

## Letter to the Editor

**Genetic variants in thymic stromal lymphopoietin are associated with atopic dermatitis and eczema herpeticum**

To the Editor:

Atopic dermatitis (AD) is a chronic skin disease affecting up to 20% of children in industrialized countries. A rare but serious complication of AD is eczema herpeticum (EH). We recently reported that subjects with AD with EH (ADEH) have more severe  $T_H2$ -polarized disease with greater allergen sensitization and more commonly have a history of food allergy, asthma, or both.<sup>1</sup> Only approximately 5% of patients with AD with herpes simplex virus (HSV) seropositivity (eg, evidence of exposure) will have EH.<sup>2</sup> This observation, coupled with the evidence that susceptibility to EH can be familial and that most subjects report recurrent EH episodes, suggests that genetic susceptibility might be important.

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine that triggers dendritic cells to induce differentiation of naive T cells into  $T_H2$  cells and is implicated in the pathogenesis of allergic diseases.<sup>3</sup> TSLP exerts its biological activities by binding to a heterodimeric receptor consisting of the IL-7 receptor  $\alpha$  chain (IL-7R $\alpha$ ) and the TSLP receptor chain (TSLPR) to initiate signal transducer and activator of transcription 3 and 5 phosphorylation.<sup>4</sup> Recent studies have demonstrated that polymorphisms of the TSLP gene appear to contribute to  $T_H2$ -polarized immunity through higher TSLP production by bronchial epithelial cells in response to viral respiratory tract infections.<sup>5</sup> In this study we hypothesized that variants in TSLP and its receptors were associated with the risk of AD, ADEH, and related subphenotypes. To test the hypothesis, we conducted genetic association studies in 2 independent and racially diverse groups of patients participating in the multicenter Atopic Dermatitis Vaccinia Network (ADVNI).<sup>1</sup> Detailed information on the participants in the ADVNI has been previously described.<sup>6</sup> Local institutional review boards and clinics approved the study, and written informed consent was obtained from all study participants.

A total of 29 single nucleotide polymorphisms (SNPs) were selected from TSLP, IL7R, and TSLPR (15, 11, and 3, respectively) for genotyping. Of these, there were 23 tagging SNPs, one recently reported TSLP functional SNP (rs3806933, -847C/T), 2 SNPs (rs1898671 and rs10062929) within the initially identified region of TSLP, and 3 newly validated TSLPR dbSNPs. Details for each SNP and minor allele frequencies (MAFs) are presented in Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). Of these, 20 SNPs were genotyped by using a custom-designed Illumina (San Diego, Calif) oligonucleotide pool assay (OPA) for the BeadXpress Reader System, and 9 SNPs were genotyped with ABI TaqMan system (Applied Biosystems, Foster City, Calif). Quality controls were performed as described previously.<sup>6</sup> The Cochran-Armitage trend test was used to test for association between each SNP and disease status by using PLINK.<sup>7</sup> A linear regression analysis was performed to test for associations between individual genetic markers and log-adjusted total serum IgE (tIgE) concentrations and log-adjusted Eczema Area and Severity Index (EASI) scores, and a multiple logistic regression model was used to test for SNP-SNP interactions among cases only between

TSLP and its receptors by using SAS version 9.1 software (SAS Institute, Inc, Cary, NC).

