Sex-stratified Linkage Analysis Identifies a Female-specific Locus for IgE to Cockroach in Costa Ricans

Gary M. Hunninghake^{1,2,3*}, Jessica Lasky-Su^{1,3,4*}, Manuel E. Soto-Quirós⁵, Lydiana Avila⁵, Catherine Liang¹, Stephen L. Lake^{1,3}, Thomas J. Hudson⁶, Mitzi Spesny⁵, Eduardo Fournier⁵, Jody S. Sylvia¹, Nelson B. Freimer⁷, Barbara J. Klanderman^{1,3}, Benjamin A. Raby^{1,2,3,8}, and Juan C. Celedón^{1,2,3,8}

¹Channing Laboratory, and ²Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts; ³Harvard Medical School, Boston, Massachusetts; ⁴Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; ⁵Division of Pediatric Pulmonology, Hospital Nacional de Niños, San José, Costa Rica; ⁶Ontario Institute for Cancer Research, Ottawa, Ontario, Canada; ⁷Department of Psychiatry, University of California at Los Angeles, Los Angeles, California; and ⁸Center for Genomic Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts

Rationale: The basis for gender influences on allergen-specific IgEs is unclear.

Objectives: To perform regular and sex-stratified genome-wide linkage analyses of IgE to each of three allergens (*Ascaris lumbricoides*, *Blatella germanica* [German cockroach], and *Dermatophagoides pteronyssinus* [dust mite]) and to conduct an association study of a candidate gene in a linked genomic region.

Methods: Genome-wide linkage analyses of allergen-specific IgEs were conducted in 653 members of eight large families of Costa Rican children with asthma. An analysis of the association between single-nucleotide polymorphisms in thymic stromal lymphopoietin (*TSLP*) and IgE measurements was conducted in 417 parent-child trios in Costa Rica. Significant results were replicated in 470 families of white children in the Childhood Asthma Management Program (CAMP).

Measurements and Main Results: Among all subjects, there was suggestive evidence of linkage (LOD ≥ 2.72) to IgE to *Ascaris* (on chromosome 7q) and IgE to dust mite (on chromosomes 7p and 12q). In a sex-stratified analysis, there was significant evidence of linkage to IgE to cockroach on chromosome 5q23 (peak LOD, 4.14 at 127 cM) in female subjects. *TSLP* is located within the 1.5 LOD-unit support interval for this linkage peak and has female-specific effects on lung disease in mice. In a sex-stratified analysis, the T allele of single-nucleotide polymorphism rs2289276 in *TSLP* was associated with reductions in IgE to cockroach (in Costa Rican girls) and total IgE (in girls in Costa Rica and in CAMP; *P* value for sex-by-genotype interaction, <0.01 in both studies).

Conclusions: Consistent with findings in murine models, a variant in *TSLP* may have female-specific effects on allergic phenotypes.

Keywords: immunoglobulin E; linkage; thymic stromal lymphopoietin; single-nucleotide polymorphisms

Childhood asthma is a major public health problem in nations with a Western lifestyle, such as Costa Rica (1), where most children with asthma are atopic (2, 3). A familial predisposition to

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Genome-wide linkage analyses of allergen-specific IgE measurements have been performed, but the results of sex-stratified analyses have not been presented.

What This Study Adds to the Field

This study identifies a female-specific locus for cockroach IgE. A SNP in a gene with female-specific effects on lung disease in mice (*TSLP*) is shown to be associated with cockroach IgE in Costa Rican girls and with total IgE in girls in two populations.

produce serum immunoglobulin E (IgE) in response to allergen exposure is a hallmark of atopy (4, 5). Measurements of allergen-specific IgEs are important predictors of asthma and asthma morbidity in general and among Costa Ricans in particular (2, 3, 6, 7).

Allergen-specific IgE levels are independently heritable traits (5, 8). Gender is a major factor influencing allergen-specific IgE responses (9–11) and their heritability (12). Genome-wide analyses that did not account for potential gender effects have failed to identify unique chromosomal loci with significant evidence of linkage to any allergen-specific IgE (13–17).

