

Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis

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Background: The genetic cause of eosinophilic esophagitis (EE) has been largely unexplored until a recent genome-wide association study identified a disease susceptibility locus on 5q22, a region that harbors the thymic stromal lymphopoietin (*TSLP*) gene. However, it is unclear whether the observed genetic associations with EE are disease-specific or confounded by the high rate of allergy in patients with EE. In addition, the genetic contributions of other allergy-associated genes to EE risk have not been explored.

Objective: We aimed to delineate single nucleotide polymorphisms (SNPs) that associated with EE apart from allergy.

Methods: We used a custom array containing 738 SNPs in 53 genes implicated in allergic responses, immune responses, or both to genotype 220 allergic or 246 nonallergic control subjects and a discovery cohort of 170 patients with EE. We replicated a

statistically significant SNP association in an independent case-control cohort and examined the induction of the candidate gene in primary esophageal epithelial cells.

Results: A single SNP residing in the *TSLP* gene reached Bonferroni linkage disequilibrium-adjusted significance but only when patients with EE were compared with allergic control subjects (rs10062929; $P = 4.11 \times 10^{-5}$; odds ratio, 0.35).

A nonsynonymous polymorphism in the thymic stromal lymphopoietin receptor (*TSLPR*) gene on Xp22.3 and Yp11.3 was significantly associated with disease only in male patients with EE. Primary esophageal epithelial cells expressed *TSLP* mRNA after Toll-like receptor 3 stimulation.

Conclusion: These data collectively identify *TSLP* as a candidate gene critically involved in EE susceptibility beyond its role in promoting T_H2 responses. (J Allergy Clin Immunol 2010;126:160-5.)

Key words: Eosinophilic esophagitis, thymic stromal lymphopoietin, single nucleotide polymorphism, allergy, cytokine receptor-like factor 2, Toll-like receptor 3

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Eosinophilic esophagitis (EE) is a chronic T_H2-associated inflammatory disease of the esophagus that affects at least 4 in 10,000 persons.¹ Although symptomatically resembling gastroesophageal reflux disease, EE is clinically defined as esophageal eosinophilia (≥ 15 intraepithelial eosinophils per high-powered field) in the absence of abnormal acid reflux disease (assessed by normal pH monitoring of the distal esophagus or persistent esophagitis during high-dose acid suppression therapy).² Another distinguishing feature is the high rate of atopic diseases, including asthma, eczema, and allergic rhinitis, and sensitivities to environmental and food allergens within both pediatric and adult EE populations.^{3,4} Consistent with an immune-based mechanism of disease induction, the most effective therapies used to manage EE are food antigen avoidance and swallowed glucocorticoid treatment. However, some patients with EE are refractory to glucocorticoid treatment,⁵ suggesting an inherent resistance with a potential genetic basis.

Reports of EE and esophageal dilatation in relatives of patients with EE suggest that the incidence of EE and associated esophageal dysfunction is common among related subjects.^{1,6} Molecular analysis of the genetics behind EE was initiated in a study by Blanchard et al,⁷ which identified a characteristic gene expression profile within the esophagi of patients with EE, termed the EE transcriptome, that was distinct from that observed in other forms of chronic esophagitis. Interestingly, the EE transcriptome was consistent across sex, age, and familial or nonfamilial inheritance patterns and was independent of atopic status, suggesting a

Abbreviations used

CCED: Cincinnati Center for Eosinophilic Disorders
CCHMC: Cincinnati Children's Hospital Medical Center
EE: Eosinophilic esophagitis
GWAS: Genome-wide association study
LD: linkage disequilibrium
MAF: Minor allele frequency
OR: Odds ratio
SNP: Single nucleotide polymorphism
TLR3: Toll-like receptor 3
TSLP: Thymic stromal lymphopoietin
TSLPR: Thymic stromal lymphopoietin receptor

common disease mechanism despite phenotypic variations.^{6,7} The most highly expressed gene in the EE transcriptome is eotaxin-3 (53-fold increase), an eosinophil and mast cell chemo-attractant; indeed, eotaxin-3 levels correlate with both esophageal eosinophil and mast cell levels.⁷ Furthermore, evidence is mounting that disease pathogenesis is mediated by the interaction of T_H2 cells and esophageal epithelial cells because the T_H2 cytokine IL-13 induces a large fraction of the EE transcriptome (including eotaxin-3) in esophageal epithelial cells.^{8,9} A recent genome-wide association study (GWAS) identified 5q22 as a susceptibility locus for pediatric EE.¹⁰ Notably, although 2 genes (thymic stromal lymphopoietin [*TSLP*] and *WDR36*) were present in one haplotype block that associated with EE, evidence was provided that *TSLP* was the stronger candidate gene because of its overexpression in the esophagi of patients with EE and its known biological activity as a key regulator of allergic sensitization.¹⁰ Yet *TSLP* has previously been implicated in various atopic responses,¹¹⁻¹³ and thus it is not clear whether the EE association with *TSLP* is reflective of a general association with atopic processes or whether it is specific to EE.