As shown in Fig 1, A, and Table I, among the primary European American group, a significant association was observed for TSLP SNP rs11466749 and AD ( $P = .028$ ). Significant associations were also observed for ADEH and 2 additional TSLP SNPs (rs1898671,  $P = .001$ ; rs2416259,  $P = .021$ ) and 4 IL7R SNPs (rs12516866,  $P = .044$ ; rs10213865,  $P = .027$ ; rs1389832,  $P = .011$ ; rs10058453,  $P = .022$ ). When replication was sought in the smaller African American sample, modest associations were observed for AD and 2 TSLP SNPs (rs10043985,  $P = .034$ ; rs2289276,  $P = .018$ ) and 2 IL7R SNPs (rs12516866,  $P = .033$ ; rs1053496,  $P = .011$ ). The number of patients with ADEH in the African American sample was too small to perform meaningful tests for association. To test for association with disease severity, we performed a linear regression analysis using an additive model. Results are summarized in Fig 1, B, and Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). Associations with tIgE concentrations were observed for 6 TSLP SNPs, including 3 SNPs in the European American sample (rs10062929,  $P = .031$ ; rs11466750,  $P = .048$ ; rs2416259,  $P = .034$ ), 3 SNPs in the African American sample (rs991035,  $P = .012$ ; rs17551370,  $P = .023$ ; rs11466749,  $P = .015$ ), and 1 IL7R SNP (rs7737000,  $P = .024$ ) in the African American sample. Two TSLP SNPs associated with tIgE concentrations were also significantly associated with the EASI score, including rs2416259 in the European American sample ( $P = .011$ ) and rs991035 in the African American sample ( $P = .013$ ). To identify functional mutations for TSLPR, we sequenced the TSLPR coding regions and validated 3 dbSNPs with allele frequencies of greater than 10%: rs36139698 (P196L), rs36177645 (X201W), and rs36133495 (A238V). All 3 SNPs were modestly associated with AD in the European American sample (rs36139698,  $P = .026$ ; rs36177645,  $P = .039$ ; rs36133495,  $P = .041$ ; Table I), but this was not replicated in the African American sample. When patients with AD were limited to those of male sex, the most significant association was observed for the SNP rs36133495 ( $P = .0005$ , data not shown). Given the biological relationship between TSLP and its receptors, we tested for epistatic effects (ie, gene-gene interaction) and observed significant interactive effects for AD and ADEH between TSLP and IL7R and TSLPR (see Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The strongest interaction was observed for AD between TSLP SNP rs2289276 and IL7R SNP rs12516866 (odds ratio, 1.86;  $P = .007$ ) among European American subjects and between TSLP SNP rs1898671 and IL7R SNP rs10213865 (odds ratio, 5.12;  $P = .005$ ) among African American subjects.

In this study we observed significant associations for TSLP and IL7R tagging SNPs and AD and ADEH among a European American sample, with replication of associations between TSLP and IL7R SNPs in an independent African American sample. A SNP (rs1898671) in TSLP showed the strongest association with a lower risk of ADEH among European American subjects ( $P = .001$ ), which remained significant after a Bonferroni correction for multiple testing ( $P = .015$ ). Although the function of this variant is unknown, 2 SNPs (rs3806933 and rs2289276) in the promoter region of TSLP are in strong linkage disequilibrium (LD) with rs1898671, and all 3 markers are localized in a single LD block (Fig 1, C). Elsewhere, these markers have been