As is the case with allergen-specific IgEs, serum total IgE shows significant sexual dimorphism in humans (9–11). We conducted sex-stratified, genome-wide linkage analyses and demonstrated distinct genetic architectures of total IgE among male and female subjects in Costa Rica (18). We hypothesized that, as demonstrated for total IgE, gender would play a prominent role in genome-wide linkage analyses of allergen-specific IgE. In this study, we report results of regular and sex-stratified, genome-wide linkage analyses of serum IgE to each of three common allergens (*Ascaris lumbricoides* [*Ascaris*], *Blatella germanica* [German cockroach], and *Dermatophagoides pteronyssinus* [dust mite]) in 653 members of eight extended families of Costa Rican children with asthma. We have identified a novel female-specific locus (chromosome 5q21-q32) for IgE to cockroach. Within this genomic region, we genotyped variants in a compelling candidate gene with experimental evidence of female-specific effects on lung disease (thymic stromal lymphopoietin [*TSLP*]) (19–23). We report an association between a single-nucleotide polymorphism (SNP) in *TSLP* and IgE to cockroach in Costa Rican girls and total IgE in girls with asthma in Costa Rica and in the Childhood Asthma Management Program (CAMP). This work was partially presented in abstract form (24).

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*These authors contributed equally to this article.

Correspondence and requests for reprints should be addressed to Juan C. Celedón, M.D., Dr.P.H., Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. E-mail: juan.celedon@channing.harvard.edu

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METHODS

For details, *see* the online supplement.

Costa Rica

Extended pedigrees. The population of the Central Valley of Costa Rica is a genetic isolate (25, 26) of mixed Spanish and Amerindian ancestry with a prevalence of asthma that is among the highest in the world (1). For linkage analysis, eight large families of Costa Rican children with asthma were recruited as previously described (18). In brief, inclusion criteria for the eight probands included age at least 6 years but less than or equal to 14 years, physician-diagnosed asthma and more than two respiratory symptoms or asthma attacks in the previous year, increased airway responsiveness, more than one sibling with physician-diagnosed asthma, and at least six great-grandparents born in the Central Valley of Costa Rica.

Parent-child trios. The protocols for subject recruitment and data collection for parent-child trios have been previously described (3, 27). Children included in the study had asthma (defined as physician-diagnosed asthma and more than two respiratory symptoms or asthma attacks in the previous year) and high probability of having more than six great-grandparents born in the Central Valley (confirmed by our study genealogist in 94.8% of children [$n = 416$]). The latter criterion was required to increase the likelihood that children would be descendants of the founder population of the Central Valley (28). Of the 439 participating children, 426 had DNA that passed quality control and are included in this analysis along with their parents.

Study procedures. All study participants (with the exception of parents in trios, who only provided a blood sample) completed a protocol that included a questionnaire and collection of blood samples for DNA extraction and measurements of serum total and allergen-specific IgE. Levels of serum total and allergen-specific IgE were determined by the UniCAP 250 system (Pharmacia and Upjohn, Kalamazoo, MI), with samples measured in duplicate.

Written consent was obtained from adults. Written parental consent was obtained for children, for whom written assent was also obtained. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, MA).

The Childhood Asthma Management Program

CAMP was a multicenter clinical trial of the effects of antiinflammatory medications in children with mild to moderate asthma. All recruited children had asthma defined by symptoms greater than two times per week, the use of an inhaled bronchodilator at least twice weekly or the use

of daily medication for asthma, and airway responsiveness (29, 30). Children with severe asthma or other clinically significant conditions were excluded (29). Of the 1,041 children enrolled in the original clinical trial, 968 children and 1,518 of their parents contributed DNA samples. This analysis was restricted to 483 families of white (non-Hispanic) children.

Study procedures. Serum total IgE was measured by radioimmunosorbent assays from blood samples collected during the screening sessions of CAMP (29). Allergen-specific IgEs were not measured in CAMP.

CAMP was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the other CAMP study centers.

