

***TSLP* polymorphisms are associated with asthma in a sex-specific fashion**

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Abstract

Background: Single nucleotide polymorphisms (SNPs) in thymic stromal lymphopoietin (*TSLP*) have been associated with IgE (in girls) and asthma (in general). We sought to determine whether *TSLP* SNPs are associated with asthma in a sex-specific fashion.

Methods: We conducted regular and sex-stratified analyses of association between SNPs in *TSLP* and asthma in families of children with asthma in Costa Rica. Significant findings were replicated in whites and African-American participants in the Childhood Asthma Management Program, in African-Americans in the Genomic Research on Asthma in the African Diaspora study, in whites and Hispanics in the Children's Health Study, and in whites in the Framingham Heart Study (FHS).

Main Results: Two SNPs in *TSLP* (rs1837253 and rs2289276) were significantly associated with a reduced risk of asthma in combined analyses of all cohorts (*P* values of 2×10^{-5} and 1×10^{-5} , respectively). In a sex-stratified analysis, the T allele of rs1837253 was significantly associated with a reduced risk of asthma in males only ($P = 3 \times 10^{-6}$). Alternately, the T allele of rs2289276 was significantly associated with a reduced risk of asthma in females only ($P = 2 \times 10^{-4}$). Findings for rs2289276 were consistent in all cohorts except the FHS.

Conclusions: *TSLP* variants are associated with asthma in a sex-specific fashion.

Asthma (MIM 610906) is a complex disease. Sex has been consistently shown to influence age-specific asthma prevalence (1). While many studies have evaluated the association between specific genetic variants and asthma in general, few studies have evaluated the role that sex might play in modifying these associations.

Thymic stromal lymphopoietin [*TSLP* (607003)] appears to be a key regulator of allergic asthma in mouse models (2). Whereas *TSLPR*^{-/-} (knockout) mice are protected from airway inflammation after ovalbumin challenge (3), lung-specific transgenic expression of *TSLP*, coupled with antigenic stimulation, leads to airway eosinophilic inflammation,

goblet cell metaplasia and remodeling in the murine lung (4). In humans, there are increased numbers of TSLP mRNA⁺ bronchial epithelial cells in asthmatics vs controls, and this is correlated with degree of airflow obstruction (5). Recently, He et al. (6) demonstrated an association between a genetic variant in the 5' genomic region of *TSLP* and asthma in a combined analysis of four populations.

Multiple lines of evidence suggest that sex might modify the role TSLP plays in asthma. Transgenic expression of *TSLP* in mice leads to perivascular leukocytic infiltration with prominent eosinophilia, with increased severity noted in female mice compared to male mice (7). Our group previously demonstrated that a variant in the 5' untranslated region of *TSLP* is associated with total IgE in girls in two independent populations (8). We hypothesized that genetic variation in *TSLP* would influence asthma, and that sex would modify the effect of these variants. To examine these hypotheses, we conducted regular and sex-stratified analyses of association between *TSLP* variants and asthma in a family-based study of Costa Rican children, with replication of significant findings in five ethnically diverse populations. We report sex-specific associations between two single nucleotide polymorphisms (SNPs) in *TSLP* (rs1837253 and rs2289276) and asthma.

Subjects and methods

Genetic association study

Costa Rica

The protocols for subject recruitment and data collection in children with asthma and their parents (trios) have been previously described in detail (9). Children included in the study had asthma (physician-diagnosed asthma and ≥ 2 respiratory symptoms or asthma attacks in the previous year) and high probability of having ≥ 6 great-grandparents born in the Central Valley of Costa Rica [confirmed by our study genealogist in 416 (94.8%) of the 439 participating children].

The Childhood Asthma Management Program (CAMP)

CAMP was a multicenter clinical trial of the effects of anti-inflammatory medications in children with mild to moderate asthma. All participants had asthma defined by symptoms greater than twice per week, use of an inhaled bronchodilator at least twice weekly or use of daily medication for asthma, and increased airway responsiveness (10). Of the 1 041 children enrolled in the original clinical trial, 968 children and 1 518 of their parents contributed DNA samples.