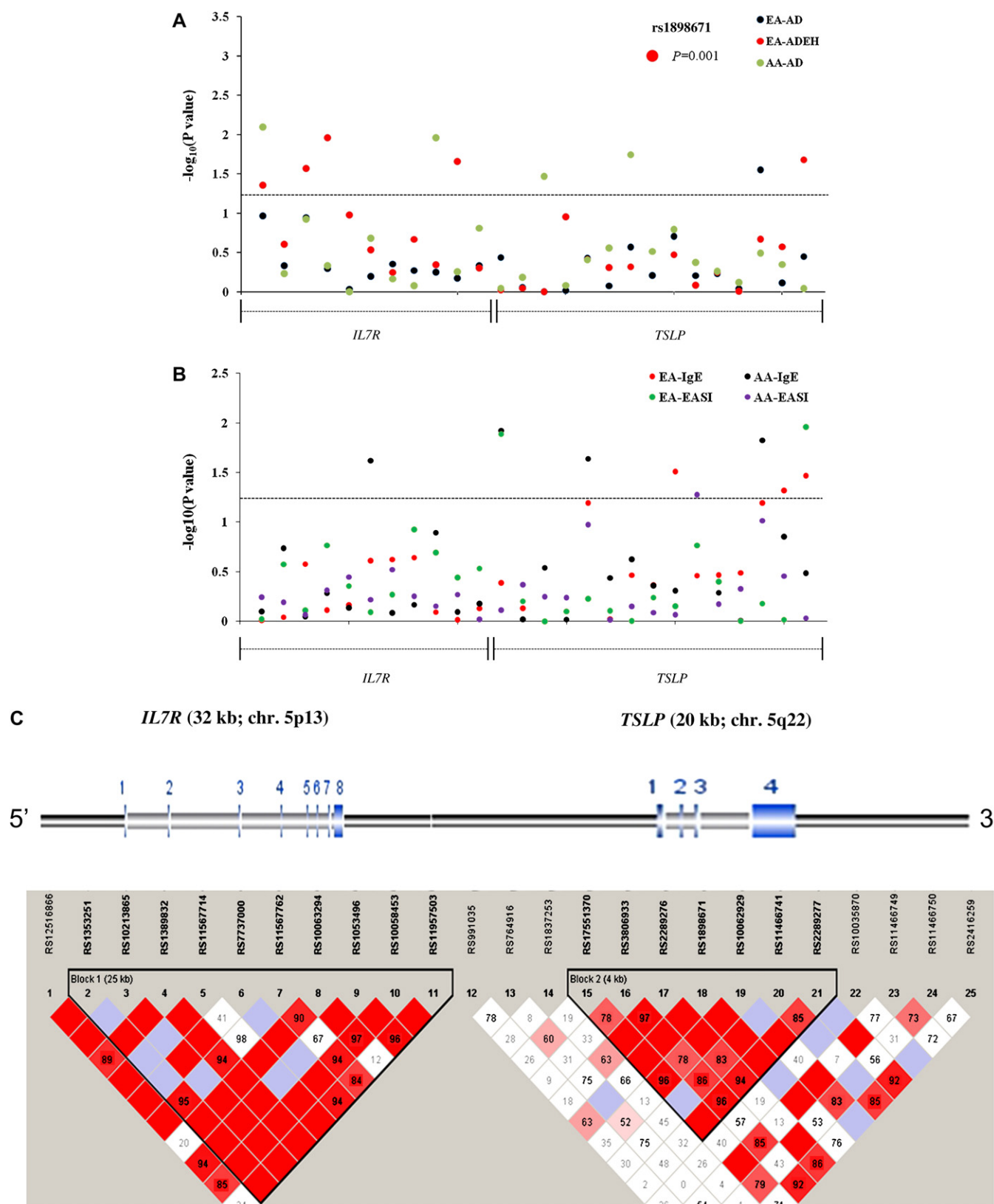


**FIG 1.** The  $-\log_{10}(P \text{ value})$  for associations of *TSLP* and *IL7R* SNPs with AD and ADEH (A) and tlgE concentrations and EASI scores (B). The x-axis represents individual SNPs. The gray line represents a  $P$  value of .05. C, Gene structure and patterns of LD (D'). Shading represents significance of LD, with red to white reflecting higher to lower LD. A square without a number corresponds to a  $D'$  value of 1. EA: European American; AA: African American.

**TABLE I.** Association tests between *TSLP*, *IL7R*, and *TSLPR* SNPs and AD and ADEH in European American (n = 444) and African American (n = 339) subjects

