

The Genetic Variants of Thymic Stromal Lymphopoietin Protein in Children with Asthma and Allergic Rhinitis

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Key Words

Asthma · Allergic rhinitis · Eosinophil · Polymorphism ·
Thymic stromal lymphopoietin

Abstract

Background: Thymic stromal lymphopoietin (TSLP) is expressed by airway epithelial cells and plays a key role in immunological events in asthma. Data on the genetic variants of TSLP and its association with asthma and allergic rhinitis are scarce. We aimed to investigate the effects of the genetic variants of TSLP in children with asthma and allergic rhinitis. **Methods:** The genetic variants of the TSLP gene were determined by sequencing 25 asthmatic and 25 healthy children. In an association study, a population of 506 asthmatics and 157 healthy controls was screened for the following single-nucleotide polymorphisms (SNPs): rs3806933 and rs2289276 in the promoter region; rs11466741, rs11466742, and rs2289278 in intron 2; rs10073816, rs11466749, and rs11466750 in exon 4, and rs11466754 in 3'-UTR. **Results:** In Multifactor Dimensionality Reduction analysis, presence of the rs11466749 AA genotype with atopy was significantly associated with a diagnosis of asthma (testing set accuracy: 0.720 and cross valida-

tion: 9/10). Two functional SNPs showed a gender-specific association with allergy, i.e. the rs3806933 CC genotype with asthma in boys ($p = 0.032$, nonsignificant after multiple testing) and the rs2289276 CC genotype with higher eosinophil numbers in asthmatic girls ($p = 0.003$). The presence of allergic rhinitis in asthmatic children strengthened the association of the rs11466749 GG genotype with asthma ($p = 0.001$), and rs2289276 was significantly associated with lower FEV₁ levels in asthmatics without allergic rhinitis ($p = 0.003$). **Conclusion:** Variants in the gene encoding the TSLP protein may have differential effects on asthma phenotypes depending on gender, atopy, and the presence of allergic rhinitis.

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Introduction

Allergic rhinitis (AR) and asthma are closely related inflammatory diseases associated with a dysregulated production of Th2 type cytokines [1]. Recent studies have shown that thymic stromal lymphopoietin (TSLP) produced by epithelial cells activates myeloid dendritic cells to induce a Th2 response [2–4].

TSLP is a 140-amino-acid, interleukin (IL)-7-like cytokine originally identified as a growth factor in the supernatants of murine thymic stromal cell lines. In contrast to murine TSLP, human TSLP does not support the growth and activation of B cells. TSLP is mainly expressed by primary skin keratinocytes, smooth muscle cells, and lung fibroblast and bronchial epithelial cells but not by hematopoietic cells, except mast cells [5].

Recently, there has been a growing interest in the role of TSLP in allergic diseases, and the TSLP protein has been detected in lesions of acute and chronic atopic dermatitis but not in normal or nonlesioned skin [2, 6, 7]. Several studies have demonstrated that a high expression of TSLP is associated with airway inflammation in mice and humans. Murine studies have shown that lung-specific expression of the TSLP transgene induces allergic airway inflammation including leukocyte infiltration, goblet cell hyperplasia, fibrosis, and increased production of IgE [8]. In humans, TSLP has been found to be increased in asthmatic airways and its expression has been correlated with Th2-attracting chemokines and the severity of the disease [9]. Proinflammatory cytokines such as tumor necrosis factor- α and IL-1 β , together with IL-4 and IL-13, induce the expression of TSLP by human airway epithelial cells and keratinocytes [10].

Following the demonstration of the physiological role of TSLP, some studies have analyzed its genetic associations. In those studies, several TSLP variants have been shown to be associated with childhood and adult asthma, bronchial hyperresponsiveness, lung function, and specific IgE in several ethnically diverse populations [3, 11–16].

Compared to data on asthma, data on the role of TSLP in AR is limited. A high expression of TSLP has been detected in epithelium from patients with AR and it has been associated with infiltration of dendritic cells, eosinophils, and enhancement of the barrier function with an increase in tight junction proteins claudin-1, claudin-4, claudin-7, and occludin [17, 18]. Recently, Bunyavanich et al. [19] studied the associations between single-nucleotide polymorphisms (SNPs) of the TSLP gene and AR in children with asthma in 3 independent cohorts.

A number of studies have shown that TSLP is critical in Th2 commitment of T cells in allergic diseases; some studies have examined genetic variations of TSLP and its association with allergic diseases [3, 12, 16, 20, 21], especially in AR and thus in one airway [17–19, 21, 22].

In this study, we aimed to determine SNPs in the TSLP gene and analyze the association of SNPs with the phenotypes of asthma and AR in Turkish children.