Children's Health Study (CHS)

The Children's Health Study (CHS) is an ongoing cohort study of genetic and environmental factors related to asthma and lung function growth in Hispanic and (non-Hispanic) white children in southern California (11). Based on questionnaire responses, children were characterized as having doctor-diagnosed asthma at study entry or during active follow-up (cases), or as never having a diagnosis of asthma (controls). The CHS genome-wide association study

(GWAS) was based on a nested case-control sample of 1 206 asthmatics and 1 566 controls selected from within the cohort (12).

Genomic Research on Asthma in the African Diaspora (GRAAD)

African-American children and adults with and without asthma were recruited from the greater Baltimore-Washington D.C. metropolitan area as part of the Genomic Research on Asthma in the African Diaspora (GRAAD). Asthma was defined as self-reported physician-diagnosed asthma. Of the 500 control subjects, 450 were known to have no history of asthma (asthma status was unknown in 50 subjects participating in a study of human pigmentation) (13). Sixty-three individuals were removed from the analysis [because of low call rate, ancestry misclassification, gender discrepancies, and duplicate samples suggested by identity by state (IBS)] testing, resulting in a total of 464 cases and 471 controls.

Framingham Heart Study (FHS)

The Framingham Heart Study (FHS) conducted clinical examinations on three generations of white adults of European descent, and research participants provided DNA samples that have recently been genotyped for GWASs (14). Asthma was classified based on self-report of a physician's diagnosis, and according to this definition, there were 961 cases and 6 516 controls.

Each study was approved by the institutional review board of the corresponding institution and informed consent was obtained for all study participants. Additional study protocol details are presented in an online Data S1 section.

Genotyping and data management

Of the 439 participating parent-child trios in Costa Rica, 417 (95%) are included in this analysis (13 had DNA that did not pass quality control, and 9 were excluded because of Mendelian inconsistencies). Using data from European Americans (CEU) in the International HapMap project (15), we applied a linkage disequilibrium (LD)-tagging algorithm [minor allele frequency (MAF) $\geq 10\%$ and $r^2 \geq 0.8$] to capture common variation in *TSLP* and its 10-kb flanks (16). For this study, additional SNPs were genotyped to improve LD coverage, to evaluate previous associations (rs1837253) (6) and to evaluate reported functional variation (rs3806933) (17) within the genomic region of *TSLP*. All SNPs were genotyped with the SEQUENOM iPLEX platform (Sequenom, San Diego, Calif) (18). The eight polymorphic SNPs successfully genotyped capture $\geq 84\%$ of the HapMap SNPs with MAF $\geq 10\%$ in *TSLP* and its 10 kb flanks in CEU trios at an $r^2 \geq 0.8$. In Costa Rica, duplicate genotyping was performed on $\sim 5\%$ of the samples to assess genotype reproducibility, and only one discordant genotype was detected, with genotype completion rates $>98.0\%$ for all loci.

Based on the results of the association study in Costa Rica, two SNPs in *TSLP* (rs1837253 and rs2289276) were tested for association with asthma in five additional populations. Detailed information about genotyping and data

management for each population is presented in an online Data S1 section.

Statistical analysis

The analysis of asthma was conducted in all cohorts under an additive model, first in all subjects and then after stratification by sex. The family-based association analyses in Costa Rica and CAMP were performed with the FBAT (Family-based Association Test) statistic implemented in GoldenHelix PBAT v3.6 (19). In the CHS and FHS, the association analyses were conducted using logistic regression. In GRAAD, the association analysis was conducted in PLINK (20).

We report two-sided *P*-values for our initial findings in Costa Rica (i.e. rs1837253 in males and rs2289276 in females), and one-sided *P*-values for replications in the same direction of association in other cohorts. To evaluate the significance of genotype-by-sex interactions in Costa Rica, we tested the significance of sex as a modifier parameter using the program UNPHASED (21). Combined *P*-values were calculated using both Fisher's method (22) and a weighted Z-score method. Additional details about statistical analyses are presented in an online Data S1 section (23).

