

A thymic stromal lymphopoietin gene variant is associated with asthma and airway hyperresponsiveness

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Background: The epithelial cell–derived protein thymic stromal lymphopoietin stimulates dendritic and mast cells to promote proallergic T_H2 responses. Studies of transgenic expression of thymic stromal lymphopoietin and its receptor knockout mice have emphasized its critical role in the development of allergic inflammation. Association of genetic variation in thymic stromal lymphopoietin with IgE levels has been reported for human subjects.

Objective: The aim of this study was to evaluate the relationship between variants of thymic stromal lymphopoietin and asthma and related phenotypes.

Methods: We selected 6 single nucleotide polymorphisms in thymic stromal lymphopoietin and genotyped 5565 individuals from 4 independent asthma studies and tested for association with asthma, atopy, atopic asthma, and airway hyperresponsiveness by using a general allelic likelihood ratio test. *P* values were corrected for the effective number of independent single nucleotide polymorphisms and phenotypes. **Results:** The A allele of rs1837253, which is 5.7 kb upstream of the transcription start site of the gene, was associated with protection from asthma, atopic asthma, and airway hyperresponsiveness, with the odds ratios and corrected *P* values for each being 0.79 and 0.0058; 0.75 and 0.0074; and 0.76 and 0.0094, respectively. Associations between thymic stromal lymphopoietin and asthma-related phenotypes were the most

statistically significant observations in our study, which has to date examined 98 candidate genes. Full results are available online at <http://genapha.icapture.ubc.ca/>.

Conclusions: A genetic variant in the region of the thymic stromal lymphopoietin gene is associated with the phenotypes of asthma and airway hyperresponsiveness. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Airway hyperresponsiveness, association study, asthma genetics, atopy, polymorphisms, thymic stromal lymphopoietin

Asthma is a syndrome characterized by airway hyperresponsiveness (AHR), which results in reversible episodes of airway obstruction associated with inflammation of the bronchial mucosa. An exaggerated production of IgE antibodies in response to common aeroallergens (atopy) is often the basis for the airway inflammation. An imbalance between T_H1 and T_H2 immune cell responses resulting in a skewing toward the T_H2 phenotype is a major factor contributing to the inflammation of the airways and the development of asthma.^{1,2} The thymic stromal lymphopoietin (*TSLP*) gene codes for an IL-7–like cytokine TSLP that induces myeloid dendritic cells to stimulate the differentiation of naive CD4⁺ T cells to T_H2 cells. In murine models, TSLP plays a critical role in the initiation and maintenance of allergic airway inflammation.³⁻⁵

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Abbreviations used

AHR:	Airway hyperresponsiveness
CAPPS:	Canadian Asthma Primary Prevention Study
LD:	Linkage disequilibrium
OR:	Odds ratio
SAGE:	Study of Asthma Genes and the Environment
SLSJ:	Saguenay-Lac-Saint-Jean and Québec City Familial Asthma Collection
SNP:	Single nucleotide polymorphism
TSLP:	Thymic stromal lymphopoietin
TSLPR:	Thymic stromal lymphopoietin receptor

Accumulating evidence supports *TSLP* as a candidate gene for asthma and allergic diseases:

1. *TSLP* is expressed in the airway epithelium, the entry site for allergens.
2. The *TSLP* receptor is a heterodimer consisting of the IL-7 receptor α chain and a common γ -like receptor chain called the *TSLP* receptor (*TSLPR*).^{3,6,7} Two nonsynonymous single nucleotide polymorphisms (SNPs) in the *interleukin 7 receptor gene* (*IL7R*) have been reported to be associated with inhalational allergy.⁸
3. *TSLPR* knockout mice (*TSLPR*^{-/-}) have demonstrated resistance to “asthma,”⁹ and overexpression of *TSLP* in the lungs and epidermis of transgenic mice induced allergic inflammation.^{9,10}
4. There is evidence to suggest that overexpression of *TSLP* may have sex-specific effects.^{11,12}
5. *TSLP* overexpression interacts with allergen exposure to produce airway inflammation by stimulating both the innate and adaptive immune responses.¹³

In addition, a previous report showed that the number of cells expressing *TSLP* mRNA is increased in the epithelium of human beings who have asthma.¹⁴ The expression of *TSLP* in human airway epithelial cells is induced by rhinovirus,¹⁵ an infection that increases the risk of asthma.¹⁶ Recently *TSLP* was identified as a strong candidate in a human linkage analysis. The T allele of the rs2289276 SNP in *TSLP* was associated with lower levels of cockroach allergen-specific IgE and total IgE in girls, and the association with total IgE was replicated in an independent sample.¹⁷ However, the findings were limited to females, and asthmatic phenotypes were not studied.