Genotyping and Data Management

The Genome Quebec Innovation Centre performed genomewide genotyping of 380 autosomal short-tandem repeat (STR) markers (average spacing, 8.2 cM) in 667 individuals with Applied Biosystems (Foster City, CA) 3700 and 3730 analyzers using DNA extracted from blood samples using Puregene kits (Gentra Systems, Minneapolis, MN). An additional nine STR markers on chromosome 7 were genotyped at Brigham and Women's Hospital as previously described (31).

RELPAIR (Version 2.0; University of Michigan, <http://csg.sph.umich.edu/boehnke/relpair.php>) was used to determine pedigree relationships on the basis of the genome scan marker data (32, 33). Mendelian inconsistencies at individual markers were resolved using PEDCHECK (34). Pedigree genotype inconsistencies were observed on average less than 0.5% per STR marker.

Using data from European Americans in the International HapMap project (35), we applied a linkage disequilibrium-tagging algorithm (minor allele frequency $\geq 10\%$ and $r^2 \geq 0.8$) to capture common variation in *TSLP* and its 10-kb flanks (36). SNPs were genotyped with the SEQUENOM iPLEX platform (Sequenom, San Diego, CA) (37). Of the seven SNPs that were successfully genotyped, one (rs11539838) was monomorphic. The remaining six SNPs capture at least 72% of the HapMap SNPs, with minor allele frequency of at least 10% in *TSLP* and its 10-kb flanks in European American trios at $r^2 \geq 0.8$. One of these SNPs (rs2289276) was selected for replication studies in families of white children in CAMP and genotyped using the Taqman genotyping assay (Applied Biosystems) (38). In Costa Rica and CAMP, duplicate genotyping was performed on approximately 5% of the samples. Only one discordant genotype was detected, with genotype completion rates greater than 98.0% for all loci.

Statistical Analysis

Estimates of narrow-sense heritability (h^2_N) and multipoint variance components linkage analysis for IgE to each of the allergens of interest were performed using SOLAR, version 2.12 (Southwest Foundation for

TABLE 1. GENOMEWIDE LINKAGE ANALYSIS* OF IgE TO ASCARIS, COCKROACH, AND DUST MITE†

Chromosome	Location (cM)	All Subjects‡ (LOD score)			Male Subjects (LOD score)			Female Subjects (LOD score)		
		IgE to <i>Ascaris</i>	IgE to Cockroach	IgE to Dust Mite	IgE to <i>Ascaris</i>	IgE to Cockroach	IgE to Dust Mite	IgE to <i>Ascaris</i>	IgE to Cockroach	IgE to Dust Mite
1	184							1.58		
4	70								1.66	
5	127								4.14	
7	12								1.83	
7	30						1.68			
7	54				1.96					
7	56			1.92						
7	153	2.72§								
7	173								2.79	
11	89								2.42	
12	152									1.67
12	161			1.90						
16	135							1.78		
17	25		1.83							
17	28								3.24	
19	51	1.68								
19	68				1.57					

* Multipoint linkage analysis by variance component models implemented in SOLAR. Multipoint LOD scores ≥ 1.5 are reported.

† Measurements for the three allergen-specific IgE levels were analyzed as continuous variables and transformed to a $\log_{10}$ scale for analysis.

‡ Models for all subjects were adjusted for sex.

§ Resultant LOD score after genotyping nine additional short-tandem repeat markers (increase from original LOD score of 2.23 at 145 cM).

Biomedical Research, San Antonio, TX) (39). Because values for each allergen-specific IgE were nonnormally distributed, the adjusted LOD score option in SOLAR was used for genomewide linkage analyses (40), which were conducted in all subjects and repeated after stratification by sex (18). Covariates considered in the linkage analyses included sex, age, ever-smoking, pack-years of cigarette smoking, age squared, and pack-years squared. Covariates that were significant ($P < 0.05$) were retained in the final models.

