids, which decrease esophageal inflammation more generally but do not target specific inflammatory triggers^{11,27}.

We propose that TSLP genotyping can play a key role in guiding the creation of optimized, individualized

Table 6 Patient Demographics for EoE Biopsies

| TSLP risk genotype | Age (years, months) | Gender | EoE Status | Eosinophils per high powered field | Identified food allergen triggers |
|--------------------|---------------------|--------|------------|------------------------------------|-----------------------------------|
| Heterozygous | 6.6 | M | Inactive | 0 | soy, meat, fruit, oat |
| Heterozygous | 10.8 | M | Inactive | 0 | meat, fruit, vegetables, oat |
| Heterozygous | 9.1 | M | Inactive | 0 | soy, meat, vegetables |
| Heterozygous | 11.9 | M | Inactive | 0 | milk, wheat, chicken |
| Heterozygous | 8.4 | F | Active | 20 | tree nuts, meat |
| Heterozygous | 14.11 | M | Active | 45 | milk |
| Heterozygous | 4.6 | M | Active | 75 | milk, meat, vegetable, rice |
| Homozygous | 7.2 | M | Inactive | 0 | milk, soy, rice |
| Homozygous | 11.6 | M | Inactive | 3 | milk |
| Homozygous | 6.3 | F | Active | 15 | wheat, meat, vegetables |
| Homozygous | 15.5 | F | Active | 15 | none identified |
| Homozygous | 5.4 | M | Active | 80 | milk, egg, peanut |



Fig. 1 Inducible Expression of TSLP is enhanced in EoE Patients who are Homozygous for the TSLP Risk Polymorphism. OVA induces TSLP in primary cultures. Cultures that are homozygous (white box) had greater induction of TSLP than heterozygous (gray box) for SNP in TSLP gene (* $p = 0.03$)

treatment plans for pediatric EoE patients with a high-disease burden. Specifically, a patient whose disease has been difficult to control who has one or more copies of the TSLP risk allele is significantly more likely to have multiple food allergen triggers. This information will aid clinical decision making, and may identify patients for whom dietary treatment may be more (or less) effective compared to medication management. Our findings also suggest that patients who carry the TSLP risk allele and choose to pursue dietary treatment may benefit from elimination of more than one food, as more than half of these patients have more than two food allergen triggers. Pharmacologic therapy may also be considered here and

may be a more desirable option for those patients with the risk allele that do not wish to undergo multiple endoscopies during their treatment courses in an attempt to identify all of their food triggers. On the other hand, a diet with only one food eliminated may be indicated in patients without the risk allele. A future prospective study is still needed to confirm validity. However, this research suggests the use of genetic testing to help determine which dietary treatment to initiate. If a future prospective study validates the use of genetic analysis, this could help to reduce the cost and the risk associated with the establishment of a targeted elimination diet, a process that often requires multiple upper endoscopies while foods are sequentially introduced and eliminated. This is not only a very costly process, but it is also associated with the typical procedural risks, and, in some clinical settings, is virtually impossible to achieve if upper endoscopies are not readily available. This study also shows that in children with multiple food allergen triggers, meat and egg are often the food triggers associated with milk sensitivity, suggesting that the classical elimination diet may not be the most appropriate in this subgroup of patients with genetic risk.

A limitation of this study is that it is retrospective in nature. These initial data provide evidence to support pursuing a prospective study to validate this relationship.

In summary, the presence of the TSLP risk allele is associated with having multiple EoE food allergen triggers. This genotypic-phenotypic correlation is a critical and novel finding that may result in more targeted and individualized therapy.

Study Highlights

What is current knowledge

- EoE is commonly triggered by specific food allergens.
- Limited data exist supporting treatment guidelines for pediatric EoE.
- The EoE genotypic-phenotypic correlation is not known.

What is new here

- TSLP risk is associated with multiple food triggers.
- TSLP risk may be linked to immune dysregulation & severity of non-IgE mediated food allergy.
- Milk and meat are common food allergen triggers in pediatric EoE.

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Conflicts of interest

Guarantors of the article: Dr Antonella Cianferoni.

Specific authors' contributions: L.M.F. planned study, collected and interpreted data, drafted manuscript, manuscript, approved final draft submitted; P.M.C. planned study, collected and interpreted data, drafted manuscript, approved final draft submitted; S.G. and B.A.: collected and interpreted data; approved final draft submitted; A.J.B. collected and interpreted data; drafted manuscript; approved final draft submitted; G.T.F. collected data; drafted manuscript; approved final draft submitted; S.S.A. interpreted data; drafted manuscript; approved final draft submitted; M.-L.W. planned study; collected and interpreted data; approved final draft submitted; C.A.L. planned study; interpreted data; drafted manuscript; approved final draft submitted; A.B.M. planned study; collected and interpreted data; drafted manuscript; approved final draft submitted; P.M.A.S. collected and interpreted data; drafted manuscript; approved final draft submitted; H.H. collected and interpreted data; approved final draft submitted; J.M.S. planned study; collected and interpreted data; drafted manuscript; approved final draft submitted; A.C. planned study, collected and interpreted data, drafted manuscript, approved final draft submitted.

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