We hypothesized that there would be associations of *TSLP* variants with allergy phenotypes, such as asthma and AHR. We included SNPs in and around *TSLP* for associations with 4 asthma/allergy phenotypes in a combined total of 5565 individuals from 4 asthma studies. After correction for multiple testing, we found evidence for association of a *TSLP* variant (rs1837253) with asthma, atopic asthma, and AHR.

METHODS

Populations for genetic association studies

Four well characterized independent studies of individuals affected with asthma were brought together for this study (Table 1). All DNA samples were collected with informed consent obtained in compliance with the Research Ethics Board of each recruiting center.

Canadian Asthma Primary Prevention Study

(CAPPS). Subjects were a total of 549 children at high risk for developing asthma and their parents who, during the second and third trimester of

pregnancy, were enrolled in an asthma prevention study and were recruited from 2 Canadian cities, Vancouver and Winnipeg.¹⁸ The children have been followed since birth and have been assessed by a pediatric allergist for the presence of asthma and allergies. All phenotypes used in the analysis were derived from the 7-year follow-up (380 children/families).¹⁹

Study of Asthma Genes and the Environment

(SAGE). A total of 723 children and their parents were recruited from a population-based sample of 16,320 children born in the Province of Manitoba, Canada, in 1995.^{20,21} In 2002, a 1-page health survey was mailed to families. Children were then stratified according to the self-reported presence of asthma ($n = 392$), allergies ($n = 192$), or neither ($n = 3002$). Children with neither condition were further stratified by rural or urban residence. All children in the asthma and allergy stratum were invited to participate in the nested case-control study, as were a representative sample of controls from both urban and rural environments. Children were assessed for asthma and other allergic phenotypes by a pediatric allergist.

Saguenay-Lac-Saint-Jean and Québec City Familial Asthma Collection (SLSJ). This collection is composed of 306 families from the Saguenay-Lac-Saint-Jean ($n = 227$) and the Québec City ($n = 79$) regions of Québec, Canada.²²⁻²⁷

Busselton Health Study Population. Residents of the town of Busselton in the southwest of Western Australia have been involved in a series of health surveys since 1966. Subjects attended 1 of 6 cross-sectional surveys from 1966 to 1981 and the follow-up survey in 1994. From this population, a nested case-control study was designed consisting of individuals who participated in 1 or more surveys and had a methacholine challenge. Cases and controls were designated on the presence (679 cases) or absence of asthma (870 controls).^{28,29}

Subjects were considered to have asthma ($n = 679$) if they answered yes to the question, “Has your doctor ever told you that you had asthma/bronchial asthma?” at any survey. All other subjects were considered controls ($n = 870$). After genotyping, sample quality control, and removal of related individuals and duplicate samples, there were 644 asthma cases and 751 controls available for the analysis. The panel includes a sample of individuals with and without allergy as determined by skin prick tests to common allergens. Methacholine challenge tests using a modification of the Yan method³⁰ were performed on all participants of the 1994 follow-up survey.

Phenotypes

Asthma. Asthma was defined as doctor-diagnosed asthma at 7 years of age in the CAPPS and SAGE cohorts; as doctor-diagnosed asthma, present asthma, or past documented clinical history of asthma in the SLSJ sample; and as self-reported doctor-diagnosed asthma in the Busselton study. In the Busselton study in the joint analysis, the self-reported asthma phenotype may be more sensitive, but less specific, when compared with physician-diagnosed asthma in the CAPPS, SAGE and SLSJ samples. As such, our asthma phenotype was harmonized but, due to differences in the study designs, we were unable to strictly standardize the definition. We note that the first and only Genome Wide Association Study³¹ used the same combination of self reported and doctor diagnosed asthma, and they too combined childhood and adult asthma samples.

Atopy. Atopy was defined as at least 1 positive response (wheal diameter ≥ 3 mm greater than the negative control at 10 minutes).

AHR. Airway hyperresponsiveness was defined as $PC_{20} < 3.2$ mg/mL for CAPPS and SAGE subjects and < 8 mg/mL for SLSJ subjects, or $PD_{20} \leq 3.9$ μ mol for Busselton subjects. In the Busselton study, controls are subjects who completed a methacholine challenge test, but whose PD_{20} was > 20 . Because AHR is more prevalent in children,^{32,33} for the childhood samples (CAPPS and SAGE), we used $PC_{20} < 3.2$ mg/mL, because it yields the greatest sum of sensitivity and specificity in children.³³

Atopic asthma. Atopic asthma was defined as individuals diagnosed with both asthma and atopy.