Results

Baseline characteristics of participants in the Costa Rican, CAMP (whites and African-Americans), CHS, GRAAD, and FHS studies are shown in Table 1 (for all subjects and after

stratification by sex). We analyzed data from four cohorts where a diagnosis of asthma was made in childhood [Costa Rica, CAMP (white and African-American), and CHS], one cohort in which asthma was ascertained in childhood and adulthood (GRAAD), and one cohort in which asthma was ascertained in adulthood (FHS). The study includes two cohorts of whites (FHS and CAMP), two African-American cohorts (CAMP and GRAAD), one Hispanic cohort (Costa Rica), and a mixed white and Hispanic cohort (CHS). The minor allele frequencies (MAFs) and LD patterns in Costa Ricans are comparable to that of CEU (white) trios from the HapMap (see Fig. 1). Whereas the MAF of rs1837253 was similar in all cohorts, the MAF of rs2289276 was lower in African-Americans in GRAAD than in subjects in the other cohorts (see Table S1).

Association analysis in Costa Rica

There was no significant association between any SNP in *TSLP* and asthma in all Costa Rican children. There was a statistically significant interaction between SNP rs1837253 and sex (*P*-value 0.004) on asthma. Although there was no statistically significant interaction between SNP rs2289276 and sex on asthma (*P*-value 0.36), we had limited statistical power to detect such an interaction on a binary trait (asthma). Because we previously demonstrated evidence for a significant interaction between SNP rs2289276 and sex on total IgE (8) (a trait related to asthma), we repeated the analyses for both SNPs after stratification by sex. In this analysis,

Table 1 Baseline Characteristics of Participants in the Costa Rica, Childhood Asthma Management Program (CAMP), Children's Health (CHS), Genomic Research on Asthma in the African Diaspora (GRAAD), and the Framingham Heart (FHS) Studies

Population	Median (interquartile range) or Count (%)			
	Subject (<i>n</i>)	Age, years	Asthma	Sex, female
All Subjects				
Costa Ricans	417	8.7 (8–10)	417 (100%)	157 (38%)
CAMP (non-Hispanic whites)	403	8.6 (7.0–10)	403 (100%)	149 (40%)
CAMP (African-Americans)	67	8.7 (8–10)	67 (100%)	37 (45%)
CHS (mixed white and Hispanic)	2772	7.6 (6–10)	1206 (44%)	1283 (46%)
GRAAD (African-Americans)	935	31 (11–44)	464 (50%)	728 (54%)
FHS (non-Hispanic whites)	7477	48.6 (19–89)	961 (12.9%)	4070 (54%)
Males				
Costa Ricans	260	8.7 (8–10)	260 (100%)	
CAMP (non-Hispanic whites)	254	8.4 (7–10)	254 (100%)	
CAMP (African-Americans)	37	9.1 (8–11)	37 (100%)	
CHS (mixed white and Hispanic)	1489	7.3 (6–10)	665 (24%)	
GRAAD (African-Americans)	406	25 (9–42)	211 (52%)	
FHS (non-Hispanic whites)	3407	48.7 (19–89)	401 (11.8%)	
Females				
Costa Ricans	157	8.7 (8–10)	157 (100%)	
CAMP (non-Hispanic whites)	149	9.0 (7–11)	149 (100%)	
CAMP (African-Americans)	30	9.1 (8–11)	30 (100%)	
CHS (mixed white and Hispanic)	1283	9.1 (7–10)	541 (20%)	
GRAAD (African-Americans)	529	34 (17–45)	253 (48%)	
FHS (non-Hispanic whites)	4070	48.6 (19–86)	560 (13.8%)	