In the present study we sought to identify genetic variants that associated with EE using a broad-spectrum candidate gene approach involving a panel of 738 single nucleotide polymorphisms (SNPs) in allergy-associated molecules, especially epithelial gene products, including *TSLP*. Furthermore, we aimed to identify genetic variants that associated with EE independent of allergy. To address this question, we genetically compared patients with EE with a set of clinically defined allergic and nonallergic control groups. Compared with all genetic variants tested, we demonstrate that genetic variants within *TSLP* are associated with EE independent of allergy. Additionally, we present further evidence for the importance of *TSLP* in patients with EE by demonstrating that a genetic variant in the thymic stromal lymphopoietin receptor (*TSLPR*) also contributes to EE susceptibility. Furthermore, we show that primary esophageal epithelial cells express *TSLP* mRNA in response to Toll-like receptor 3 (TLR3) activation.

METHODS

Study participants

The discovery EE case cohort consisted of 172 patients who were recruited by the Cincinnati Center for Eosinophilic Disorders (CCED). DNA samples were collected at the time of endoscopy or at follow-up from blood or saliva specimens. Patients were identified with clinically diagnosed EE (≥ 24 eosinophils per high-power field). All patients with EE self-reported race as white. The male/female ratio was 2.18, and the mean age at diagnosis was 9.39 years (SD, 8.60 years). Approximately 85% of the discovery cohort was

composed of patients characterized in a recent EE GWAS.¹⁰ The percentages of patients with diagnosed asthma, allergic rhinitis, or eczema were 31%, 53%, and 39%, respectively. Approximately 50% of these patients with EE were receiving acid-suppressive therapy, and 18% were taking swallowed glucocorticoid treatment at the time of endoscopy. The discovery control cohorts were recruited through the Greater Cincinnati Pediatric Clinic Repository and Genomic Control Cohort at Cincinnati Children's Hospital Medical Center (CCHMC). All consenting patients visiting various clinics at CCHMC, including the allergy, immunology, and pulmonary clinics, provided buccal swabs or saliva samples for DNA isolation. Information regarding patient history was collected through questionnaires pertaining to allergy symptoms. All control subjects had self-reported race as white. The allergic control cohort ($n = 227$) was defined as nonasthmatic subjects having clinically diagnosed atopic rhinitis or atopic dermatitis or a self-reported history of environmental allergies, hay fever, or eczema. The nonallergic control group ($n = 246$) had no personal or family history of asthma or a personal history of environmental allergies, allergic rhinitis, or atopic dermatitis.

The replication EE case cohort consisted of 122 independent patients with similar characteristics as the EE discovery cohort. This cohort was also collected at the CCED by using the procedures described above. All patients with EE in the replication cohort self-reported race as white, and 72% of these patients were included in the EE GWAS. The replication control cohort consisted of 119 subjects. This cohort was comprised of consenting CCHMC employees who reported race as white with no self-reported personal history of gastroesophageal reflux disease or other gastrointestinal symptoms. The percentage of subjects within this group with self-reported allergy was approximately 44%. These studies were approved by the CCHMC Institutional Review Board.

Candidate gene and SNP selection

For the discovery phase, we used a custom Illumina SNP chip ($n_{\text{SNP}} = 768$) designed to interrogate genes with a role in allergy-related signaling pathways, epithelial cell function, or both based on current literature searches as part of National Institutes of Health grant U19 AI070235. The *TSLP* gene was submitted for inclusion in the initial chip design by Dr Yong-Jun Liu (University of Texas, M.D. Anderson Cancer Center, Houston, Tex); a total of 9 *TSLP* SNPs were included on the chip. In addition to candidate gene SNPs, 30 ancestry informative markers were also included. Genotyping with the Illumina GoldenGate Assay (<http://www.illumina.com>) system was performed at the CCHMC Genetic Variation and Gene Discovery Core. Genotypes were assigned by using Illumina's BeadStudio 2 Software (San Diego, Calif). SNPs that exhibited differences in minor allele frequencies (MAFs > 0.1) between plates were manually examined to ensure consistent clustering patterns. For the replication set, *TSLP* SNPs were genotyped with the ABI TaqMan allelic discrimination assays (Applied Biosystems, Foster City, Calif). The initial *TSLPR* sequencing was performed with the ABI 3700 DNA Analyzer. *TSLPR* SNPs were genotyped with the ABI TaqMan allelic discrimination assays (Applied Biosystems) in the replication case-control cohorts, as well as in additional DNA samples from patients with EE and healthy control subjects collected through the CCED.