Materials and Methods

Study Population

Children with Asthma

The characteristics of the patients in the asthma group have been detailed previously [23–25]. Briefly, we enrolled Caucasian children aged 6–18 years who were diagnosed with asthma at the Pediatric Allergy and Asthma Unit of Hacettepe University School of Medicine, Ankara, Turkey, between 2002 and 2009. Examination by a pediatric allergist according to GINA guidelines confirmed the asthma diagnoses [26]. All children had evidence of reversible airway obstruction as defined by at least a 12% improvement in FEV₁ following bronchodilator administration, therapeutic response to antiasthma treatment, or an abnormal result on the methacholine bronchoprovocation test (PC20 <8 mg/dl). Spirometric measurements, total IgE, and eosinophil counts were obtained. Skin testing was performed as detailed previously [25]. This study was approved by the Ethics Committee of Hacettepe University and parents provided written informed consent for participation in this study.

Healthy Children

The characteristics of the patients in the control group have been detailed previously [25]. Briefly, the control group was comprised of Caucasian Turkish school children who presented between 2005 and 2006 to the outpatient department of the same hospital. They presented for reasons such as minor trauma or for their regular follow-up. They responded negatively to an established and validated asthma questionnaire (ISAAC) [27], had never had a diagnosis of asthma or allergic bronchitis by a physician, and did not have a history of wheezing. They all had normal pulmonary function tests. All children underwent skin prick tests and had their total IgE measured in serum.

Identification of Genetic Variants of the TSLP Gene

DNA was extracted from whole blood using modified methods of Poncz et al. [28]. Briefly, after hypertonic lysis of cells and proteinase K digestion of proteins, phenol-chloroform extraction and ethanol precipitation were performed for the extraction of DNA. To determine the functional polymorphisms of the TSLP gene, the promoter region (up to –1,000 bp) and exonic regions within the exon-intron boundaries of the TSLP gene were amplified from the DNA samples of 25 healthy individuals and 25 asthmatic patients via polymerase chain reaction (PCR) and subjected to sequencing analysis. Primers were designed according to the genomic sequence of TSLP with the accession number AC008572.6. The primers for PCR and sequencing are listed in online supplementary table S1 (see www.karger.com/doi/10.1159/000358488 for all online suppl. material). Numbers from the dbSNP of NCBI (<http://www.ncbi.nlm.nih.gov/SNP/>) were used to define polymorphisms. A sequencing reaction was performed using a Big Dye Terminator Cycle Sequencing Kit (version 3.2; ABI Prism, Foster City, Calif., USA) with an ABI Prism 310 Sequence Detection System. Sequencing analysis revealed 9 SNPs, i.e. rs3806933 and rs2289276 polymorphisms in the promoter region, rs11466741, rs11466742, and rs2289278 in intron 2, rs10073816, rs11466749, and rs11466750 in exon 4, and finally rs11466754 in 3'-UTR of the TSLP gene (long form). According to the initial results of the sequence analysis, 2 sets of linkage (100%) were determined. The first was between rs11466749, rs11466750, and rs11466754 and the second was between rs2289276 and rs11466741.

Table 1. Characteristics of the study population

	Healthy children (n = 157)	Asthmatics (n = 506)	p
Age, years	10.4 (7.9–12.4)	9.2 (7.5–11.8)	>0.05
Gender			
Male	78 (50)	309 (61)	0.019
Female	79 (50)	196 (39)	
Age at asthma onset, years	–	5.0 (3.0–7.6)	
FEV ₁ , % predicted	101 (90–108)	89 (79–99)	<0.001
IgE, kU/l	37.5 (18–77)	136 (53–367)	<0.001
Skin prick test positivity	25 (16)	297 (59)	<0.001
Eosinophil count, mm ³	138 (96–260)	300 (170–510)	<0.001
Family history of allergic disease	20 (13)	168 (33)	<0.001
Exposure to cigarette smoke	91 (58)	198 (39)	<0.001
Exposure to animals	18 (12)	41 (8)	>0.05
Values are presented as medians (IQR) or numbers (%) unless otherwise specified.			

Four SNPs were chosen for screening, i.e. rs3806933 and rs2289276 (linkage with rs11466741, $D = 0.250$, $r^2 = 0.99$) in the promoter region, and rs10073816 and rs11466749 (linkage with rs11466750 and rs11466754, $D = 0.056$, $r^2 = 0.99$) in exon 4. Since the frequencies of the SNPs rs11466742 and rs2289278 were lower than 5%, they were not analyzed any further.

Association Study

Screening of 4 SNPs, i.e. rs3806933, rs2289276, rs10073816, and rs11466749, in 157 healthy controls and 506 asthmatic children was performed using Competitive Allele-Specific PCR with the KASPar™ SNP Genotyping System (KBiosciences, UK).

The KASP SNP genotyping system is a homogeneous fluorescent FRET (or fluorescent resonance energy transfer)-based system which allows the detection of SNPs without the need for a separation step and also has the power of competitive allele-specific PCR.