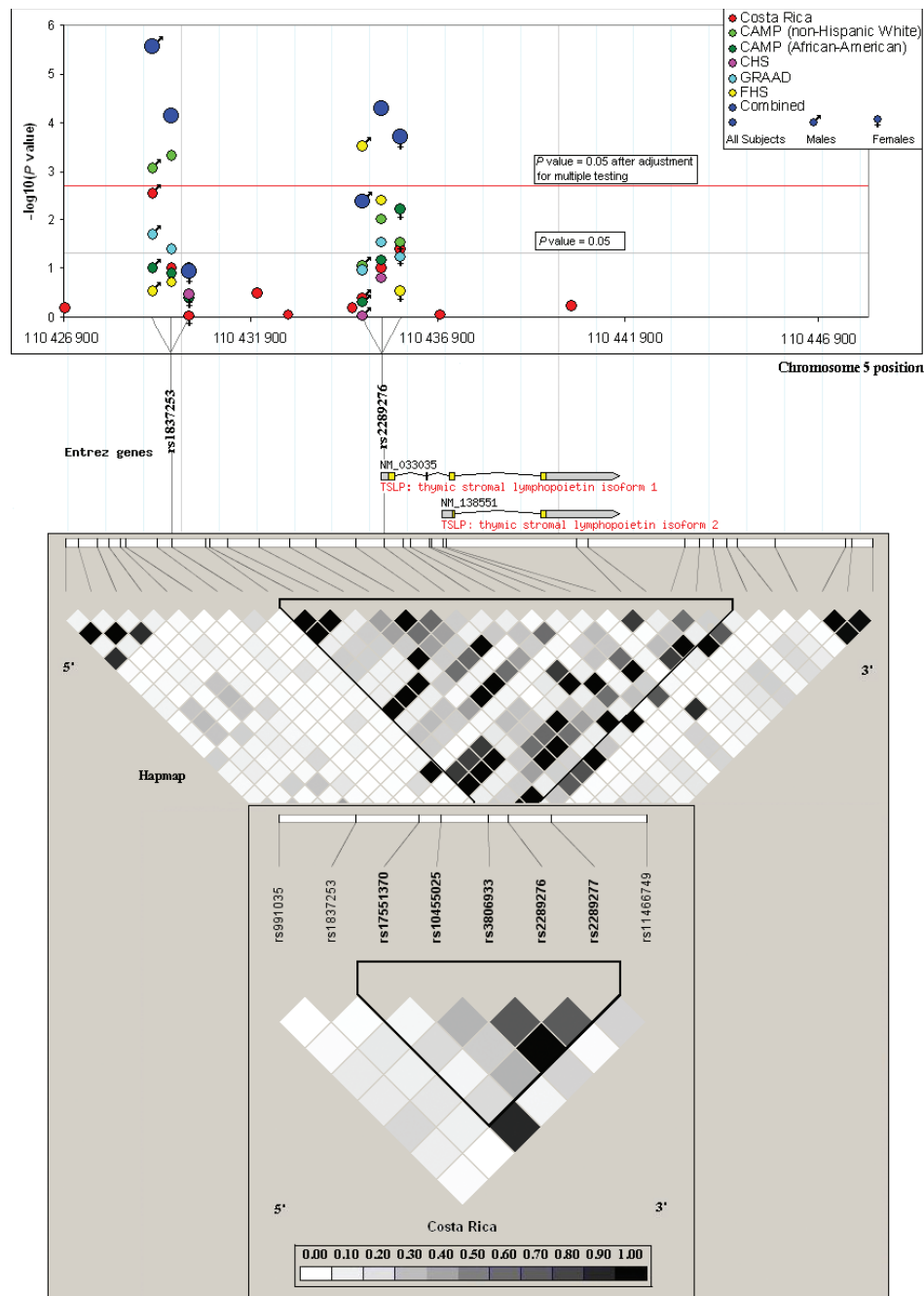


Figure 1 The data in the upper portion of the figure represents the results of genetic association between asthma and single nucleotide polymorphisms (SNPs) in *TSLP* genotyped in Costa Rica (in red), Childhood Asthma Management Program (CAMP-in light green for white subjects, in dark green for African-American subjects), the Children's Health Study (CHS-in pink), the Genome Research on the African-American Diaspora (GRAAD-in light blue) and in the Framingham Heart Study (FHS-in yellow). The combined P -values are represented in blue for all subjects for rs1837253 and

rs2289276. For SNP rs1837253 and rs2289276, the colored symbols 'o' represent all subjects, '♂' represents male subjects, and '♀' represents female subjects. The gray horizontal bar represents a nominal significance level (P -value 0.05), and the red horizontal bar represents a P -value of 0.05 after adjustment for multiple comparisons (P -value 0.0023). The bottom portion of the figure represents the pairwise (r^2) linkage disequilibrium (LD) patterns for the genotyped region in CEPH (Centre d'étude du polymorphisme humain), Costa Rican parents.