Statistical analyses

All analyses were performed separately among the allergic and nonallergic control cohorts. No *TSLP* SNPs failed Hardy-Weinberg equilibrium in the control dataset ($P < 10^{-3}$), were removed for poor genotype calling (missing rate $> 10\%$), or were excluded with MAFs of less than 10%. A single *TSLP* SNP was removed before analysis because of poor clustering. To adjust for multiple testing, we applied a Bonferroni adjustment after correcting for linkage disequilibrium (LD) correlation among the SNPs that passed quality control. This approach is less conservative than the standard Bonferroni adjustment because it incorporates an LD block-based correction to reduce the total number of independent tests, thus preventing type I error inflation.¹⁴ Filtering of subjects with greater than 20% missing genotypic information over the chip resulted in the removal of 2 patients with EE and 7 allergic control subjects before analysis, yielding a total of 170 cases and 220 allergic and 246 nonallergic control

TABLE I. *TSLP* SNPs in patients with EE compared with allergic, nonallergic, or both control subjects

SNP*	BP†	Patients with EE (n = 170)		Allergic control subjects (n = 220)		Nonallergic control subjects (n = 246)			Allergic and nonallergic control subjects (n = 466)		
		MAF‡ cases	MAF control subjects	OR§	P value	MAF control subjects	OR	P value	MAF control subjects	OR	P value
rs3806932	110433574	0.33	0.40	0.72	3.27E-02	0.41	0.70	1.55E-02	0.41	0.70	9.02E-03
rs3806933	110434641	0.31	0.41	0.63	3.29E-03	0.40	0.70	1.56E-02	0.40	0.66	2.38E-03
rs2289276	110435406	0.24	0.25	0.94	7.40E-01	0.27	0.83	2.69E-01	0.26	0.86	3.43E-01
rs1898671	110435901	0.45	0.33	1.61	1.26E-03	0.35	1.48	7.35E-03	0.34	1.57	5.52E-04
rs10062929	110436078	0.08	0.17	0.35	4.11E-05	0.13	0.54	1.27E-02	0.15	0.45	5.79E-04
rs2289277	110436966	0.31	0.42	0.61	1.90E-03	0.40	0.69	1.05E-02	0.41	0.64	1.35E-03
rs11466749	110440484	0.11	0.19	0.48	9.29E-04	0.15	0.71	1.12E-01	0.17	0.59	8.47E-03
rs11466750	110440793	0.09	0.18	0.43	3.37E-04	0.13	0.63	5.25E-02	0.15	0.52	3.08E-03

Presented are the associated *P* values for *TSLP* SNPs between patients with EE versus 3 control groups (allergic, nonallergic, or combined).

*dbSNP Build 130 rs number.

†BP, Base pair; chromosomal location National Center for Biotechnology Information (NCBI) Build 36.

‡MAF in patients with EE or the specified control group.

§OR for the specified control group compared with patients with EE.

subjects that entered the analyses. Principal component analysis was performed with 30 ancestry informative markers and the EIGENSTRAT software (<http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm>) to account for potential population stratification/confounding or admixture in these samples. The principal component score for each subject was included as a covariate in all models along with age and sex in logistic regression models. As a general association screen, we tested for the additive models of single-SNP analysis, which assumes that each copy of the risk allele will increase disease prevalence. Unconditional logistic regression was used to calculate *P* values and odds ratios (ORs) for each SNP by using the software PLINK (version 1.05).¹⁵ Statistical analyses of the *TSLP* SNPs (dominant model) and the *TSLP* SNPs (Cochran-Armitage trend test) in the replication cohorts were also performed in PLINK. The Fisher method,¹⁶ which calculates the combined probability from independent tests addressing the same hypothesis, was used in the meta-analysis of the discovery and replication cohorts.