Statistical Analyses

Statistical analyses were performed with SPSS 15 for Windows (Chicago, Ill., USA). Comparisons of quantitative variables such as age, eosinophil counts, IgE levels and FEV₁ (% predicted) were made with parametric or nonparametric tests depending on the normalcy of the distribution. The χ^2 test or Fisher's exact test was used for the comparison of categorical variables as appropriate. The genotype frequencies of each SNP were tested for concordance with Hardy-Weinberg equilibrium using Pearson's χ^2 test. For all analyses, after correction for multiple testing, $p < 0.05/4 = 0.0125$ was considered statistically significant.

An interaction analysis was implemented using the open-source Multifactor Dimensionality Reduction (MDR) software package (version 1.0.0, available at www.epistasis.org). Using the MDR constructive induction function, we constructed all possible interactions among different genotype variants belonging to the TSLP gene. The genetic data were collapsed into 2 categories with high and low risk in each cell. An interaction dendrogram was applied to visualize the nature of the dependencies. Differences in

SNPs and haplotype distributions of the rs3806933, rs10073816, and rs114666749 genotypes were analyzed using models, and they were adjusted for gender, atopy, and family history of allergic diseases as covariates.

Results

A total of 506 children with asthma and 157 healthy controls were included in this study. The characteristics of the study and control groups are summarized in table 1. Four SNPs were screened in healthy and asthmatic children. In the codominant model, the rs11466749 AA genotype was more frequent in asthmatics compared to healthy children ($p = 0.035$) (table 2). According to multiple testing, the difference between asthmatic and healthy children became significant in the A-dominant model (GA+AA vs. GG) of the rs11466749 genotype ($p = 0.01$).

Asthmatic girls with the CC genotype at rs2289276 had higher levels of eosinophils compared to those with the CT+TT genotype [$390/\text{mm}^3$ (IQR 200–607) vs. $241/\text{mm}^3$ (IQR 130–432); $p = 0.003$] (fig. 1). This difference was not observed in asthmatic boys.

MDR analyses showed that the AA genotype at rs11466749 was significantly associated with the diagnosis of asthma [testing set accuracy (TSA): 0.720 and cross validation (CV): 9/10] among atopic children (fig. 2). Children with presence of the AA+AG genotype at rs11466749 and atopy showed the same TSA and CV values for the diagnosis of asthma.

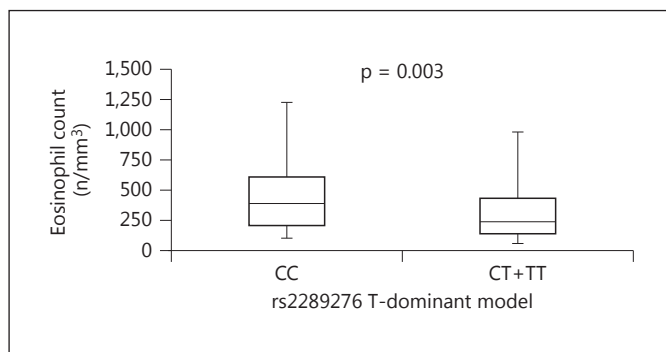


Fig. 1. The eosinophil count was significantly higher in asthmatic girls with the rs2289276 CC genotype than in those with the CT+TT genotype [390/mm³ (IQR 200–607) vs. 241/mm³ (IQR 130–432); $p = 0.003$].

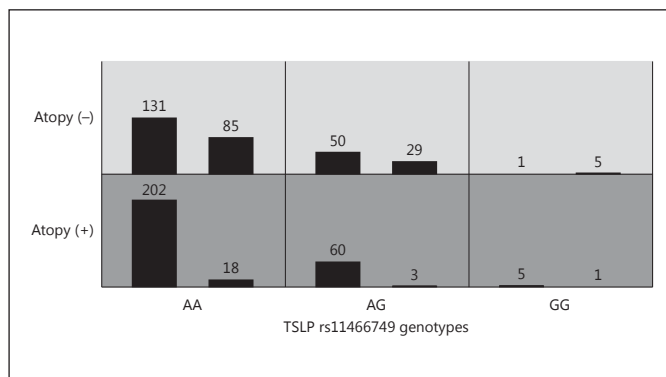


Fig. 2. Children carrying the rs11466749AA genotype and atopy (positive skin prick test) were significantly associated with the diagnosis of asthma (testing set accuracy: 0.720 and CV: 9/10). Children with the rs11466749 AA+AG genotype and atopy showed the same TSA (0.720) and CV (9/10) values.

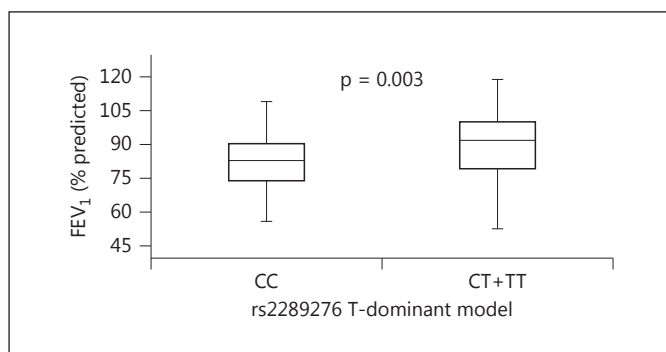


Fig. 3. Children with asthma but without AR with the rs2289276 T-dominant CC genotype had lower FEV₁ than those with the CT+TT genotype [83 (IQR 74–90) vs. 92 (IQR 79–100); $p = 0.003$].