| Gene         | Marker     | Base change | Role       | European American subjects            |             |                                                          |             | African American subjects             |             |
|--------------|------------|-------------|------------|---------------------------------------|-------------|----------------------------------------------------------|-------------|---------------------------------------|-------------|
|              |            |             |            | Patients with AD* vs healthy subjects |             | ADEH <sup>+</sup> subjects vs ADEH <sup>-</sup> subjects |             | Patients with AD* vs healthy subjects |             |
|              |            |             |            | OR (95% CI)                           | P value     | OR (95% CI)                                              | P value     | OR (95% CI)                           | P value     |
| <i>TSLP</i>  | rs10043985 | A/C         | Promoter   | NA                                    | NA          | NA                                                       | NA          | <b>0.5 (0.3-1.0)</b>                  | <b>.034</b> |
|              | rs2289276  | C/T         | 5'-UTR     | 1.3 (0.8-1.9)                         | .270        | 1.2 (0.7-2.1)                                            | .480        | <b>1.8 (1.1-3.1)</b>                  | <b>.018</b> |
|              | rs1898671  | C/T         | Intron     | 0.9 (0.7-1.3)                         | .619        | <b>0.6 (0.4-0.9)</b>                                     | <b>.001</b> | 1.3 (0.8-2.1)                         | .306        |
|              | rs11466749 | A/G         | 3'-UTR     | <b>0.6 (0.4-1.0)</b>                  | <b>.028</b> | 0.7 (0.3-1.3)                                            | .213        | 0.7 (0.5-1.3)                         | .321        |
|              | rs2416259  | T/C         | Downstream | 0.8 (0.5-1.3)                         | .356        | <b>2.0 (1.1-3.6)</b>                                     | <b>.021</b> | 1.0 (0.4-2.1)                         | .906        |
| <i>IL7R</i>  | rs12516866 | G/T         | Promoter   | 0.7 (0.4-1.1)                         | .108        | <b>0.6 (0.3-1.0)</b>                                     | <b>.044</b> | <b>0.8 (0.5-1.2)</b>                  | <b>.033</b> |
|              | rs10213865 | A/C         | Intron     | 0.7 (0.5-1.1)                         | .114        | <b>0.6 (0.3-1.0)</b>                                     | <b>.027</b> | 0.7 (0.4-1.1)                         | .119        |
|              | rs1389832  | C/T         | Intron     | 1.1 (0.7-1.7)                         | .508        | <b>2.0 (1.1-3.7)</b>                                     | <b>.011</b> | 1.2 (0.7-2.0)                         | .464        |
|              | rs1053496  | C/T         | Downstream | 1.1 (0.7-1.7)                         | .561        | 1.2 (0.7-2.1)                                            | .453        | <b>1.8 (1.1-2.9)</b>                  | <b>.011</b> |
|              | rs10058453 | T/C         | Downstream | 0.9 (0.6-1.4)                         | .674        | <b>1.9 (1.1-3.4)</b>                                     | <b>.022</b> | 1.2 (0.7-1.8)                         | .555        |
| <i>TSLPR</i> | rs36139698 | C/T (P196L) | Exon       | <b>0.6 (0.4-1.0)</b>                  | <b>.026</b> | 0.9 (0.5-1.5)                                            | .064        | 1.1 (0.7-1.8)                         | .584        |
|              | rs36177645 | A/G (X210W) | Exon       | <b>1.5 (1.0-2.3)</b>                  | <b>.039</b> | 1.6 (0.9-2.7)                                            | .096        | 1.0 (0.6-1.6)                         | .916        |
|              | rs36133495 | C/T (A238V) | Exon       | <b>1.6 (1.0-2.7)</b>                  | <b>.041</b> | 1.2 (0.6-2.5)                                            | .553        | 0.9 (0.5-1.4)                         | .547        |

Odds ratios (ORs) and P values were calculated under the dominant model. Boldfaced odds ratios and P values are statistically significant.

UTR, Untranslated region.

\*ADEH<sup>+</sup> subjects plus ADEH<sup>-</sup> subjects.

associated with an increase in promoter activity and enhanced activator protein 1 (AP-1) binding to the regulatory element of *TSLP*.<sup>5</sup> SNP rs2416259 was also associated with risk of ADEH, as well as associated phenotypes, high tIgE concentrations, and EASI scores. Of interest, SNP rs2289276, an SNP previously associated with specific IgE levels to cockroach antigen and total IgE concentration,<sup>8</sup> was associated with AD in our study. Three validated coding dbSNPs in *TSLPR* were significantly associated with AD in European American subjects, one of which is a non-sense SNP (rs36177645 X201W), which might lead to premature termination of peptides; investigation into the effects on gene expression are underway. Potential epistatic effects between SNPs in *TSLP* and its 2 receptors, *IL7R* and *TSLPR*, were observed for both AD and ADEH. Associations for SNP-SNP interactions were stronger than the single-marker associations.