TSLP expression in stimulated esophageal epithelial cells

Primary esophageal epithelial cells were cultured from patients' esophageal biopsy specimens, as previously described.⁹ Cells were grown in hydrocortisone-free media 24 to 48 hours before stimulation. Cells were then treated with 10 or 100 μ g/mL poly I:C (InvivoGen, San Diego, Calif) for 3 or 8 hours. RNA was isolated with TRIzol (Invitrogen, Carlsbad, Calif), and cDNA was synthesized with iScript (Bio-Rad Laboratories, Hercules, Calif). RT-PCR analysis for *TSLP* expression was performed with the following primers: forward, 5'-cccaggctattcggaaactca-3'; reverse, 5'-acgccacaatcctgtatgtg-3'. Graphed data were normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression from 3 to 4 independent experiments performed in duplicate.

RESULTS

In our effort to identify EE-specific genetic susceptibility loci, we used a custom Illumina SNP genotyping chip to screen for variants within candidate genes involved in allergy, epithelial cell function, or both. The Bonferroni LD-adjusted *P* value required for statistical significance in this study was determined to be 3×10^{-4} to account for multiple testing. Because the coincidence of allergy is high (approximately 70%) in patients with EE, we first chose to investigate the specificity of any potential associations of candidate gene SNPs by stratifying the discovery control cohort into 2 phenotypically defined groups based on a history of allergy (see the Methods section). When comparing patients with

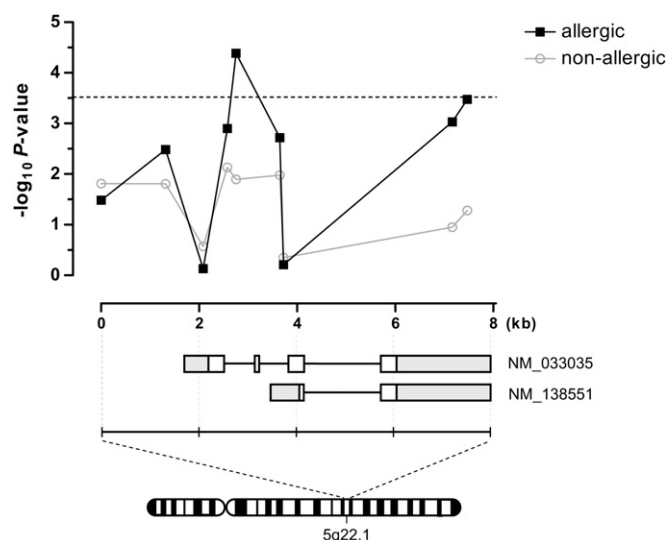


FIG 1. Associations of *TSLP* SNPs with EE are independent of allergy phenotypes. *Upper panel*, The associated $-\log_{10} P$ values for the genotyped *TSLP* SNPs from each analysis (patients with EE vs allergic or nonallergic control subjects) are plotted based on relative base pair location. The dashed line represents the Bonferroni threshold for significance ($P = 3 \times 10^{-4}$). *Lower panel*, The *TSLP* SNPs reside within an approximate 8-kb interval on 5q22.1 encoding the *TSLP* gene isoforms (NM_033035 and NM_138551). Represented are exons (white boxes), introns (bold lines), and untranslated regions (gray boxes).

EE with the allergic control subjects, we found that only *TSLP* harbored variants that reached statistical significance (Table I and Fig 1 and see Table E1 in this article's Online Repository at www.jacionline.org). Indeed, 7 SNPs in *TSLP* exhibited association with EE ($4.11 \times 10^{-5} \leq P \leq 3.29 \times 10^{-3}$). However, when comparing patients with EE with the nonallergic control subjects, no genes exhibited variants that reached statistical significance; the strongest observed *TSLP* SNP association only reached a *P* value of 7.35×10^{-3} in this analysis. When the 2 control groups were combined (allergic and nonallergic), the association strengthened, most likely because of the increase in the sample size of the control group. Interestingly, polymorphisms in *IL4*, a known allergy susceptibility gene, were associated with EE (best $P = 1.33 \times 10^{-3}$; OR, 1.91) using the nonallergic control

TABLE II. Replication of *TSLP* SNP association with EE in 2 independent case-control cohorts