Tagging SNP selection, genotyping, and data cleaning

Five common SNPs (minor allele frequency ≥ 0.05) were selected by using pairwise linkage disequilibrium (LD) measures³⁴ to capture the genetic

TABLE I. Clinical characteristics of subjects from the 4 study samples

Study design	Family-based trios				Case-control	
Sample	CAPPS	SAGE	SLSJ	Total	Busselton	Total
Families	545	723	306	1574		
Individuals genotyped (parents, children, affected, and unaffected)	1316	1466	1234	4016	1549	1549
Complete trios					No. of cases	No. of controls
Asthma*†	57	139	379	575	644	751
Atopy*†	135	190	362	687	620	575
AHR*†	170	120	278	568	213	814
Atopic asthma*†	43	92	305	440	382	400
Characteristics						
Sex (male:female)‡	1.05:1	1.26:1	1:1.32		1:1.46	1:1.26
White ethnicity, n (%)	301 (79)	525 (73)	100		100	100

*Number of complete trios (genotypes on both parents and affected child) with this phenotype classification who are eligible for the analysis (ie, passed all quality controls) in each study.

†In the Busselton study, this indicates the number of cases with the phenotype and the number of controls for that phenotype.

‡In the Busselton study, the sex ratio is for asthma cases and controls.

variation within *TSLP* and a 10-kb interval upstream from the transcription start site and downstream of the 3' untranslated region. An additional SNP (rs2289276) was subsequently reported in the literature and was added to the study. Simultaneously we genotyped 150 SNPs previously tested for association with at least 1 asthma-related phenotype, an additional 30 coding non-synonymous SNPs with a minor allele frequency ≥ 0.05 , and 761 tagging SNPs that interrogate the bulk of the genetic variation in 98 genes.^{22,35} We genotyped samples with the Illumina Bead Array System in accordance with the manufacturer's protocol³⁶ (San Diego, Calif) and according to Lincoln et al.³⁷ For 669 samples in the CAPPS and SAGE cohorts, where there was insufficient genomic DNA to complete the genotyping, we used DNA templates generated using a Whole Genome Amplification method with the RepliG Midi kit (Qiagen catalog no. 150045, Qiagen, Hilden, Germany). We retained markers for analysis if they had a minimum call rate of 90%, a maximum of 4 mendelian errors, and a maximum of 1 reproducibility error, and showed consistency with Hardy-Weinberg equilibrium at the level $P > .001$. An additional SNP (rs2289276) was genotyped by TaqMan, using the manufacturer's protocol (Applied Biosystems, Foster City, Calif) with the following specifications: 5 ng DNA per reaction, with a total reaction volume of 5 μ L and drying of DNA template overnight at room temperature.

In the family-based samples, relationships between samples were confirmed by comparing pairwise average number of alleles identical by state to what is expected. A total of 24 families (13 CAPPS, 8 SAGE, and 3 SLSJ) were excluded because of either nonpaternity or unresolved DNA switches. At most 1 mendelian inconsistency was observed for 99.1% of the SNPs in the remaining families. Four sets of twins were identified in the CAPPS cohort (2 monozygotic and 2 dizygotic). The dizygotic twins were retained. A single sibling was chosen from the monozygotic pairs for inclusion in the analysis. In the case-control sample (the Busselton study), we identified 73 parent-offspring relationships and 52 sibling pairs. To address these relationships, we eliminated 125 samples. Moreover, we identified 2 samples that were likely of Asian descent, inclusion of which (in a case-control design) could contribute to spurious results because of population differences in allele frequency. We also identified 2 duplicate samples (identical at all loci), but because the 2 samples differed in phenotype (1 case and 1 control), both samples were removed. This resulted in 644 asthma cases and 751 controls available for the analysis.

Statistical analysis and correction for multiple testing

Associations between SNPs and the 4 phenotypes were tested by using a general additive allelic likelihood ratio test χ^2 test as implemented in UNPHASED,^{38,39} which uses a retrospective conditional likelihood similar to the Transmission Disequilibrium Test⁴⁰ for the family-based studies and

a standard retrospective case-control likelihood for case-control designs. In the family studies, the likelihood is the probability of observing the joint genotype distribution (mother, father, and child), conditional on the affection status of the child. The likelihoods from family and case-control designs can then be combined in a joint analysis of the data. The allelic model is a standard model for Transmission Disequilibrium Test designs, in which the transmission, and nontransmission, of parental alleles are compared with the expected distribution under the assumption of mendelian segregation and random mating. Both alleles are expected to be equally transmitted from parent to offspring. Deviation from this distribution is known as *transmission distortion*, the most likely explanation of which is disease association. For each SNP, the common allele is the referent, and an odds ratio (OR) is provided for the minor allele. The test statistic is distributed as a χ^2 with 1 degree of freedom. We chose this approach because it easily allows for the joint analysis of trio and case-control samples while maintaining protection against population stratification in the family samples. We used only complete affected trios for analyses in the family-based studies to protect against potential bias⁴¹ and population stratification. For the analysis of the CAPPS and SAGE panels, which are independent trios, we used the no-linkage option; because the SLSJ panel has multiple affected sibling pairs, we used the default, which assumes linkage, for the analysis of the SLSJ and combined panels. We included a factor covariate to account for the confounding effects of different population allele frequencies and between sample heterogeneity by using the Busselton panel as the referent group, because it had a population-based study design. Estimates of OR used the common allele as the referent. Stratified analyses were conducted to evaluate the sensitivity of the OR estimates to the inclusion of non-white samples in the analyses.