Association of TSLP Genotypes with Asthma and AR

The frequency of the AA genotype at rs11466749 was higher in patients with AR compared to those without (0.826 vs. 0.706; $p = 0.001$) (table 3).

There was a tendency towards a higher eosinophil count in girls but not in boys with asthma and AR carrying the CC genotype at rs2289276 [440 (IQR 352–594) in the CC genotype vs. 305 (IQR 172–663) in CT+TT; $p = 0.088$]; this tendency was lost in girls with asthma but without AR.

Despite the lack of an association between TSLP genotypes and FEV₁, in the total group of children with asthma, the FEV₁ was significantly lower in children with the CC genotype at rs2289276 than in those with the CT+TT genotype among children with asthma who did not have AR [83 (IQR 74–90) vs. 92 (IQR 79–100); $p = 0.003$] (fig. 3). The same analysis failed to show any difference in asthmatic boys with or without AR.

Discussion

In this study we investigated the effects of the genetic variants of the TSLP gene on allergic phenotypes in children with asthma and AR. We found that the CC genotype at rs3806933 and the AA genotype at rs11466749 are associated with asthma in children. The association at rs3806933 showed a gender-specific association with asthma only in males. The MDR analysis showed that atopic children carrying the A allele at rs11466749 have an increased risk of asthma. In addition, the AA genotype at rs11466749 showed an association with AR in children with asthma. A second functional SNP rs2289276 was significantly associated with asthma phenotypes in a gender-stratified manner; the rs2289276 CC genotype was associated with higher eosinophil numbers in asthmatic girls and lower FEV₁ levels in asthmatic girls without AR.

TSLP is an epithelial cell-derived cytokine that activates dendritic cells to induce naive CD4⁺ T cells to differentiate into Th2 type cells and plays important roles in the initiation and maintenance of allergic immune responses [1–5]. A number of association studies for the TSLP gene have been published [3, 11, 12, 16]. Harada et al. [3] reported that a genetic variant at rs3806933 located in the promoter region is associated with asthma. The SNP rs3806933 (–847 C > T) in the promoter region of long-form TSLP creates a binding site for the transcription factor activating protein (AP)-1, and in vitro functional analyses demonstrated that the SNP enhanced

Table 2. Comparison of rs3806933, rs11466749, and rs10073816 polymorphisms in codominant and dominant models between asthmatic and healthy children

	Healthy children*	Asthmatics*	p
rs3806933 codominant			
CC	41 (0.190)	26 (0.220)	0.048
TC	101 (0.470)	67 (0.568)	
TT	73 (0.340)	25 (0.212)	
rs3806933 T dominant			
CC	21 (0.134)	109 (0.222)	0.016
TC+TT	136 (0.866)	381 (0.778)	
rs10073816 codominant			
GG	20 (0.132)	102 (0.204)	>0.05
GA	79 (0.520)	244 (0.487)	
AA	53 (0.349)	155 (0.309)	
rs10073816 A dominant			
GG	20 (0.132)	102 (0.204)	0.046
GA+AA	132 (0.868)	399 (0.796)	
rs11466749 codominant			
AA	112 (0.727)	369 (0.744)	0.035
GA	35 (0.227)	121 (0.244)	
GG	7 (0.045)	6 (0.012)	
rs11466749 A dominant			
AA+GA	147 (0.955)	490 (0.988)	0.010
GG	7 (0.045)	6 (0.012)	

* n (frequency).

AP-1 binding to the regulatory element. The functional variant increased the promoter-reporter activity of long-form TSLP in response to poly (I:C) stimulation in normal human bronchial epithelial cells. Thus, functional genetic polymorphism of the TSLP gene appears to contribute to Th2-polarized immunity through a higher TSLP production by bronchial epithelial cells in response to viral respiratory infections [3]. The same group also reported that rs3806933 was associated with both childhood atopic asthma and adult asthma [12]. In the present study, we showed that rs3806933 was associated with asthma and this association became stronger in a gender-specific fashion in males with the rs3806933 CC genotype.