SNP	Discovery cohort*				Replication cohort†				Combined <i>P</i> value‡
	MAF cases	MAF control subjects	OR	<i>P</i> value	MAF cases	MAF control subjects	OR	<i>P</i> value	
rs3806933	0.31	0.40	0.66	2.38E-03	0.31	0.41	0.66	6.10E-02	1.43E-03
rs2289276	0.24	0.26	0.86	3.43E-01	0.26	0.23	1.17	5.01E-01	4.74E-01
rs10062929	0.08	0.15	0.45	5.79E-04	0.09	0.19	0.36	3.31E-04	3.16E-06
rs2289277	0.31	0.41	0.64	1.35E-03	0.36	0.43	0.75	1.69E-01	2.14E-03
rs11466749	0.11	0.17	0.52	3.08E-03	0.11	0.21	0.46	5.00E-03	1.86E-04
rs11466750	0.09	0.15	0.70	9.02E-03	0.10	0.19	0.46	9.00E-03	8.48E-04

*The discovery cohort was composed of 170 patients with EE and 466 allergic and nonallergic control subjects, as described in Table I.

†The replication cohort was composed of either 87 patients with EE and 114 allergic and nonallergic control subjects or 87 patients with EE and 122 allergic and nonallergic control subjects for SNP rs10062929.

‡Combined *P* values from the discovery and replication cohorts using the Fisher method.

subjects but not the allergic control subjects (best $P = .27$; see Table E1), emphasizing the importance of using phenotypically matched control subjects for identifying risk variants associated with primary disease. Moreover, these data highlight the specificity of the *TSLP* genetic association with EE.

We next aimed to replicate the observed genetic association with *TSLP* variants using a completely independent replication cohort of patients with EE and control subjects (Table II). Although the replication control cohort was composed of both allergic and nonallergic subjects, 3 *TSLP* SNPs were significantly associated with EE (Table II, $P < .5$) in this independent analysis. In a meta-analysis of the associated *TSLP* variants from both the discovery and replication cohorts, the association of rs10062929 strengthened (combined $P = 3.16 \times 10^{-6}$) approximately 2 logs above the threshold of the Bonferroni adjustment. Moreover, the combined *P* values for rs11466750 and rs11466749 were nominally significant (combined $P = 8.48 \times 10^{-4}$ and 1.86×10^{-3} , respectively).

The prevalence of EE in male subjects is approximately 2.5-fold higher than in female subjects. To determine whether *TSLP* variants contribute to this sex bias, we performed a sex-stratified analysis of patients with EE and allergic control subjects from the discovery cohort and determined the SNPs with the greatest change in *P* value in either the male or female cohorts (see Table E2 in this article's Online Repository at www.jacionline.org). Of the 9 *TSLP* SNPs, only rs10062929 and rs11466749 also associated in the female EE cohort ($P = .016$ and $P = .048$ in patients with EE vs female atopic control subjects, respectively), whereas the *P* values of most variants in *TSLP* remained unchanged in the male-only analyses.

Interestingly, *TSLPR* is encoded on a pseudoautosomal region on Xp22.3 and Yp11.3, which further underscores the potential significance of finding SNPs in the *TSLP/TSLPR* pathway observed among male patients with EE.¹⁷ We were interested in testing the hypothesis that variants in *TSLPR* might also associate with EE in a sex-specific manner. Therefore we genotyped 3 coding SNPs recently validated from direct sequencing of the *TSLPR* gene (P.G., unpublished data): rs36139698, rs36177645, and rs36133495. Of these, the SNP rs36133495 (Ala to Val) was significantly associated with disease risk in a cohort of male patients with EE and healthy male control subjects (Val/Val: 27% in male patients with EE vs 15% in male control subjects; $P = .039$; OR, 2.05; Fig 2 and Table III). Conversely, rs36133495 was not significantly associated in a female case-control analysis ($P = .929$; OR, 1.22). Taken together, these data present evidence that the *TSLP* signaling pathway might contribute to the male bias seen in patients with EE.

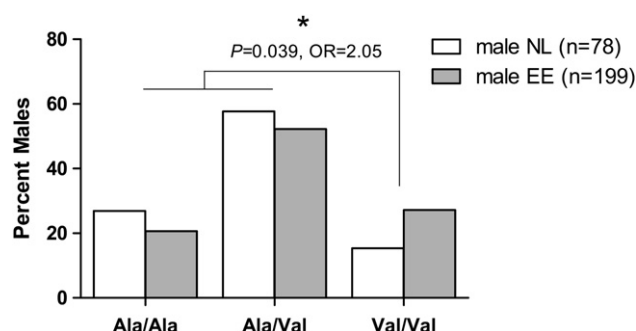


FIG 2. A nonsynonymous SNP in *TSLPR* associates with EE in a sex-specific manner. A nonsynonymous SNP (rs36133495) in *TSLPR*, which results in an Ala to Val coding change, shows a sex-based association with increased disease risk in male patients with EE, with Val/Val and Ala/Val subjects at higher risk (OR, 2.05) compared with Ala/Ala subjects. Genotyped were 199 male patients with EE and 78 male healthy control subjects (NL).