Table 2 Association between *TSLP* SNPs and asthma in all subjects participating in this study and a comparison with previously reported findings

Six populations Evaluated for this Study					Four Populations Evaluated by He et al. (16)												
Costa Rica	CAMP	CHS	GRAAD	FHS	Combined*	CAPPS	SAGE	SLSJ	Busselton	Combined*	Overall Combined*						
rs1837253	Non-Hispanic White Children	Mixed Hispanic and White Children	African-American Mixed Adolescents and Adults	Non-Hispanic White Adults		Mixed White Asian Children	Mixed White Aboriginal Children	Non-Hispanic White Adults	Non-Hispanic White Adults								
												T:U ratio (FBAT) P-value)†	OR (Case-control) P-value)‡	T:U ratio (FBAT) P-value)†	OR (Case-control) P-value)‡	OR (Case-control) P-value)‡	OR (Case-control) P-value)‡
0.85 (NS)	0.67 (5E-04)	0.74 (NS)	0.90 (0.04)	0.95 (0.04)	0.96 (NS)	0.80 (NS)	1.09 (NS)	0.69 (0.003)	0.79 (0.003)	0.63 (NS)	7E-07 (3E-05)						
rs2289276	Hispanic Children	African-American Children	Mixed Hispanic and White Children	Non-Hispanic White Adults		Mixed White Asian Children	Mixed White Aboriginal Children	Non-Hispanic White Adults	Non-Hispanic White Adults								
												T:U ratio (FBAT) P-value)†	OR (Case-control) P-value)‡	T:U ratio (FBAT) P-value)†	OR (Case-control) P-value)‡	OR (Case-control) P-value)‡	OR (Case-control) P-value)‡
0.88 (NS)	0.78 (0.01)	0.67 (0.07)	0.94 (NS)	0.95 (0.03)	0.86 (0.004)	0.65 (0.08)	1.16 (NS)	1.02 (NS)	1.13 (NS)	0.63 (0.90)	6E-04 (0.002)						

Cohorts include (for this study) Costa Rica, Busselton, Busselton Health Study Population; CAPPS, (previously evaluated) Canadian Asthma Primary Prevention Study; CAMP, Childhood Asthma Management Program; CHS, Children's Health; FBAT, Family-based Association Test; FHS, Framingham Heart Studies; GRAAD, Genomic Research on Asthma in the African Diaspora; SAGE, Study of Asthma and Genes and the Environment; SLSJ, Saguenay-Lac-Saint-Jean and Quebec City Familial Asthma Collection.

*Combined *P*-values were obtained using Fisher's method referencing the inverse association of the minor allele of the SNP (using one-sided alternatives) and asthma. Combined *P*-values from a weighted Z-score method are in parenthesis.

T:U ratio = ratio of transmitted to untransmitted alleles. *P*-value attained from a family-based association test.

*Odds Ratio (genotypic) and *P*-value from either case-control association test, or from a population-based association test.

For ease of exposition only P -values ≤ 0.1 (and their respective effect estimates) within individual populations are displayed.

refer to the direction of effect with respect to the minor allele (an inverse association).

Table 3 Sex-specific association between *TSLP* SNP and asthma in the Costa Rica, Childhood Asthma Management Program (CAMP), Children's Health (CHS), the Genomic Research on Asthma in the African Diaspora (GRAAD), and the Framingham Heart Studies (FHS)