Mice models using either lung-specific expression of the TSLP transgene or intranasal administration of TSLP have denoted that TSLP may induce a weak innate response, which is insufficient for full airway inflammatory disease [8, 29, 30]. However, when TSLP is combined with antigenic stimulation a complete airway inflammation characterized by the infiltration of eosinophils, CD4+ Th2 cells, airway remodeling, goblet cell metaplasia, and

Table 3. TSLP genotypes and their relation with AR in asthmatic children

	Asthmatics		p
	AR (-)*	AR (+)*	
rs3806933			
CC	26 (0.215)	31 (0.233)	>0.05
TC	56 (0.463)	68 (0.511)	
TT	39 (0.322)	34 (0.256)	
rs2289276			
CC	46 (0.368)	49 (0.353)	>0.05
TC	55 (0.440)	68 (0.489)	
TT	24 (0.192)	22 (0.158)	
rs10073816			
GG	26 (0.211)	33 (0.239)	>0.05
GA	57 (0.463)	69 (0.500)	
AA	40 (0.325)	36 (0.261)	
rs11466749			
AA	89 (0.706)	114 (0.826)	0.001
GA	37 (0.294)	20 (0.145)	
GG	0 (0.000)	4 (0.029)	

* n (frequency).

mucus overproduction is developed. The link between antigen and TSLP may be a modifying factor for asthma phenotypes, especially atopic asthma. As mentioned in the study of Headley et al. [29], a TSLP-dependent response against harmless environmental allergens may cause spontaneous lung disease in mice expressing the lung-specific TSLP transgene. The studies summarized above show that Th2 type inflammation is induced by the modifying effect of TSLP in conjunction with antigen in mice models, and our findings showing an increased risk of asthma in atopic children carrying the A allele at rs11466749 of the TSLP gene support the interaction of TSLP with allergens in Th2-driven inflammation in humans.

Previous studies have shown that TSLP expression is increased in human asthmatic airways and a number of cells expressing TSLP in airways have correlated inversely with FEV₁ or FEV₁/FVC [4, 31]. Harada et al. [12] found a significant correlation between the rs2289278 genotype and FEV₁/FVC measurements in Japanese patients with asthma. In our study the rs2289276 CC genotype was significantly associated with a lower FEV₁ in asthmatic children without AR. In addition, the same functional SNP rs2289276 CC genotype correlated with higher blood eosinophil numbers in girls with asthma but not in healthy girls. Hunninghake

et al. [11] found that the T allele of TSLP rs2289276 was inversely associated with cockroach-specific IgE in Costa Rican girls and it was also inversely associated with total IgE levels in girls both in Costa Rica and in the CAMP study. They also showed that the TSLP rs1837253 T allele was associated with a reduced risk of asthma in boys and the rs2289276 T allele was associated with a reduced risk of asthma in girls [32]. The study by Harada et al. [12] showed binding of the AP-2 alpha protein, which is a possible transcription suppression factor, to the rs2289276 sequences, with a higher affinity of binding to the C allele (protective allele). In this study, even though rs2289276, rs3806933, and rs2289278 were not associated with gender, eosinophil counts, or IgE concentrations, rs2289276, and rs3806933 were significantly associated with childhood atopic asthma and rs2289278 with FEV₁/FVC in adult asthma. Our results show that the rs2289276 CC genotype is significantly associated with higher blood eosinophil counts in asthmatic girls and lower FEV₁ in children with asthma but without AR. This differential effect of rs2289276 on eosinophil-related inflammation and bronchoconstriction was observed in a gender-stratified model and in the absence of AR in children with asthma. The mechanisms of these observations remain to be studied in the future. The gene encoding the TSLP receptor component TSLPR has a sex chromosome location in humans (Xp22.3 and Yp11.3) [33] and this may be related to the gender-specific difference in TSLP polymorphisms. It is also possible to have gender-specific hormonal regulation of transcription.

The differences between studies may be attributed to ethnic differences in genotype distribution, the heterogeneity of the phenotypes, and the multifactorial etiology of allergic diseases. Another possible cause of the genetic variations that may show opposite effects on certain phenotypes of different cohorts is the amount of allergens that the individuals are exposed to. These findings emphasize the complexity of the relationships between genetic variants and environmental exposures, and the fact that specific environmental exposure among susceptible individuals with a specific genetic predisposition may have opposite effects on different, albeit related, phenotypes (such as atopy and airway reactivity).

There are a limited number of studies concerning increased TSLP expression in cell cultures and nasal epithelium from AR patients [17–19]. mRNA and protein expression of TSLP have been found to be increased in the nasal mucosa of patients with AR compared to normal controls, and TSLP protein expression has been strongly

correlated with IL-4 and the severity of AR [34]. One study investigated the association of SNPs in TSLP with allergy in children with asthma [19]. That study found that the T allele of TSLP rs1837253 was associated with reduced odds ratios for AR in a male-specific fashion in 3 cohorts of children with asthma. We found that among asthmatic children the frequency of the rs11466749 AA genotype was higher in the presence of AR versus its absence. However, children with the rs11466749 GG genotype are very rare and there was not a single patient with the GG genotype among asthmatic children without AR and there were only 4 children among asthmatic children with AR. Therefore, this observation needs to be confirmed in a larger study population.

Similar to ours, the Costa Rican, CAMP, and BAMSE studies found some significant associations with asthma or AR in a gender-specific fashion. These findings suggest that the effect of TSLP genotypes on allergic inflammation may show different patterns depending on gender, atopic status, and AR in asthmatic children. The CC genotype at rs2289276 was significantly associated with higher blood eosinophil numbers and lower FEV₁ in non-atopic asthmatics without AR. There may be a link between high eosinophil counts and lower FEV₁ measurements that is related to this specific TSLP genotype.