Our strategy for correction for multiple testing was influenced by the study design. It is recognized that a Bonferroni correction does not take into account the correlation between tagSNPs and would result in a significant overcorrection and subsequent loss of power.⁴² Applying a global multiple correction factor to each SNP in this study would have an undesirable effect: densely typed genes would tend to show greater trends of association merely because they use a greater proportion of the total SNP resources. We used a gene-based approach by applying a correction only with respect to the number of SNPs in that gene and its neighborhood and the number of phenotypes tested (Nyholt 2004). P values were adjusted for both the number of independent SNPs ($N = 4.83$) and phenotypes ($N = 3$) tested⁴²; thus, the unit that is tested is the gene.^{43,44} We chose this approach because it has been suggested that inconsistencies arising from population differences can be resolved by use of a gene-based approach rather than either a SNP-based or a haplotype-based approach.^{43,44} For each gene investigated, an effective number of independent SNPs was calculated by using the definition of Li and Ji,⁴⁵ as implemented in Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD).⁴² We included only the 5 SNPs originally selected for study in this calculation

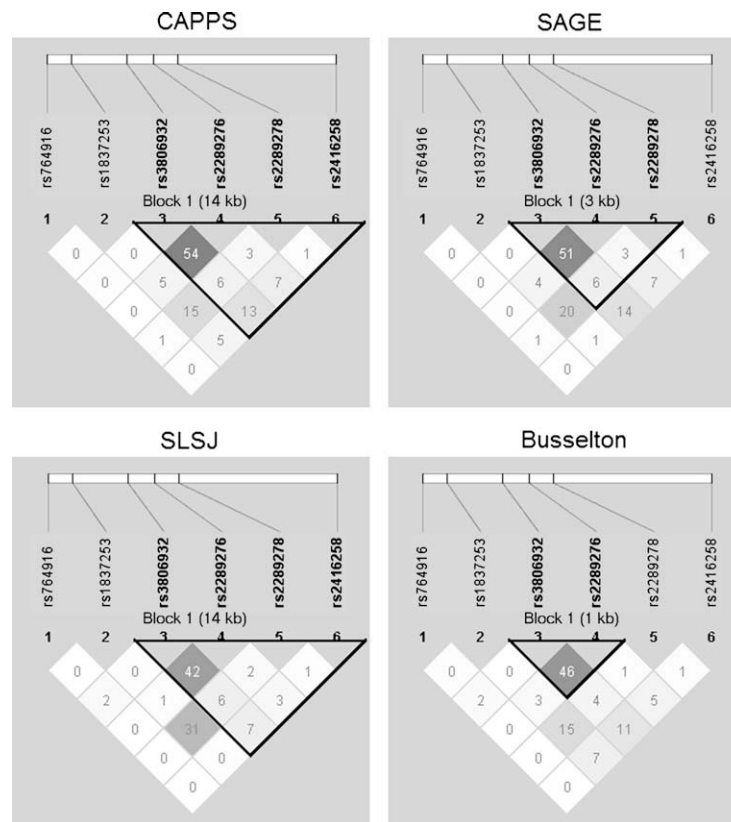


FIG 1. Pairwise linkage disequilibria of *TSLP* SNPs evaluated by the r^2 value. The r^2 values are calculated pairwise, and the r^2 value appears in the corresponding box. Upper left, CAPPS; lower left, SAGE; upper right, SLSJ; lower right, Busselton study. Only 1 common haplotype was identified in each population, and a triangle identifies the block boundary.

because the additional SNP was genotyped at the request of the reviewers. By using a similar procedure, the Matrix Spectral Decomposition approach, we estimated the number of independent phenotypes to be 3 in each sample and in the combined analysis.

To determine whether the genetic effect of *TSLP* differs between males and females (ie, effect modification), sex was included in the model; the statistical significance of the effect modification was evaluated by using the test modifier parameter as implemented in UNPHASED. Under the null hypothesis of no sex effects, the OR will be equal for males and females. If the OR for females differs significantly from males with the same allele, this would provide statistical evidence for effect modification.

RESULTS

LD study

The LD patterns (Fig 1) of the 6 *TSLP* SNPs for each population sample were similar; the pairwise r^2 values ranged from 0.00 to 0.28, indicating minimal LD among the 6 genotyped SNPs.