A gene-for-gene replication of associations was observed in the African American sample for the traits analyzed (AD, tIgE concentration, and EASI score) and *TSLP* and *IL7R* SNPs. Because of the smaller African American ADEH sample, it was not possible to test for replication to the ADEH trait. However, the SNPs significantly associated with AD, tIgE concentration, and EASI score in the African American subjects were not for the same SNPs as those associated with these 3 traits in the European American subjects. Failure to observe SNP-for-SNP replication in ethnically diverse populations is not uncommon,<sup>9</sup> and potential reasons include variation in allele frequencies, genetic structure, and heterogeneity of the phenotype and environment. Heterogeneity of the phenotype is unlikely in this case because all cases were characterized by using identical protocols at multiple centers.<sup>1</sup> Previously, we evaluated the potential effect of admixture on the case-control design using ancestry informative markers,<sup>6</sup> but we observed no significant population stratification in this sample. However, nearly half (44.8%) of the SNPs genotyped in the current study had substantially different MAFs between the 2 populations, highlighting considerable genetic heterogeneity between the 2 ethnic groups, and the LD structure differed considerably in these 2 samples (data not shown). Very

few of the markers genotyped in this study are likely to be causal but rather are tagging SNPs in LD with an unknown disease-causing variant or variants. That said, it is well accepted that African Americans are more genetically diverse than populations of European ancestry and have shorter LD blocks, and it is therefore not surprising that different sets of tagging SNPs might be associated with the trait of interest compared with European American subjects. We contend that by capturing all of the potential risk-conferring variants, a gene-based approach, rather than an SNP-for-SNP approach, might provide evidence for genetic analysis at the functional level.<sup>10</sup>

Other confounders could influence the observed associations in this study, both between case groups and across ethnic groups. Most cases of ADEH are caused by HSV-1, despite the fact that HSV-1 seropositivity is high in the general population.<sup>1</sup> In the ADVN study 93% of the ADEH group was seropositive for HSV-1 compared with seropositivity of just over 50% for patients with AD without ADEH (ADEH negative, 52%) and control subjects (54%).<sup>1</sup> To test for the possibility that the associations observed between *TSLP*, *IL7R*, and *TSLPR* were for manifestations of an HSV exacerbation in an AD population rather than the disease markers, we investigated the association between HSV infection as determined by both HSV-1 and HSV-2 positivity and the associated SNPs among control subjects, none of which showed enhanced associations compared with the associations with ADEH (data not shown). Because there were no statistical differences in HSV infection between ADEH-negative and control subjects,<sup>1</sup> we also tested for association between the associated SNPs and ADEH-negative status and found that the associations with AD remained (data not shown). These results suggest that the associations between AD and ADEH and *TSLP*, *IL7R*, and *TSLPR* are independent of HSV infection, although a more robustly powered sample is needed to verify these findings. We acknowledge that this study was marginally powered overall, largely because of the rarity of the trait of interest, ADEH, which occurs in less than 5% of subjects with AD. Given the candidate gene nature of this study and the strong biological

justification for interrogating the role of *TSLP* variants and variants in its receptors in the risk of ADEH, AD, or both, the Bonferroni correction for multiple tests is likely too conservative. Nevertheless, one of the most compelling associations was between ADEH and a SNP (rs1898671) in *TSLP* that is in strong LD with a functional variant previously associated with enhanced AP-1 binding to the regulatory element of *TSLP*,<sup>5</sup> an association that remained significant after correcting for multiple testing.