We selected one gene within the 1.5 LOD-unit support interval for the linkage peak to cockroach-specific IgE in female subjects for further analysis. *TSLP* was selected because it was the only gene within the genomic region of interest that had previously demonstrated a role in allergic disease in a sex-specific fashion (23). Association analyses were performed with the FBAT (Family-based Association Test) statistic implemented in PBAT version 5 (41). Sex-by-genotype interactions were tested using the FBAT-interaction statistic in PBAT (42). Analysis was limited to additive models. Measurements of IgE to cockroach were nonnormally distributed and rank-transformed before analysis. Given the

nonmeasurement of IgE to cockroach in CAMP, we evaluated a correlated trait (total IgE, which was measured in both populations) in replication studies. In Costa Rica, cockroach-specific IgE is significantly correlated with ($\log_{10}$ transformed) total serum IgE (Spearman's correlation coefficient, 0.56; $P < 0.0001$). Total IgE levels were transformed to a $\log_{10}$ scale and adjusted for age (and sex where appropriate) for data analysis in Costa Rica and CAMP. For total IgE, we used $P < 0.05$ in both populations to define statistical significance in the setting of multiple tests (43).

RESULTS

Characteristics of Members of Extended Families of Children with Asthma

Of the 667 members of extended pedigrees with genotypic data, allergen-specific IgE measurements were missing on 14 individuals. Table E1 in the online supplement shows the characteristics of the

Figure 1. Results of genomewide multipoint linkage analysis for allergen-specific IgE levels in eight pedigrees of Costa Rican children with asthma. In each figure, results are presented for all subjects and after stratification by sex. Blue lines and red lines denote suggestive ($\text{LOD} \geq 1.90$) and significant ($\text{LOD} \geq 3.3$) LOD scores, as suggested by Lander and Kruglyak (44). Measurements for each of the three allergen-specific IgE levels were transformed to a $\log_{10}$ scale. Models for all subjects were adjusted for sex. (A) *Ascaris* serum IgE. For *Ascaris*-specific IgE, results are for linkage after the inclusion of nine additional markers on chromosome 7. (B) Cockroach serum IgE. (C) Dust mite serum IgE. (A, B) Blue lines, all individuals; red lines, females only; green lines, males only.

653 members of the eight families included in the linkage analysis. There was significant variability among participating families with regard to number of members, gender, values of each allergen-specific IgE, and percentage of subjects with asthma.

Heritability Estimates and Genomewide Linkage Analysis for Serum Allergen-specific IgEs

Among all subjects, estimates of h^2_N of IgE to *Ascaris*, IgE to cockroach, and IgE to dust mite were 0.33 (SE, 0.072; $P = 2.2 \times 10^{-13}$), 0.39 (SE, 0.069; $P = 1.6 \times 10^{-17}$), and 0.38 (SE, 0.075; $P = 3.6 \times 10^{-13}$), respectively. Sex was the only covariate significantly associated with each of the IgEs. After stratification by sex, estimates of the h^2_N of IgE to *Ascaris*, IgE to cockroach, and IgE to dust mite among female subjects were 0.34 (SE, 0.12; $P = 3.8 \times 10^{-5}$), 0.52 (SE, 0.11; $P = 3.8 \times 10^{-10}$), and 0.28 (SE, 0.11; $P = 9.6 \times 10^{-5}$), respectively. Among male subjects, estimates of the h^2_N of IgE to *Ascaris*, IgE to cockroach, and IgE to dust mite were 0.47 (SE, 0.11; $P = 8.2 \times 10^{-9}$), 0.43 (SE, 0.11; $P = 7.0 \times 10^{-6}$), and 0.49 (SE, 0.13; $P = 3.4 \times 10^{-6}$), respectively. Thus, the estimated h^2_N of IgE to *Ascaris* and IgE to dust mite was lower in female subjects than in male subjects, but that of IgE to cockroach was higher in female subjects than in male subjects.