Association study

In a combined analysis of participants in all 4 population samples, after correcting for multiple comparisons, the A allele of rs1837253, which is 5.717 kb upstream of the transcription start site of *TSLP*, was significantly associated with protection from asthma (OR = 0.79; adjusted P = .0058), atopic asthma (OR = 0.75; adjusted P = .0074), and AHR (OR = 0.76; adjusted P = 0.0094; Table II). We found the associations of *TSLP*

rs1837253 with asthma, atopic asthma, and AHR to be the most statistically significant observations in our studies to date⁴⁶ (Fig 2).

Analyses of individual samples are consistent with the results from the combined analysis. The A allele of rs1837253 showed an association of borderline significance (adjusted P values) with protection from asthma in the SLSJ sample (P = .0053; adjusted P = .0758) and the Busselton study (P = .0056; adjusted P = .0815; Table II). There were several associations of borderline statistical significance in both the combined and individual analyses before adjustment for multiple comparisons (detail in Table II). To define the ORs better, we stratified by ethnicity. When we then examined the evidence for association in white subjects, the most prevalent ethnic group in our studies, we obtained similar results (data not shown). All association results are available online at <http://genapha.icapture.ubc.ca/>.

On the basis of the reported sex-specific association of the *TSLP* (T allele of rs2289276) with allergen-specific IgE¹⁷ and sex-specific hepatic dysfunction caused by overexpression of *TSLP* in transgenic mice,^{11,12,17} we hypothesized that there may be sex-specific allelic effects. However, we found no significant sex effects in the combined analysis or individual populations for any phenotype, except an increased OR (8.16; 95% CI, 1.46-45.84) in females versus a male baseline OR (0.98) for AHR (rs764916, C allele, P = .0170) in the CAPPS sample. For rs2289276, we found no evidence for association with asthma or a related phenotype. We tested for sex modification and found no evidence

TABLE II. Associations of *TSLP* SNPs with asthma, atopy, atopic asthma, and AHR in the separate and combined analyses

SNP and minor allele		CAPPS				SAGE				SLSJ				Busselton				Combined		
		Phenotypes	Trios	OR*	P value	Corrected P value	Trios	OR*	P value	Corrected P value	Trios	OR*	P value	Corrected P value	Case/ Controls	OR*	P value	Corrected P value	OR*	P value
rs1837253A	Asthma	27	0.80	0.5045	1.0000	79	1.09	0.6861	1.0000	157	0.69	0.0053	0.0758	642/750	0.79	0.0056	0.0815	0.79	0.0004	0.0058
	Atopy	71	0.86	0.4629	1.0000	113	1.10	0.5554	1.0000	168	0.71	0.0212	0.3045	618/574	0.92	0.3576	1.0000	0.89	0.0869	1.0000
	Atopic A**	21	0.71	0.3520	1.0000	53	0.94	0.8026	1.0000	142	0.66	0.0056	0.0801	381/400	0.77	0.0240	0.3478	0.75	0.0005	0.0074
	AHR	89	0.96	0.8488	1.0000	71	0.78	0.2689	1.0000	129	0.73	0.0586	0.8423	212/813	0.68	0.0025	0.0363	0.76	0.0007	0.0094
rs2289278G	Asthma	11	2.00	0.2437	1.0000	32	0.89	0.7388	1.0000	90	0.88	0.4721	1.0000	644/750	0.91	0.5298	1.0000	0.92	0.4430	1.0000
	Atopy	30	1.83	0.0840	1.0000	52	1.65	0.0536	0.7693	105	0.88	0.4982	1.0000	619/575	0.82	0.2224	1.0000	1.02	0.8667	1.0000
	Atopic A**	10	1.75	0.3627	1.0000	20	1.09	0.8348	1.0000	84	0.85	0.4231	1.0000	382/800	0.75	0.1876	1.0000	0.86	0.2777	1.0000
	AHR	38	1.10	0.7576	1.0000	28	0.83	0.6013	1.0000	76	1.15	0.5220	1.0000	213/814	0.91	0.6907	1.0000	1.01	0.9280	1.0000
rs2416258G	Asthma	23	1.00	1.0000	1.0000	67	0.85	0.4911	1.0000	100	1.03	0.8628	1.0000	643/750	0.95	0.6457	1.0000	0.96	0.6025	1.0000
	Atopy	65	0.83	0.4247	1.0000	96	0.87	0.4495	1.0000	121	0.92	0.6493	1.0000	620/574	1.29	0.0299	0.4327	1.06	0.4787	1.0000
	Atopic A**	20	0.92	0.8348	1.0000	45	0.86	0.5789	1.0000	103	0.95	0.7758	1.0000	382/399	1.18	0.2523	1.0000	1.04	0.6837	1.0000
	AHR	68	1.14	0.5687	1.0000	55	0.81	0.3848	1.0000	100	0.84	0.3484	1.0000	213/813	0.82	0.2076	1.0000	0.87	0.1700	1.0000
rs3806932C	Asthma	42	0.66	0.1138	1.0000	92	0.98	0.9270	1.0000	230	0.99	0.9183	1.0000	638/747	0.88	0.0879	1.0000	0.91	0.0914	1.0000
	Atopy	93	0.72	0.0783	1.0000	134	0.92	0.5595	1.0000	257	1.04	0.7381	1.0000	617/570	0.81	0.0112	0.1621	0.87	0.0182	0.2635
	Atopic A**	31	0.55	0.0510	0.7360	61	1.02	0.9136	1.0000	212	1.01	0.9534	1.0000	380/397	0.77	0.0126	0.1823	0.86	0.0353	0.5104
	AHR	115	1.00	1.0000	1.0000	82	0.91	0.6255	1.0000	199	1.08	0.5412	1.0000	211/810	0.94	0.6027	1.0000	0.99	0.8808	1.0000
rs764916C	Asthma	8	3.00	0.1480	1.0000	16	0.89	0.8083	1.0000	63	1.09	0.7179	1.0000	644/750	1.09	0.6546	1.0000	1.11	0.4538	1.0000
	Atopy	28	1.14	0.7149	1.0000	30	1.06	0.8618	1.0000	68	1.20	0.4592	1.0000	620/574	1.36	0.1406	1.0000	1.23	0.1190	1.0000
	Atopic A**	8	3.00	0.1480	1.0000	13	1.00	1.0000	1.0000	57	1.04	0.8946	1.0000	382/399	1.45	0.1453	1.0000	1.26	0.1659	1.0000
	AHR	37	2.08	0.0308	0.4442	17	0.80	0.6370	1.0000	48	1.43	0.2361	1.0000	213/813	0.78	0.3901	1.0000	1.18	0.2969	1.0000
rs2289276T	Asthma	32	0.65	0.1638	1.0000	46	1.16	0.5860	1.0000	198	1.02	0.8510	1.0000	644/751	1.13	0.6063	1.0000	0.97	0.6401	1.0000
	Atopy	68	0.86	0.5474	1.0000	91	0.91	0.6350	1.0000	197	1.12	0.3488	1.0000	620/575	0.86	0.1138	1.0000	0.94	0.3151	1.0000
	Atopic A**	23	0.65	0.2213	1.0000	29	1.33	0.3972	1.0000	153	1.02	0.8892	1.0000	382/400	0.88	0.2669	1.0000	0.94	0.4194	1.0000
	AHR	104	1.04	0.7962	1.0000	40	0.81	0.4654	1.0000	138	1.11	0.4985	1.0000	213/814	0.92	0.4980	1.0000	0.99	0.8663	1.0000