In the lung and skin *TSLP* is produced primarily by epithelial cells in response to T_H2 cytokines or TLR3 agonists, subsequently targeting dendritic cells to develop T_H2 -polarizing activity, including cytokine and chemokine production^{13,18}; however, it has been observed that activated mast cells can also express *TSLP* to similarly drive T_H2 responses.^{12,19,20} Indeed, we have recently observed increased expression of *TSLP* mRNA in the esophagi of patients with EE compared with that seen in control subjects.¹⁰ Accordingly, we aimed to determine whether primary esophageal epithelial cells could produce *TSLP*. Notably, exposure to the TLR3 ligand poly I:C (a double-stranded RNA mimetic) robustly increased *TSLP* mRNA expression in as little as 3 hours after treatment with either 10 or 100 $\mu\text{g/mL}$ poly I:C (Fig 3). These data suggest that the esophageal epithelium is a source of *TSLP* expression in patients with EE.

DISCUSSION

Herein we report that *TSLP* is the most dominant genetic variant associated with EE risk using a large panel of SNPs within relevant allergy and epithelial gene products. We demonstrate that the genetic association of EE with *TSLP* occurs largely independent of allergy, providing compelling evidence in support of our recent GWAS analysis that identified the 5q22 locus as a risk locus for EE susceptibility.¹⁰ It is notable that the direction of the disease risk (OR < 1) was similar in the discovery and replication cohorts in our present study and recent EE GWAS, supporting the genetic association to this region. However, given that the

TABLE III. A sex-specific association of a *TSLPR* SNP in male patients with EE

SNP*	Male patients with EE (n = 199), male control subjects (n = 78)				Female patients with EE (n = 105), female control subject (n = 78)			
	MAF cases	MAF control subjects	OR	P value†	MAF cases	MAF control subjects	OR	P value†
rs36133495	0.53	0.44	2.05	.039	0.47	0.42	1.22	.929

*dbSNP Build 130 rs number.

†P value using a dominant model of association.

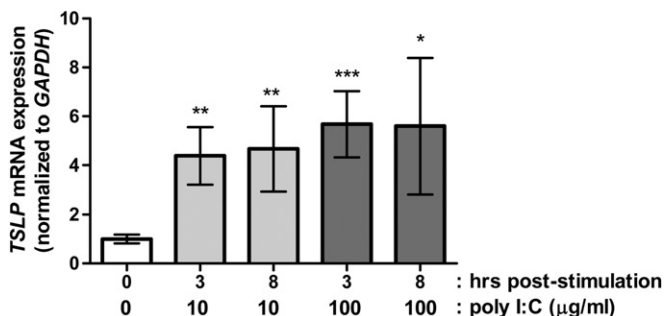


FIG 3. TLR3 signaling stimulates *TSLP* expression in primary esophageal epithelial cells. Treatment of primary esophageal epithelial cells with the TLR3 agonist poly I:C induces a robust increase in *TSLP* mRNA levels. Data shown are the means $\pm$ SEMs from 4 to 5 independent experiments performed in duplicate. * $P < .05$, ** $P < .01$, and *** $P < .001$. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

more common allele is associated with disease susceptibility, the causative allele in EE is unlikely to have been identified. That the same genetic region (5q22) has been linked to blood eosinophilia further implicates a potential relationship between *TSLP* and eosinophilic disease.²¹

In exploring potential mechanisms for the male predilection observed in the population with EE, we performed a sex-stratified analysis for *TSLP* and *TSLPR* polymorphisms. Indeed, a sex-specific interaction was supported for *TSLP* variants, as well as a nonsynonymous SNP within *TSLPR* on Xp22.3/Yp11.3 in male patients with EE. Bioinformatic analysis of the variant suggests that the mutant *TSLPR* has increased stability compared with the wild-type protein, which could conceivably prolong *TSLP*-induced signal transduction.²² In a recent study by Mullighan et al,²³ comparative genomic hybridization analysis in patients with B-progenitor acute lymphoblastic leukemia identified a fusion event between exon 1 of the P2RY8 receptor and *TSLPR*, resulting in a constitutive activation of the Janus kinase–signal transducer and activator of transcription pathway, indicating the profound ability of the *TSLP* pathway to induce disease.