Asthma	rs number	Alleles	Location	Initial Identification of Association									
				Replication Populations									
				Costa Rica	CAMP	CHS	GRAAD	FHS	Combined*	Combined*	Combined*	Combined*	Combined*
				Hispanic	Non-Hispanic White	African-American	Mixed Hispanic and White	African-American	Non-Hispanic White				
				T:U ratio (FBAT) <i>P</i> -value†	T:U ratio (FBAT) <i>P</i> -value†	T:U ratio (FBAT) <i>P</i> -value†	OR (Case-control) <i>P</i> -value‡	OR (Case-control) <i>P</i> -value‡	OR (Population-based) <i>P</i> -value‡				
Males	rs1837253	C>T	5' genomic	0.65 (0.006)	0.63 (9E-04)	0.64 (0.1)	0.84 (0.02)	0.92 (0.02)	0.94 (NS)	-3E-06 (-5E-04)	-6E-05 (0.003)		
Males	rs2289276	C>T	5' Untranslated	0.95 (NS)	0.82 (NS)	1 (NS)	1 (NS)	0.94 (NS)	0.74 (5E-04)	-0.004 (0.01)			
Females	rs1837253	C>T	5' genomic	1.37 (0.03)	0.77 (NS)	0.9 (NS)	0.97 (NS)	0.96 (NS)	0.98 (NS)	0.1 (0.06)			
Females	rs2289276	C>T	5' Untranslated	0.77 (0.04)	0.71 (0.03)	0.27 (0.006)	0.87 (0.06)	0.94 (0.06)	0.97 (NS)	-2E-04 (0.009)	-6E-04 (0.02)		

TSLP, Thymic stromal lymphopoietin; SNP, Single nucleotide polymorphism.

*Combined *P*-values were obtained using Fisher's method referencing the inverse association of the minor allele of the SNP (using one-sided alternatives) and asthma. Combined *P*-values from a weighted Z-score method are in parenthesis.

†T:U ratio = ratio of transmitted to untransmitted alleles. *P*-value attained from a family-based association test.

‡Odds Ratio (genotypic) and *P*-value from either case-control association test, or from a population-based association test.

For ease of exposition only *P*-values ≤0.1 (and their respective effect estimates) within individual populations are displayed, *P*-values >0.1 are listed as NS = non-significant. Negative signs refer to the direction of effect with respect to the minor allele (an inverse association).

SNP rs1837253 was significantly inversely associated with asthma in boys and SNP rs2289276 was significantly inversely associated with asthma in girls (see Table 2). We then tested for association between these two variants and asthma in additional cohorts.

Association analyses of asthma

In the analysis of all cohorts, the T allele of SNP rs1837253 was significantly inversely associated with asthma in whites in CAMP, in the CHS and in GRAAD (see Table 2) but not in African-Americans in CAMP or in the FHS (P values of 0.4 and 0.2, respectively). The T allele of SNP rs2289276 was significantly inversely associated with asthma in whites in CAMP, in the GRAAD, and in the FHS (see Table 2) but not in African-Americans in CAMP or in the CHS (P values of 0.07 and 0.4, respectively). Because the direction of the association between the minor allele of either of the two SNPs (rs1837253 and rs2289276) and asthma was consistent across all six cohorts, we conducted a combined analysis. In this analysis, there was a significant inverse association with asthma for the minor alleles of rs1837253 and rs2289276 (see Table 2 and Fig. 1), which remained significant after correction for multiple testing.

Comparison with previously published data

We compared our findings to the previously published findings for the minor alleles of rs1837253 and rs2289276 and asthma in the Canadian Asthma Primary Prevention Study (CAPPS), Study of Asthma and Genes and the Environment (SAGE), Saquenay-Lac-Saint-Jean and Quebec City Familial Asthma Collection (SLSJ), and Busselton Health Study Population (Busselton) cohorts (6). All but one of the ten cohorts (SAGE) demonstrated an inverse association between SNP rs1837253 and asthma [Fisher's combined P -value = 7×10^{-7} (see Table 2)]. More variability was noted in the association between SNP rs2289276 and asthma (see Table 2).

Sex-stratified replication studies

After stratification by sex, SNP rs1837253 was significantly inversely associated with asthma in boys, and SNP rs2289276 was significantly inversely associated with asthma in girls (see Table 3). On the basis of these findings, we tested for association between these two variants and asthma in additional cohorts.