This study had some limitations. First, it included a hospital-based cohort and the results may differ from those of population-based studies. However, our center is the biggest of the region and accepts patients directly or with referral throughout the country. There were no important differences with respect to ethnicity or other confounding factors. Another problem was the multiple testing. Since we evaluated 4 different SNPs in this study, the p value had to be divided by a factor of 4 for acceptance of the results as significant. For this reason, we reported only the significant results according to multiple testing.

In conclusion, our results show that genetic variants in the gene encoding the TSLP protein may have differential effects on asthma phenotypes, AR, and allergic inflammation depending on gender- and atopy-specific models.

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References

- ▶ 1 Grossman J: One airway, one disease. *Chest* 1997;111:11–16.
- ▶ 2 Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerman A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan RA, Liu YJ: Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3: 673–680.
- ▶ 3 Harada M, Hirota T, Jodo AI, Doi S, Kameda M, Fujita K, Miyatake A, Enomoto T, Noguchi E, Yoshihara S, Ebisawa M, Saito H, Matsumoto K, Nakamura Y, Ziegler SF, Tamari M: Functional analysis of the thymic stromal lymphopoietin variants in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2009;40:368–374.
- ▶ 4 Shikotra A, Choy DF, Ohri CM, Doran E, Butler C, Hargadon B, Shelley M, Abbas AR, Austin CD, Jackman J, Wu LC, Heaney LG, Arron JR, Bradding P: Increased expression of immunoreactive thymic stromal lymphopoietin in patients with severe asthma. *J Allergy Clin Immunol* 2012;129:104–111.e1–9.
- ▶ 5 Ito T, Wang YH, Duramad O, Hori T, Deslespesse GJ, Watanabe N, Qin FX, Yao Z, Cao W, Liu YJ: TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005; 202:1213–1223.
- ▶ 6 Le TA, Takai T, Vu AT, Kinoshita H, Chen X, Ikeda S, Ogawa H, Okumura K: Flagellin induces the expression of thymic stromal lymphopoietin in human keratinocytes via Toll-like receptor 5. *Int Arch Allergy Immunol* 2011;155:31–37.
- ▶ 7 Iyengar SR, Hoyte EG, Loza A, Bonaccorso S, Chiang D, Umetsu DT, Nadeau KC: Immunologic effects of omalizumab in children with severe refractory atopic dermatitis: a randomized, placebo-controlled clinical trial. *Int Arch Allergy Immunol* 2013;162:89–93.
- ▶ 8 Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, Gyarmati D, Aye T, Campbell DJ, Ziegler SF: Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047–1053.
- ▶ 9 Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, Robinson D, Zhang G, Zhao J, Lee TH, Corrigan C: Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005;174:8183–8190.
- ▶ 10 Bogiatzi SI, Fernandez I, Bichet JC, Marloie-Provost MA, Volpe E, Sastre X, Soumelis V: Cutting edge: proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes. *J Immunol* 2007;178:3373–3377.
- ▶ 11 Hunninghake GM, Lasky-Su J, Soto-Quirós ME, Avila L, Liang C, Lake SL, Hudson TJ, Spesny M, Fournier E, Sylvia JS, Freimer NB, Klanderman BJ, Raby BA, Celedón JC: Sex-stratified linkage analysis identifies a female-specific locus for IgE to cockroach in Costa Ricans. *Am J Respir Crit Care Med* 2008;177: 830–836.
- ▶ 12 Harada M, Hirota T, Jodo AI, Hitomi Y, Sakashita M, Tsunoda T, Miyagawa T, Doi S, Kameda M, Fujita K, Miyatake A, Enomoto T, Noguchi E, Masuko H, Sakamoto T, Hizawa N, Suzuki Y, Yoshihara S, Adachi M, Ebisawa M, Saito H, Matsumoto K, Nakajima T, Mathias RA, Rafaels N, Barnes KC, Himes BE, Duan QL, Tantisira KG, Weiss ST, Nakamura Y, Ziegler SF, Tamari M: Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *Am J Respir Cell Mol Biol* 2011;44:787–793.
- ▶ 13 Masuko H, Sakamoto T, Kaneko Y, Iijima H, Naito T, Noguchi E, Hirota T, Tamari M, Hizawa N: Lower FEV₁ in non-COPD, non-asthmatic subjects: association with smoking, annual decline in FEV₁, total IgE levels, and TSLP genotypes. *Int J Chron Obstruct Pulmon Dis* 2011;6:181–189.