We also demonstrate that *TSLP* mRNA expression is induced in primary esophageal epithelial cells after activation of the TLR3 pathway; it is notable that viral gastroenteritis–like symptoms often precede the onset of EE. *TSLP* was originally identified as a pro-survival factor that could induce pre-B-cell differentiation and proliferation.²⁴ Constitutive phosphorylation of signal transducer and activator of transcription 5 has been linked with lymphoproliferative diseases and malignancies.²⁵ Perhaps *TSLP* overexpression could contribute to both the inflammatory and hyperproliferative states observed in the esophageal epithelium of patients with EE.

Beyond elucidating a role for *TSLP* in patients with EE, these results have broad implications for the discovery of rare disease risk variants. Specifically, these results demonstrate the

importance of appropriate control cohort selection. Consideration of comorbid diseases might provide insight into the role of genetic risk variants, particularly when a rare disease phenotype (EE) tracks with a more common phenotype (allergy). In the present study we show that use of well-characterized control populations in genetic association studies can overcome relatively small sample sizes to identify true risk variants.

In conclusion, we have identified a specific genetic contribution of *TSLP* to EE susceptibility, a finding that provides key insight into disease pathogenesis and likely explains, at least in part, the male sex bias in this disease. We propose that activation of the innate immune system in the esophageal epithelium involving *TSLP* is likely to have a key role in the subsequent adaptive allergic response ultimately triggered by food antigens. These findings present *TSLP* as a potential molecular target for therapeutic intervention and thus highlight the key role of innate immunity in the development of specific allergic disease.

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Key messages

- Polymorphisms in *TSLP* are risk factors for EE independent of underlying allergy phenotypes.
- A sex-specific association between SNPs in *TSLP*, as well as a nonsynonymous SNP in *TSLPR*, suggests a mechanism for the male predilection of EE.
- Primary esophageal epithelial cells express *TSLP* mRNA in response to TLR3 signaling.

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ALLERGY ARCHIVES

THE EOSINOPHIL, A HISTORICAL RETROSPECTIVE: TOXIC FUNCTION



Gerald J. Gleich, MD
(provided by Dr G. J. Gleich).

The question of whether blood and tissue eosinophils characterizing human disease function to the benefit or detriment of the host was addressed in the investigations of Gleich (1931-) at the Mayo Clinic. In 1973, he identified, isolated, and characterized the major basic protein (MBP) in the crystalline core of the guinea pig eosinophil granule.¹ Demonstration of MBP as the eosinophil-derived agent that damaged tegument of parasitic helminths and murine ascites tumor cells raised the possibility that it might damage cells from organs infiltrated with eosinophils. Gleich's extended studies centered on determining whether MBP could damage normally functioning cells and whether concentrations of MBP in the toxic range were present in fluids bathing organs.

Findings indicated that MBP damaged cells from organs whose dysfunction was frequently associated with an infiltration of eosinophils.² *In vitro* injurious damaging concentrations present in body fluids from patients with eosinophil-associated disorders pointed to the possibility that the eosinophil may impair organ function in disease states. That concept could explain positive associations between eosinophilia and organ dysfunction in asthma, vasculitis, and hypereosinophilic syndrome and beneficial effects of glucocorticoids, which at therapeutic doses reduced eosinophil numbers in blood and presumably in tissues.

At about the same time, Austen and his associates were studying modulating effects of eosinophil granular enzymes on allergic reactions. To read about their investigative findings, please visit www.jacionline.org/content/allergy_archives for an Allergy Archive summary entitled "Immune and Hypersensitivity Relevance."

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METHODS

Statistical analysis

Quality control filtering and adjustment for population stratification were performed before sex stratification, as described. To test for genotype-by-sex interaction, we first performed split-sample analysis, in which the male and female subjects were run separately to determine whether the association was strong in one of the sexes. We then performed formal genotype-by-sex interaction analysis in PLINK to ensure that different strengths of association were not due to differences in sample size.