*Odds Ratio; **Atopic asthma.

for effect modification in the combined analysis; however, we did find evidence to suggest that there may be sex effects in the childhood cohorts (CAPPS and SAGE) for the asthma, atopic asthma, and atopy phenotypes. However, the direction of the effects was not consistent between the samples (Table III).

Associated SNP and proxies in the International HapMap Database

The rs1837253 polymorphism showed the most significant association in our study. A search of the HapMap database <http://www.hapmap.org/> (accessed December 15, 2007) failed to identify any perfect proxies ($r^2 = 1$) among 3045 SNPs in a 2-Mb genomic segment encompassing the *TSLP* locus. There were no correlated SNPs, even when the moderate criterion of $r^2 > 0.4$ was used.

DISCUSSION

In this large international study of 4 populations, the A allele of rs1837253 demonstrates protective effects for atopic asthma (OR = 0.75; adjusted P = .0074) and for AHR (OR = 0.76; adjusted P = .0094) in the SLSJ and Busselton samples that capture adult persistent asthma. Protective effects are also seen in the CAPPS and SAGE cohorts, which are enriched for transient wheezing, but they did not reach statistical significance. Our comprehensive study, which to date has examined 98 candidate genes for asthma and related phenotypes,⁴⁶ provides further evidence to support the hypothesis that common genetic variants within *TSLP* are associated with the phenotypes of asthma, atopic asthma, and AHR. In fact, the associations with *TSLP* are the most statistically significant observations in our studies to date⁴⁶ (Fig 2). Given the heterogeneity in populations, study designs, and age at onset (childhood and adult) present in the 4 studies, the robustness and consistency of the *TSLP* associations is especially notable. Further, studying childhood asthma and adult asthma together has the

benefit of identifying genetic susceptibility alleles common to both disease subtypes and may lead to the early identification of children whose symptoms are likely to persist into adulthood.