- ▶ 14 Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, Baurley JW, Eng C, Stern DA, Celedón JC, Rafaels N, Capurso D, Conti DV, Roth LA, Soto-Quirós M, Togias A, Li X, Myers RA, Romieu I, Van Den Berg DJ, Hu D, Hansel NN, Hernandez RD, Israel E, Salam MT, Galanter J, Avila PC, Avila L, Rodriguez-Santana JR, Chapela R, Rodriguez-Cintron W, Diette GB, Adkinson NF, Abel RA, Ross KD, Shi M, Faruque MU, Dunston GM, Watson HR, Mantese VJ, Ezurum SC, Liang L, Ruczinski I, Ford JG, Huntsman S, Chung KF, Vora H, Li X, Calhoun WJ, Castro M, Sienna-Monge JJ, del Rio-Navarro B, Deichmann KA, Heinzmann A, Wenzel SE, Busse WW, Gern JE, Lemanske RF Jr, Beaty TH, Bleeker ER, Raby BA, Meyers DA, London SJ; Mexico City Childhood Asthma Study (MCAAS), Gilliland FD; Children's Health Study (CHS) and HARBORS Study, Burchard EG; Genetics of Asthma in Latino Americans (GALA) Study, Study of Genes-Environment and Admixture in Latino Americans (GALA2), Study of African Americans, Asthma, Genes and Environments (SAGE), Martinez FD; Childhood Asthma Research and Education (CARE) Network, Weiss ST; Childhood Asthma Management Program (CAMP), Williams LK; Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE), Barnes KC; Genetic Research on Asthma in African Diaspora (GRAAD) Study, Ober C, Nicolae DL: Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011;43:887–892.
- ▶ 15 Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Doi S, Fujita K, Miyatake A, Enomoto T, Miyagawa T, Adachi M, Tanaka H, Niimi A, Matsumoto H, Ito I, Masuko H, Sakamoto T, Hizawa N, Taniguchi M, Lima JJ, Irvin CG, Peters SP, Himes BE, Litonjua AA, Tantisira KG, Weiss ST, Kamatani N, Nakamura Y, Tamari M: Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. *Nat Genet* 2011;43:893–896.
- ▶ 16 He JQ, Hallstrand TS, Knight D, Chan-Yeung M, Sandford A, Tripp B, Zamar D, Bossé Y, Kozyskyj AL, James A, Laprise C, Daley D: A thymic stromal lymphopoietin gene variant is associated with asthma and airway hyperresponsiveness. *J Allergy Clin Immunol* 2009; 124:222–229.
- ▶ 17 Kamekura R, Kojima T, Koizumi J, Ogawara N, Kurose M, Go M, Harimaya A, Murata M, Tanaka S, Chiba H, Himi T, Sawada N: Thymic stromal lymphopoietin enhances tight-junction barrier function of human nasal epithelial cells. *Cell Tissue Res* 2009;338: 283–293.
- ▶ 18 Zhu DD, Zhu XW, Jiang XD, Dong Z: Thymic stromal lymphopoietin expression is increased in nasal epithelial cells of patients with mugwort pollen sensitive-seasonal allergic rhinitis. *Chin Med J* 2009;122:2303–2307.
- ▶ 19 Bunyavanich S, Melen E, Wilk JB, Granada M, Soto-Quirós ME, Avila L, Lasky-Su J, Hunninghake GM, Wickman M, Pershagen G, O'Connor GT, Weiss ST, Celedón JC: Thymic stromal lymphopoietin (TSLP) is associated with allergic rhinitis in children with asthma. *Clin Mol Allergy* 2011;9:1.
- ▶ 20 Sherrill JD, Gao PS, Stucke EM, Blanchard C, Collins MH, Putnam PE, Franciosi JP, Kushner JP, Abonia JP, Assa'ad AH, Kovacic MB, Biagini Myers JM, Bochner BS, He H, Hershey GK, Martin LJ, Rothenberg ME: Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis. *J Allergy Clin Immunol* 2010;126:160–165.e3.
- ▶ 21 Wang WL, Li HY, Zhang MS, Gao PS, He SH, Zheng T, Zhu Z, Zhou LF: Thymic stromal lymphopoietin: a promising therapeutic target for allergic diseases. *Int Arch Allergy Immunol* 2013;160:18–26.
- ▶ 22 Xu M, Guo Y: Expression of thymic stromal lymphopoietin in nasal mucosa of a mouse model with allergic rhinitis (in Chinese). *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2009;23:794–795, 799.
- ▶ 23 Sackesen C, Karaaslan C, Keskin O, Tokol N, Tahan F, Civelek E, Soyer OU, Adaloglu G, Tuncer A, Birben E, Oner C, Kalayci O: The effect of polymorphisms at the CD14 promoter and the TLR4 gene on asthma phenotypes in Turkish children with asthma. *Allergy* 2005;60:1485–1492.