Table 1 and Figure 1 show the main results of the genomewide linkage analysis of serum allergen-specific IgEs in large families of Costa Rican children with asthma. Among all subjects, there was suggestive evidence of linkage (LOD ≥ 1.90) (44) to IgE to *Ascaris* on chromosome 7q (peak LOD, 2.23 at 145 cM), which was increased when the analysis was repeated after inclusion of nine additional STR markers (peak LOD, 2.72 at 153 cM). A sex-stratified analysis revealed only modest evidence of linkage to IgE to *Ascaris* in male and female subjects (peak LOD for male subjects, 1.96 on chromosome 7 at 54 cM; peak LOD for female subjects, 1.78 on chromosome 16 at 135 cM). Among all subjects, no genomic region showed at least suggestive evidence of linkage to IgE to cockroach (peak LOD, 1.83 on chromosome 17 at 25 cM). However, results of the sex-stratified linkage analysis of IgE to cockroach were markedly different between female and male subjects. Although no genomic region showed at least suggestive evidence of linkage in male subjects, there was significant linkage (LOD, ≥ 3.3) (44) to IgE to cockroach on chromosome 5 (peak LOD, 4.14 at 127 cM) in female subjects. The 1.5 LOD-unit support interval for this linkage peak encompasses chromosomal region 5q21-q32 and includes a candidate gene for atopy (*TSLP*). Three additional genomic regions showed suggestive evidence of linkage to cockroach-specific IgE (chromosomes 7q [LOD, 2.79 at 173 cM], 11q [LOD, 2.42 at 89 cM], and 17p [LOD, 3.24 at 28 cM]) in female subjects. Among all subjects, only one genomic region (chromosome 7p at 56 cM) showed suggestive evidence of linkage to IgE to dust mite. A sex-stratified analysis revealed only modest evidence of linkage to IgE to dust mite on chromosome 7p (peak LOD, 1.68 at 30 cM) in male subjects and chromosome 12q (peak LOD, 1.67 at 152 cM) in female subjects.

Family-based Association Study of TSLP

Baseline characteristics of children with asthma in Costa Rica and white (non-Hispanic) children with asthma in CAMP are presented in Table 2. Of the 426 participating parent-child trios in Costa Rica, 9 were excluded from this analysis because of Mendelian inconsistencies. Of the 483 white families in CAMP, 13 were removed from this analysis because of Mendelian inconsistencies, leaving 470 families and 503 probands. Parental genotypes were in Hardy-Weinberg equilibrium ($P > 0.01$) for all of the SNPs in Costa Rica and CAMP.

No SNP in *TSLP* was significantly associated with IgE to cockroach in all subjects in Costa Rica. However, the T allele of

TABLE 2. BASELINE CHARACTERISTICS OF CHILDREN WITH ASTHMA IN COSTA RICA* AND WHITE (NON-HISPANIC) CHILDREN WITH ASTHMA IN THE CHILDHOOD ASTHMA MANAGEMENT PROGRAM

Variable	Median (IQR) or Count (%)	
	Costa Ricans (n = 417)	CAMP (n = 503)
Age, yr	8.7 (7.7–10.4)	8.6 (7.0–10.5)
Gender, female	157 (37.7)	192 (38.2)
Total serum IgE, IU/ml	414 (117–962)	399 (159–1,066) [†]
Eosinophil count, cells/ml ³	530 (290–805) [‡]	402 (211–700) [†]
Positive [‡] IgE to <i>Ascaris</i>	162 (38.9)	Not measured
Positive [‡] IgE to dust mite	319 (76.5)	Not measured
Positive [‡] IgE to cockroach	174 (41.7)	Not measured

Definition of abbreviations: CAMP = Childhood Asthma Management Program; IQR = interquartile range.

* Index children in parent-child trios from the Central Valley of Costa Rica.

[†] Information missing on a subject in Costa Rica for total eosinophil count (n = 1). Information missing on some subjects in CAMP for total serum IgE (n = 7) and total eosinophil count (n = 9).

[‡] IgE values to each allergen (*Blattella germanica*, *D. pteronyssinus*, and *A. lumbricoides*) were considered positive if ≥ 0.35 IU/ml.

a SNP in the 5' untranslated region of *TSLP* (rs2289276) was inversely associated with IgE to cockroach in Costa Rican girls ($P = 0.03$). Although this is not statistically significant, there was a positive association between the T allele of rs2289276 and IgE to cockroach in Costa Rican boys ($P = 0.18$). Although there was no significant evidence of a sex-by-genotype interaction for SNP rs2289276 on IgE to cockroach ($P = 0.19$), there was limited statistical power to detect such an interaction.