Although significantly associated with asthma, atopic asthma, and AHR, rs1837253 showed no association with atopy, defined as positive skin prick test results. However, it should be noted that we did find evidence for association (unadjusted P = .0182) with atopy and rs3806932 in the combined analysis. The fact that experimental asthma and allergic dermatitis can be produced in *TSLP* transgenic animals lacking T cells and IgE,^{9,10,47} coupled with the observation that *TSLP* derived from epithelial cells can directly activate mast cells in human beings,⁴⁸ supports the concept that *TSLP* can initiate local, organ-specific, allergic inflammation in the absence of systemic markers of atopy. Induction of *TSLP* in the epithelium can also occur via nonallergic stimuli, including rhinovirus and dsRNA.¹⁵ However, the report of an association of *TSLP* variants with levels of allergen-specific IgE and total IgE also supports its potential role in classic IgE-mediated mechanisms.¹⁷

Associations of rs1837253 with asthmatic phenotypes indicate that it is either a causal SNP or in tight LD with a causal SNP. The prediction that SNP rs1837253 will disrupt transcription factor binding sites⁴⁹ indicates that the SNP may be functional. Transcription factors capable of binding the *TSLP* promoter in the epithelium include nuclear factor- κ B and signal transducer and activator of transcription 6, which have been implicated in asthma pathogenesis.¹⁵ We speculate that transcription factor regulation by variants in and around *TSLP*⁵⁰ alters *TSLP* expression, activating *TSLP*-driven airway inflammation and remodelling.¹³ Furthermore, there were no SNPs correlated with rs1837253, even when the moderate criterion of $r^2 > 0.4$ was used. A second *TSLP* SNP (rs2289276) has been reported to be associated with IgE in a sex-specific manner.¹⁷ We found evidence to suggest that there may be sex-specific effects; however, these effects are not consistent across samples (Table III). The 2 SNPs rs2289276