In summary, we demonstrate evidence of association between markers in *TSLP* and its receptors, *IL7R* and *TSLPR*, and the risk of AD and its most serious complication, ADEH, in a multicenter case-control study. Our findings suggest that *TSLP* might be an important candidate for AD, AD severity, and ADEH. This is the first study to implicate *TSLP* as a potential causal gene for ADEH. A clearer understanding of these risk factors might improve our ability to identify patients at greatest risk for ADEH, which might ultimately lead to early intervention and improved surveillance in this vulnerable population.

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Pei-Song Gao, MD, PhD<sup>a</sup>  
Nicholas M. Rafaels, MS<sup>a</sup>  
Deguang Mu, MD, PhD<sup>a,h</sup>  
Tracey Hand, MS<sup>a</sup>  
Tanda Murray, MHS<sup>b</sup>  
Mark Boguniewicz, MD<sup>c</sup>  
Tissa Hata, MD<sup>d</sup>  
Lynda Schneider, MD<sup>e</sup>  
Jon M. Hanifin, MD<sup>f</sup>  
Richard L. Gallo, MD<sup>d</sup>  
Li Gao, MD, PhD<sup>a</sup>  
Terri H. Beaty, PhD<sup>b</sup>  
Lisa A. Beck, MD<sup>g</sup>  
Donald Y. M. Leung, MD<sup>c</sup>  
Kathleen C. Barnes, PhD<sup>a</sup>

From <sup>a</sup>Johns Hopkins Asthma & Allergy Center, Johns Hopkins University School of Medicine, Baltimore, Md; <sup>b</sup>the Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Md; <sup>c</sup>the Department of Pediatrics, National Jewish Health, Denver, Colo; <sup>d</sup>the Division of Dermatology, University of California San Diego, San Diego, Calif; <sup>e</sup>the Division of Immunology, Children's Hospital Boston, Boston, Mass; <sup>f</sup>the Department of Dermatology, Oregon Health & Science University, Portland, Ore; <sup>g</sup>the Department of Dermatology, University of Rochester Medical Center, Rochester, NY; and <sup>h</sup>the Department of Respiratory Medicine, Tangdu Hospital, The Fourth Military Medical University, Xi'an, PR China. E-mail: kbarnes@jhmi.edu.

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## REFERENCES

1. Beck LA, Boguniewicz M, Hata T, Schneider LC, Hanifin J, Gallo R, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. *J Allergy Clin Immunol* 2009;124:260-9.
2. Tay YK, Khoo BP, Goh CL. The epidemiology of atopic dermatitis at a tertiary referral skin center in Singapore. *Asian Pac J Allergy Immunol* 1999;17:137-41.
3. Liu YJ, Soumelis V, Watanabe N, Ito T, Wang YH, Malefyt Rde W, et al. *TSLP*: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu Rev Immunol* 2007;25:193-219.
4. Pandey A, Ozaki K, Baumann H, Levin SD, Puel A, Farr AG, et al. Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. *Nat Immunol* 2000;1:59-64.
5. Harada M, Hirota T, Jodo AI, Doi S, Kameda M, Fujita K, et al. Functional analysis of the thymic stromal lymphopoietin variants in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2009;40:368-74.
6. Gao PS, Rafaels NM, Hand T, Murray T, Boguniewicz M, Hata T, et al. Filaggrin mutations that confer risk of atopic dermatitis confer greater risk for eczema herpeticum. *J Allergy Clin Immunol* 2009;124:507-13, e1-7.
7. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-75.
8. Hunninghake GM, Lasky-Su J, Soto-Quiros ME, Avila L, Liang C, Lake SL, et al. Sex-stratified linkage analysis identifies a female-specific locus for IgE to cockroach in Costa Ricans. *Am J Respir Crit Care Med* 2008;177:830-6.
9. Cui Y, Kang G, Sun K, Qian M, Romero R, Fu W. Gene-centric genomewide association study via entropy. *Genetics* 2008;179:637-50.
10. Neale BM, Sham PC. The future of association studies: gene-based analysis and replication. *Am J Hum Genet* 2004;75:353-62.