- ▶ 24 Kalayci O, Birben E, Sackesen C, Keskin O, Tahan F, Wechsler ME, Civelek E, Soyer OU, Adalioglu G, Tuncer A, Israel E, Lilly C: ALOX5 promoter genotype, asthma severity and LTC production by eosinophils. *Allergy* 2006;61:97–103.
- ▶ 25 Ercan H, Birben E, Dizdar EA, Keskin O, Karaaslan C, Soyer OU, Dut R, Sackesen C, Besler T, Kalayci O: Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. *J Allergy Clin Immunol* 2006;118:1097–1104.
- 26 Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention, revised ed. Bethesda, National Institutes of Health, National Heart Lung and Blood Institute, 2002.
- ▶ 27 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, Strachan D, Weiland SK, Williams HC: International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–491.
- ▶ 28 Poncz K, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S: Construction of human gene libraries from small amounts of peripheral blood: analysis of beta-like globin genes. *Hemoglobin* 1982;6:27–36.
- ▶ 29 Headley MB, Zhou B, Shih WX, Aye T, Comeau MR, Ziegler SF: TSLP conditions the lung immune environment for the generation of pathogenic innate and antigen-specific adaptive immune responses. *J Immunol* 2009;182:1641–1647.
- ▶ 30 Ziegler SF: Thymic stromal lymphopoietin and allergic disease. *J Allergy Clin Immunol* 2012;130:845–852.
- ▶ 31 Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, Robinson D, Zhang G, Zhao J, Lee TH, Corrigan C: Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005;174:8183–8190.
- ▶ 32 Hunninghake GM, Soto-Quiros ME, Avila L, Kim HP, Lasky-Su J, Rafaels N, Ruczinski I, Beaty TH, Mathias RA, Barnes KC, Wilk JB, O'Connor GT, Gauderman WJ, Vora H, Baurley JW, Gilliland F, Liang C, Sylvia JS, Klanderman BJ, Sharma SS, Himes BE, Bossley CJ, Israel E, Raby BA, Bush A, Choi AM, Weiss ST, Celedón JC: TSLP polymorphisms are associated with asthma in a sex-specific fashion. *Allergy* 2010;65:1566–1575.
- ▶ 33 Maglott D, Ostell J, Pruitt KD, Tatusova T: Entrez Gene: gene-centered information at NCBI. *Nucleic Acids Res* 2007;35:D26–D31.
- ▶ 34 Mou Z, Xia J, Tan Y, Wang X, Zhang Y, Zhou B, Li H, Han D: Overexpression of thymic stromal lymphopoietin in allergic rhinitis. *Acta Otolaryngol* 2009;129:297–301.

Table S1. Primers for PCR reactions and sequencing of TSLP gene

TSLP pro4F	5'-AGC GAT GAC TCC TAG GAA CTT C-3'
TSLP pro4R	5'-GATGCTGTCCTGAGCTCAGCT-3'
TSLP pro3F	5'- TCTCGATGCTTCTCCTGTCTCT-3'
TSLP pro3R	5'-AAACGCCTACTCTTGGTGGCTA-3'
TSLP pro2F-	5'-TAGCCACCAAGAGTAGGCGTTT-3'
TSLP pro2R	5'-TTCTCCTACAGGGCATTTCCTG-3'
TSLP pro1F	5'-ATTGGTCACGGAAATGCCCT-3'
TSLP pro1R	5'-TTCACCCATGCTGTTGCCTT-3'
TSLP ex1F	5'-AAG GCA ACA GCA TGG GTG AAT-3'
TSLP ex1R	5'- TCC TCA TCA GCA TCT GCA CAC-3'
TSLP ex2F	5'-TGT GCA GTT ATG GCT TGA GGG-3'
TSLP ex2R	5'-CGT CCC AGT TCC CAA TTT GCC-3'
TSLP ex3F	5'- TTG CTC TAC TCA ACC CTG ACC T-3'
TSLP ex3R	5'-TTT ACC CGG CTC GTT TGC TCT T-3'
TSLP ex4F	5'- AAG CAG GTG CTT TGG AGA CAC -3'
TSLP ex4R	5'-GTC TTC TTT GTC ACA GTT AGA G-3'
TSLP ex4F-2	5'-CTC TAA CTG TGA CAA AGA AGA C-3'
TSLP ex4R-2	5'-GCT GTA ACA AAG GTA CTT CTC AG-3'
TSLP ex4F-3	5'-CAA GTA GAT CCT GAG AAG TAC-3'
TSLP ex4R-3	5'- ATT AGA GGA ACA TGT CTA CTC CT-3'
TSLP ex4F-4	5'- ACT GCA CGT GGG AAT GCA CT-3'
TSLP ex4R-4	5'-ACT TTG AGT TGT CTC CTT TCT CC-3'
TSLP ex4F-5	5'- GGA GAA AGG AGA CAA CTC AAA-3'
TSLP ex4R-5	5'-GCA GTA GAC TTT CAT ACA GCT -3'
Ex1 R-2	5'-CCTGAAAGAAACTGACAGAA-3'
Ex4R-3A	5'-CATGTAATGTAAGCTTCCTC-3'
Ex4F-2A	5'-GAACTCAATGATAGCACCT-3'
Ex4F-3A	5'-CCTTTGTTACAGCTACTAT-3'