Among males in all cohorts, the minor allele of SNP rs1837253 was inversely associated with asthma. This inverse association attained nominal statistical significance in whites in CAMP, CHS and GRAAD (see Table 3) but not in African-Americans in CAMP or in the FHS (see Table 3). There was a significant inverse association between the minor allele of SNP rs1837253 and asthma in males in all cohorts (see Table 3 and Fig. 1), which remained significant after excluding data from the Costa Rican cohort and correction for multiple testing (see Table 3). There was no significant evidence of an inverse association between SNP rs1837253 in

female subjects from any cohort except for a weakly significant association in female Costa Ricans (see Table 3).

In contrast to our findings for SNP rs1837253 in males, the T allele of SNP rs2289276 was inversely associated with asthma in females from all cohorts, with nominal significance in CAMP (both in whites and in African-Americans) (see Table 3), and associations of borderline statistical significance in GRAAD and CHS (see Table 3). These findings were similar in direction of association but did not attain significance in the FHS (see Table 3). In the combined analysis of all cohorts, there was a significant inverse association between the T allele of SNP rs2289276 and asthma in females (see Table 3 and Fig. 1), which remained significant after exclusion of data from the Costa Rican cohort and adjustment for multiple testing (see Table 3). While an inverse (or null) association between SNP rs2289276 and asthma was noted in both sexes in all cohorts, only in the FHS was this effect greater in males (see Table 2) than in females. However, the combined evidence for an inverse association between rs2289276 and asthma in males (see Table 3) was not significant.

Discussion

Our study demonstrates that two polymorphisms (rs1837253 and rs2289276) in the genomic region of *TSLP* are inversely associated with asthma. Our findings in six populations (including > 13 000 subjects), coupled with those of He et al. (6) in four populations (including 5565 subjects), establish that an inverse association between the T allele of rs1837253 and asthma is among the most consistently replicated findings in asthma genetics and approaches a genome-wide level of significance (P -value 7×10^{-7}). The inverse association between SNP rs2289276 and asthma, while attaining a strong level of combined significance (P -value 6×10^{-4}), was more variable across populations.

This study is among the largest to evaluate the role of sex in a genetic epidemiologic study (24), and our analysis was strongly motivated by evidence from a sex-stratified genome-wide linkage analysis (8) and animal models suggesting that sex plays a prominent role in the pulmonary pathology and resultant mortality associated with the transgenic expression of *TSLP* in mice (7). To our knowledge, these are the first sex-specific genetic associations for asthma to be replicated at the SNP level in independent populations.

He et al. (6) did not report that the association between rs1837253 and asthma was significantly modified by sex. However, as effect estimates and associations were not presented the previous study, it is difficult to compare these statements to our findings. Consistent with our findings in cohorts of children or adolescents (Costa Rica, CAMP, CHS, GRAAD), the previous report noted a female-specific inverse association between rs2289276 and asthma in one population of children (SAGE) with a contrasting effect in another childhood population (CAPPS). Taken together, these findings suggest that both sex and age likely influence the association between rs2289276 and asthma. Potential reasons for a discrepant association between rs2289276 and asthma in girls

include differences in sample size (CAPPS includes only 57 parent–child trios with asthma) and phenotypic assessment of asthma across studies.

TSLP is an IL-7-like cytokine with two splice variants that function through an interaction with a heterodimeric receptor consisting of the IL-7 receptor α -chain [IL-7R α (146661)] and the TSLP receptor [TSLPR(300357)] that is related to, but distinct from, the common cytokine receptor γ chain [γ_c (308380)] (25, 26). While few studies have examined the effect of individual *TSLP* isoforms, at least one study suggests that they may play distinct roles in the asthma phenotype (17). *TSLP* is expressed in bronchial smooth muscle cells (27), basophils (28), IgE-activated mast cells and prominently in bronchial epithelial cells [(29), Fig. S1].