Consistent with the findings for IgE to cockroach, no SNP was significantly associated with total IgE among all Costa Rican children. However, a sex-by-SNP (rs2289276) interaction on total IgE was statistically significant in Costa Rica and CAMP ($P < 0.01$ in both studies). The T allele of SNP rs2289276 was inversely associated with total IgE in Costa Rican girls ($P = 0.01$) and in girls in CAMP ($p = 0.004$). There was no statistically significant association between the T allele of SNP rs2289276 and total IgE among boys in Costa Rica or CAMP (Table 3).

DISCUSSION

This is the first report of a genomewide, sex-stratified linkage analysis of serum allergen-specific IgEs in families of children with asthma. Although sex had no strong influence in our findings for IgE to *Ascaris* and IgE to dust mite, we found distinct genetic architectures for cockroach-specific IgE in male and female subjects and identified significant linkage to IgE to cockroach on chromosome 5 (peak LOD, 4.14 at 127 cM) in female subjects. The T allele of a SNP (rs2289276) in a candidate gene near this linkage peak (*TSLP*) was inversely associated with cockroach-specific IgE in Costa Rican girls. In addition, this allele was inversely associated with total IgE in girls in each of two family-based study samples (in Costa Rica and in CAMP). To our knowledge, this is the first sex-specific association with allergy-related phenotypes to be replicated in independent populations (45).

Among members of the eight large families of Costa Rican children with asthma, the estimated heritability of IgE to *Ascaris*, IgE to dust mite, and IgE to cockroach was similar in all subjects (h^2_N range, 0.33–0.39) and in male subjects only (h^2_N range, 0.43–0.49). In contrast, the estimated heritability of IgE to cockroach was higher (h^2_N , 0.52) than that of *Ascaris*-specific IgE (h^2_N , 0.34) or dust mite-specific IgE (h^2_N , 0.28) among female subjects,

TABLE 3. FAMILY-BASED ANALYSIS OF ASSOCIATION BETWEEN *TSLP* SINGLE-NUCLEOTIDE POLYMORPHISMS AND TOTAL SERUM IgE AMONG CHILDREN WITH ASTHMA IN COSTA RICA AND CHILDHOOD ASTHMA MANAGEMENT PROGRAM

rs Number	Alleles	Location	Costa Rica				CAMP					
			MAF	Z score (P value)			FBAT1 P value	MAF	Z score (P value)			FBAT1 P value
				All	Males	Females			All	Males	Females	
rs991035	A > G	5' genomic	0.35	—	—	—	—	—	—	—	—	—
rs17551370	G > A	5' genomic	0.10	—	—	—	—	—	—	—	—	—
rs10455025	A > C	5' genomic	0.31	—	—	—	—	—	—	—	—	—
rs2289276	C > T	5' untranslated	0.29	—	—	−2.19 (0.013)	0.009	0.26	—	—	−2.66 (0.004)	0.002
rs2289277	C > G	Intronic	0.41	—	—	—	—	—	—	—	—	—
rs11466749	A > G	Exonic	0.11	—	—	—	—	—	—	—	—	—

Definition of abbreviations: CAMP = Childhood Asthma Management Program; FBAT = family-based association test; FBAT1 = family-based test of interaction; MAF = minor allele frequency.

Em dashes (—) indicate nonsignificant results (P values > 0.05). For clarity only P values < 0.05 are shown. Minus signs (−) refer to negative association with the minor allele and total serum IgE.

suggesting that genetic factors play a more significant role in the production of IgE to cockroach than in the production of IgE to *Ascaris* or dust mite in female Costa Ricans.