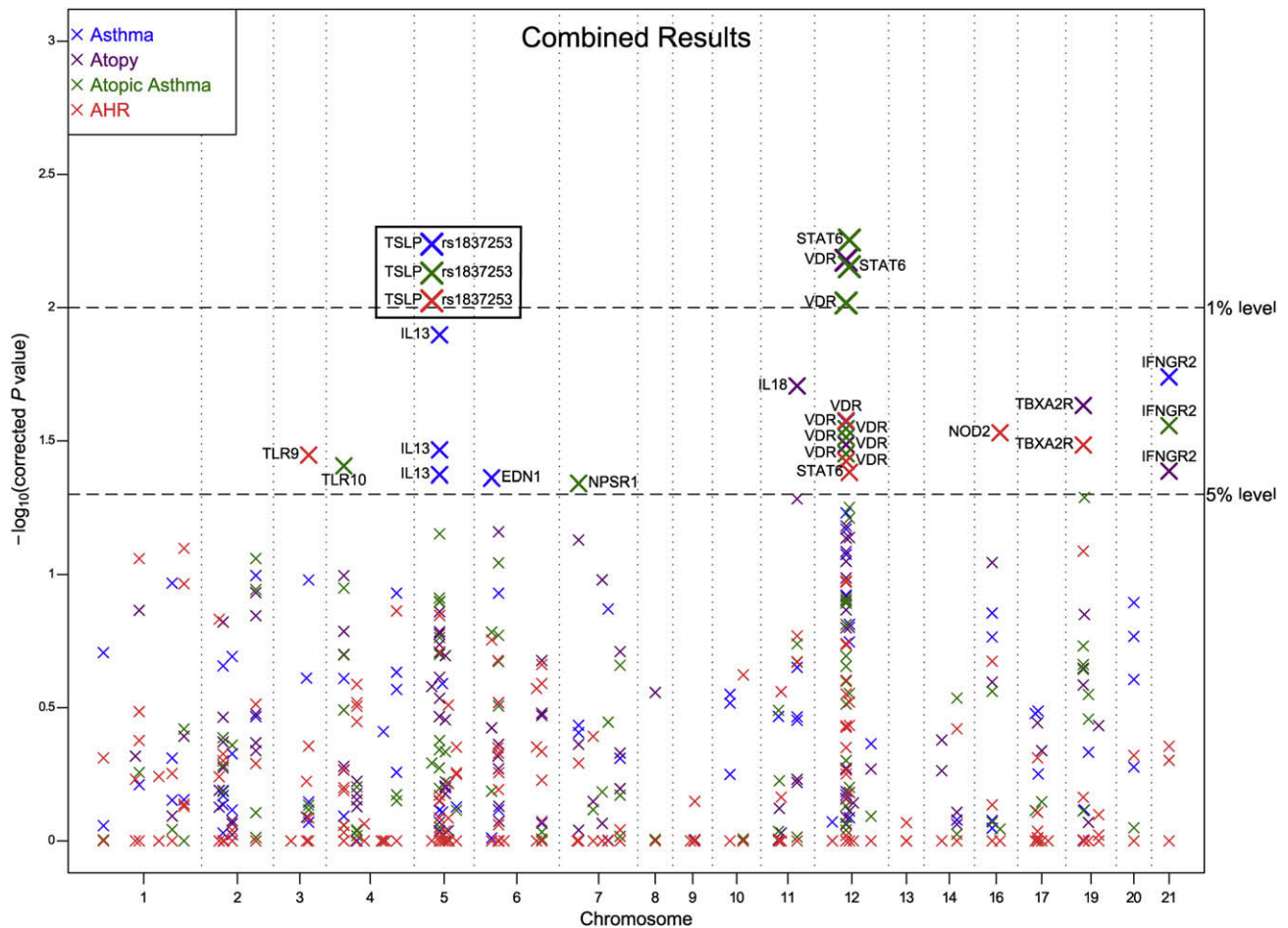


FIG 2. The combined results of the associations of SNPs in the *TSLP* gene with 4 asthma-related phenotypes. Genes are ordered on the x-axis by chromosome and position. The legend for the phenotypes is in the top right corner. Corrected *P* values are on the y-axis. SNPs with corrected *P* values <.05 are labeled with gene name; *the SNP has been previously reported in the literature; [] indicates coding nonsynonymous SNP. *EDN1*: Endothelin 1; *IL13*: Interleukin 13; *IL18*: Interleukin 18; *IFNGR2*: Interferon gamma receptor 2; *NOD2*: Nucleotide-binding oligomerization domain containing 2; *NPSR1*: Neuropeptide S receptor 1; *TLR9*: Toll-like receptor 9; *TLR10*: Toll-like receptor 10; *VDR*: Vitamin D (1,25-dihydroxyvitamin D3) receptor; *STAT6*: Signal transducer and activator of transcription 6, interleukin-4 induced; *TBXA2R*: Thromboxane A2 receptor.

TABLE III. Effect modification by sex for rs2289276 T allele with asthma, atopy, atopic asthma, and AHR in the separate and combined analyses

Phenotype	CAPPS			SAGE			SLSJ			Busseton			Combined		
	Effect			Effect			Effect			Effect			Effect		
	Male OR	Female OR	modification <i>P</i> value	Male OR	Female OR	modification <i>P</i> value	Male OR	Female OR	modification <i>P</i> value	Male OR	Female OR	modification <i>P</i> value	Male OR	Female OR	modification <i>P</i> value
Asthma	0.47	2.13	.0080	1.62	0.41	.0024	0.89	1.11	.7146	0.80	1.35	.0850	0.85	1.22	.1495
Atopy	0.77	1.29	.2372	1.16	0.53	.0020	0.98	1.02	.9312	0.79	1.18	.3631	0.88	1.05	.7421
AHR	1.03	1.04	.8075	0.84	0.85	.6788	1.07	0.95	.8898	0.81	1.23	.4192	0.92	1.09	.6120
Atopic asthma	0.46	2.17	.0158	1.88	0.38	.0165	0.91	1.06	.8631	0.68	1.57	.0513	0.79	1.28	.1532

P values in this table have not been corrected for multiple testing.

and rs1837253 show very weak levels of LD (r^2 of 0.003) in the HapMap Centre d'Etudes du Polymorphisme Humain (CEPH) Utah residents with ancestry from northern and western Europe (CEU) population. These results suggest that multiple SNPs in *TSLP* may influence asthmatic phenotypes independently, as has

been shown for several SNPs in Orosomucoid-like protein 3 (ORMDL3) that contribute to the risk of childhood asthma.³³ Because neither SNP may be functional, but rather in LD with a causal SNP or SNPs that are yet to be identified, deep resequencing and functional assays are needed.

The increasing incidence of asthma during the past 2 decades has resulted in a substantial global burden of morbidity, especially in developed countries.⁵¹ To help alleviate this worldwide problem, new methods to predict and help prevent asthma must be found. It is hoped that genetic studies will aid in the early identification of children with persistent asthma, perhaps even before airway remodeling.⁵² Our results have potential clinical significance in that they suggest novel targets for modulating allergic inflammation in the airways. Because *TSLP* is highly expressed in the airway epithelium, TSLP is accessible to small molecules or antibodies that inhibit its action. Recent studies have also shown that TSLP can induce the expression of Tumor Necrosis Factor (Ligand) superfamily, member 4 (*OX40L* or *TNFSF4*) on the surface of dendritic cells.⁵³ Blockade of OX40-L inhibits TSLP-driven atopic inflammation,⁵⁴ thus providing an additional target for local control of TSLP-driven allergic diseases.^{55,56} Ultimately, identification of individuals whose disease is related to genetic dysregulation of TSLP pathways may lead to more effective and individualized strategies for the prevention and management of asthma.

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Key messages

- Thymic stromal lymphopoietin is a candidate asthma gene. The first association of a single nucleotide polymorphism in *TSLP* with serum IgE levels has been reported. However, associations of *TSLP* variants with allergic phenotypes, such as asthma and AHR, have not yet been described.
- This study identified a *TSLP* SNP that is associated with asthma, atopic asthma, and AHR in Canadian and Australian study populations.

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