Although our data and previous work in animal models (7) suggest that sex modifies the effect of TSLP on allergic diseases, the reasons for this are unclear. Female *TSLP* transgenic mice had increased mortality attributable to a pulmonary perivascular leukocytic infiltration with prominent eosinophilia (7). A similar but less severe phenotype was present in male mice (7). This implies that diametric responses to increased *TSLP* expression in men and women are unlikely. Our finding of inverse associations with asthma in men (for SNP rs1837253) and women (for SNP rs2289276) additionally implies that altered transcription (or post-translational modification) resulting from these genotypes is likely to be complex and may depend on particular environmental factors (e.g. the association with IgE level and asthma is more consistent for *TSLP* SNP rs2289276 than for rs1837253, see Supporting Information, Table S2). The gene for the TSLP receptor (*TSLPR*) is located in pseudoautosomal region 1(Xp22.3 and Yp11.3) in humans and on chromosome 5 in the mouse. The sex chromosome location of *TSLPR* in humans cannot explain the similar sex-specificity across species. We speculate that one possible mechanism for our findings is that sex (or the hormonal regulation associated with sex) could result in the differential regulation of a transcription factor whose binding site is altered by the SNPs of interest. Another alternate and/or additional possibility is that sex might be more important determinant of the end-organ response to specific stimuli than has been previously realized. *TSLP* transgenic mice developed sex-dependent differences pulmonary morbidity and mortality despite having similar measured TSLP protein levels (Charles Alpers, personal communication).

Our study has several limitations. First, although we were able to replicate many findings in the same direction of association across populations, we cannot exclude the possibility that some of our findings are false negatives because of sample size (particularly among African-Americans in CAMP). Secondly, given lack of a significant genotype-by-sex interaction and inconsistent replication across cohorts, we have less evidence for a sex-specific association between rs2289276 and asthma. However, given a significant interaction between rs2289276 and sex on total IgE in children (8) and the fact that most discrepant findings appear to be driven by the differences between children and adults, we suspect that age may have an important role in these findings of association. Asthma is most prevalent in male children, equally prevalent

in men and women between ages 20–40 years, and most prevalent in females in adults over the age of 40 years (see Fig. S2) (30). Because the relation between sex and asthma varies with age, it is tempting but premature to link TSLP regulation to this phenomenon. Thirdly, while bioinformatic databases note that SNP rs2289276 is predicted to disrupt an exonic splicing enhancer site and that SNP rs1837253 is predicted to both remove and create a number of potential transcription factor binding sites (31), there is no current experimental evidence of the function of these SNPs. Finally, while genome-wide association studies (GWAS) of asthma have not implicated *TSLP* to date, our results suggest that large sample sizes will likely be required to detect convincing findings of association that vary by sex (and age).

In summary, we have identified a sex-specific association between two polymorphisms in the genomic region of *TSLP* and asthma. Our work provides support to the growing body of evidence that *TSLP* plays an important role in the pathogenesis of asthma. In addition, our findings provide one genetic clue to the long-standing, but puzzling, relation between sex and asthma.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Additional information on Subjects and Methods, Statistical Analysis, and Results (including Sex-Specific Association Analysis for Total IgE [Table S2] and Immunohistochemistry).

Figure S1. Human endobronchial biopsy samples from asthmatic patients were fixed, embedded, and serially sectioned. Paraffin-embedded tissues were hydrated, retrieved, and immunostained with anti-TSLP (in dark brown). Images were taken with a conventional light microscope using 200x magnification.

Figure S2. The upper portion of the figure presents the sex and age specific current asthma prevalence rates in the United States from the Centers of Disease Control, National Health Interview Survey, 2006 (<http://www.cdc.gov/asthma/nhis/06/table-4-1.htm>). The bottom portion of the figure presents the age-specific interquartile ranges of subjects from Costa Rica (in red), Childhood Asthma Management Program (CAMP-in light green for white subjects, in dark green for African-American subjects), the Children's Health Study (CHS-in pink), the Genome Research on the African-American Diaspora (GRAAD-in light blue), and in the Framingham Heart Study (FHS-in yellow).

Table S1. Minor Allele Frequencies of Two *TSLP* SNP in the Costa Rica, Childhood Asthma Management Program (CAMP), Children's Health (CHS), the Genomic Research on Asthma in the African Diaspora (GRAAD), and the Framingham Heart Studies (FHS) and the HapMap CEPH and Yoruban populations.

Table S2. Association between *TSLP* SNP and Total IgE in the Costa Rica, Childhood Asthma Management Program (CAMP), and the Genomic Research on Asthma in the African Diaspora (GRAAD) studies.

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