We report the first genomewide linkage analysis of IgE to *Ascaris* (a phenotype associated with asthma severity in Costa Rican children) (3). Among all members of large Costa Rican families, there was suggestive evidence of linkage to *Ascaris*-specific IgE on chromosome 7q, a genomic region previously linked to obesity (a trait often associated with asthma) (46, 47). Previous genomewide studies in families of subjects with asthma revealed two chromosomal regions (19p13 [LOD, 3.51] and 20q13 [LOD, 4.93]), with significant evidence of linkage to dust mite allergy (assessed by skin test positivity and not by continuous measurement of specific IgE) (48, 49). Although “usual” genomewide linkage analyses of allergen-specific IgE measurements have not revealed loci meeting criteria for genomewide significance, an analysis that took maternal imprinting into account showed significant evidence of linkage to dust mite-specific IgE on chromosome 8p22 (maximized LOD score with respect to disease-model parameters, 4.76) (50). There was little to no overlap in our findings for each allergen-specific IgE, suggesting that these phenotypes are influenced by different genomic regions.

The 1.5 LOD-unit support interval for the observed linkage peak to cockroach-specific IgE in female subjects is broad (chromosome 5q21-q32, encompassing ~87 known genes) and includes a region previously shown to be significantly linked to mite-sensitive asthma in Japanese subjects (17). Although genes within 5q21-q32 (e.g., *IL4*, *IL13*, and *CD14*) (52–54) have been previously examined, this is the first association study of *TSLP* for asthma- and allergy-related phenotypes.

Gender-specific analyses of genetic association can lead to false-positive results due to multiple testing (45). Studies that claim to identify sex-specific effects are often limited by lack of replication in independent populations, absence of replication of the findings with regard to the direction of the original association, and no formal testing for sex-by-genotype interaction. We have tested for sex-by-genotype interaction and replicated our female-specific association between *TSLP* and total IgE in two independent populations for the same SNP, in a consistent direction of association.

TSLP, a gene with two splice variants, can prime dendritic cells and stimulate production of Th2 cytokines by naive T cells (54), and *TSLP* knockout mice are protected from allergic airway responsiveness (55). Increased expression of *TSLP* in the airways of human subjects with asthma is associated with increased levels of Th2 cytokines and airway responsiveness (56). Of relevance to our findings, overexpression of *TSLP* in transgenic mice leads to prominent pulmonary pathology, including perivascular leuko-

cytic infiltration and prominent eosinophilia, with more severe effects in female mice than in male mice (23).

Our study has several limitations. First, we had strong motivation to perform sex-stratified, genomewide linkage analyses of allergen-specific IgEs (9–11, 18) and found evidence for an inverse association between the T allele of SNP rs2289276 in *TSLP* and IgE to cockroach in Costa Rican girls, but replication of this finding is needed. However, we are encouraged by our finding that this allele was also inversely associated with total IgE in Costa Rican girls and girls in CAMP. Second, evidence of a sex-by-SNP (rs2289276) interaction for total IgE in Costa Rica and CAMP may implicate that *TSLP* is involved more generally in the sex-dependent regulation of total and allergen-specific IgEs. Third, the functional effects of *TSLP* polymorphisms are not known. However, SNP rs2289276 is predicted to disrupt an exonic splicing enhancer site (57). Finally, data from the International HapMap consortium suggest that SNP rs2289276 is within a large block of linkage disequilibrium that includes *WDR36* (WD repeat-containing protein 36), a gene involved in T-cell activation. We cannot rule out the possibility that other SNPs within *WDR36* alternately or additionally explain our findings of association. Although our findings provide evidence to support a role of *TSLP* in human allergy (in a sex-specific fashion), a fine-mapping association study of chromosome 5q21-q32 is needed to firmly identify the gene(s) responsible for the observed female-specific linkage peak to IgE to cockroach.

In summary, we have identified a female-specific locus for cockroach-specific IgE on chromosome 5q21-q32 in Costa Ricans and a female-specific association between a variant in a candidate gene in this region (*TSLP*) and IgE to cockroach in Costa Rica and total serum IgE in Costa Rica and CAMP. The strong influence of gender on age-associated asthma prevalence (58) and expression of various atopic phenotypes has been well documented but largely unexplored. Our results suggest an important role of sex-specific genetic effects on allergic phenotypes.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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