TABLE E1. Top 10 SNPs most associated with EE in discovery cohort analysis

Patients with EE (n = 170) vs allergic control subjects (n = 220)									
Gene	CHR	SNP*	BP†	Minor allele	MAF cases	MAF control subjects	OR	P value	
1	<i>TSLP</i>	5	rs10062929	110436078	A	0.08	0.17	0.35	4.11E-05
2	<i>TSLP</i>	5	rs11466750	110440793	A	0.09	0.18	0.43	3.37E-04
3	<i>TSLP</i>	5	rs11466749	110440484	G	0.11	0.19	0.48	9.29E-04
4	<i>TSLP</i>	5	rs1898671	110435901	T	0.45	0.33	1.61	1.26E-03
5	<i>TSLP</i>	5	rs2289277	110436966	G	0.31	0.42	0.61	1.90E-03
6	<i>TSLP</i>	5	rs3806933	110434641	T	0.31	0.41	0.63	3.29E-03
7	<i>IL10</i>	1	rs3024500	205007454	C	0.41	0.50	0.65	5.99E-03
8	<i>IL10</i>	1	rs2222202	205012004	T	0.41	0.50	0.66	8.06E-03
9	<i>DNAH5</i>	5	rs11958133	13794223	C	0.26	0.18	1.60	8.79E-03
10	<i>IL10</i>	1	rs3024496	205008487	C	0.41	0.50	0.67	1.07E-02
Patients with EE (n = 170) vs nonallergic control subjects (n = 246)									
Gene	CHR	SNP	BP	Minor allele	MAF cases	MAF control subjects	OR	P value	
1	<i>IL4</i>	5	rs2243250	132037053	T	0.18	0.10	1.91	1.33E-03
2	<i>IL4</i>	5	rs2243282	132044453	A	0.18	0.10	1.79	3.96E-03
3	<i>IL4</i>	5	rs2243268	132041862	C	0.17	0.10	1.77	5.09E-03
4	<i>KIF3A</i>	5	rs3798130	132070045	A	0.17	0.10	1.76	5.47E-03
5	<i>TSLP</i>	5	rs1898671	110435901	T	0.45	0.35	1.48	7.35E-03
6	<i>KIF3A</i>	5	rs2299011	132070441	G	0.17	0.10	1.71	9.07E-03
7	<i>TSLP</i>	5	rs2289277	110436966	G	0.31	0.40	0.69	1.05E-02
8	<i>IL4</i>	5	rs2243274	132042731	A	0.18	0.11	1.66	1.08E-02
9	<i>ADCY2</i>	5	rs12652807	7502380	G	0.09	0.14	0.55	1.11E-02
10	<i>IL10</i>	1	rs3024500	205007454	C	0.41	0.50	0.69	1.15E-02
Patients with EE (n = 170) vs combined control subjects (n = 466)									
Gene	CHR	SNP	BP	Minor allele	MAF cases	MAF control subjects	OR	P value	
1	<i>TSLP</i>	5	rs1898671	110435901	T	0.4471	0.3411	1.57	5.52E-04
2	<i>TSLP</i>	5	rs10062929	110436078	A	0.07647	0.1516	0.45	5.79E-04
3	<i>TSLP</i>	5	rs2289277	110436966	G	0.3118	0.4093	0.64	1.35E-03
4	<i>TSLP</i>	5	rs3806933	110434641	T	0.3107	0.403	0.66	2.38E-03
5	<i>TSLP</i>	5	rs11466750	110440793	A	0.08929	0.1544	0.52	3.08E-03
6	<i>IL10</i>	1	rs3024500	205007454	C	0.1765	0.1753	0.68	3.85E-03
7	<i>IL10</i>	1	rs2222202	205012004	T	0.4118	0.4978	0.70	7.37E-03
8	<i>IL10</i>	1	rs3024496	205008487	C	0.4118	0.4946	0.70	8.47E-03
9	<i>TSLP</i>	5	rs11466749	110440484	G	0.1088	0.1688	0.59	8.47E-03
10	<i>TSLP</i>	5	rs3806932	110433574	G	0.3254	0.4056	0.70	9.02E-03

CHR, Chromosome.

*dbSNP Build 130 rs number.

†BP, Base pair; chromosomal location National Center for Biotechnology Information (NCBI) Build 36.

TABLE E2. Sex stratification for *TSLP* risk variants

SNP	BP	Female sex*		Male sex†	
		OR	P value	OR	P value
rs10062929	110436078	0.34	1.57E-02	0.35	9.50E-04
rs11466750	110440793	0.48	6.84E-02	0.38	1.69E-03
rs11466749	110440484	0.48	4.78E-02	0.48	1.02E-02
rs1898671	110435901	1.36	2.08E-01	1.77	3.40E-03
rs2289277	110436966	0.62	7.62E-02	0.59	1.14E-02
rs3806933	110434641	0.66	1.23E-01	0.61	1.60E-02

*Female patients with EE (n = 53); female allergic control subjects (n = 94).

†Male patients with EE (n = 117); male allergic control subjects (n = 126).