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## METHODS

## Genotyping and quality control

We performed genotyping on genomic DNA extracted from blood samples using the MagAttract DNA blood Mini M48 kit (Qiagen, Hilden, Germany) on a Biorobot M48, according to the manufacturer's instructions. DNA quantification was performed with Pico-Green (Molecular Probes, Eugene, Ore).

Tagging SNPs were selected to provide coverage 10 kb upstream and 10 kb downstream of the *TSLP* and *IL7R* genes in both the European American and African American groups in accordance with design score validations based on Illumina in-house measurements and the 60-bp limitation (ie, a SNP cannot be closer than 60 bp to another SNP on an OPA). All available SNPs were initially selected from the HapMap (<http://www.hapmap.org/>) to tag the LD blocks in each ethnic group (European American and African American). Tagging was based on the LDSelect algorithm,<sup>E1,E2</sup> with an MAF of 10% or greater and an  $r^2$  threshold of 0.80 (as reported in HapMap) to ensure nearly perfect LD to infer information on all SNPs captured by the tag set.

The 20 SNPs were genotyped by using the custom-designed Illumina OPA for the BeadXpress Reader System and the GoldenGate Assay with VeraCode Bead technology (San Diego, Calif), according to the manufacturer's protocol.<sup>E3</sup> An additional 74 SNPs identified as ancestry informative markers were selected for maximal difference between African American and European American populations and genotyped to assess potential confounding caused by the population substructure by using the same Illumina platform. Briefly, the GoldenGate assay uses 3 primers designed for each locus. Two are specific to each allele at the SNP site, and a third hybridizes at a downstream locus from the site. All 3 primers have regions complementary to both genome and universal PCR primer sites. A total of 250 ng of high-quality genomic DNA was plated and then activated. The activated DNA, paramagnetic particles, assay oligos, and hybridization buffer were combined in a hybridization step to allow DNA to bind to the particles. After hybridization of primers, plates were washed to reduce noise, and allele-specific oligos were extended and ligated to the downstream locus-specific primer. This mix then served as a PCR template by using the universal primers P1, P2, and

P3. P1 and P2 are Cy3 and Cy5 labeled. After downstream processing, the single-stranded dye-labeled PCR products were hybridized to their complement VeraCode bead type. Plates were then scanned in the BeadXpress Reader for fluorescence and code identification. Scanned data and oligo assignments were uploaded into Illumina's BeadStudio software for downstream genotype cluster analysis.

Nine SNPs were genotyped by using custom-designed primers and probes for the TaqMan Allelic Discrimination Assay (Applied Biosystems). Reactions were set up in 5  $\mu$ L on 96-well plates in TaqMan Universal Master Mix (Applied Biosystems) with 5 ng of DNA, 1  $\mu$ M of each primer, and 0.2  $\mu$ M of each probe. The thermal cycling reactions (50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute) were run and analyzed on a 7900HT Sequence Detection System (Applied Biosystems) with Applied Biosystems Genotyper software (SDS system, version 2.2).

Genotyping quality was high, with an average completion rate of 97% for the TaqMan genotyping and 97.2% to 98.2% (384-SNP and 96-SNP OPAs, respectively) for the BeadXpress genotyping. For the TaqMan genotyping, each plate contained 8 duplicated samples that were randomly selected, as well as 4 blank wells as negative controls, with an average error rate of 0.7%. Additionally, approximately 8% of randomly selected samples were further validated by means of direct sequencing on the 7900HT Sequence Detection System. There were no significant departures from Hardy-Weinberg equilibrium within each ethnic group ( $P > .01$ ).

## REFERENCES

- E1. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004;74:106-20.
- E2. Howie BN, Carlson CS, Rieder MJ, Nickerson DA. Efficient selection of tagging single-nucleotide polymorphisms in multiple populations. *Hum Genet* 2006;120:58-68.
- E3. Fan JB, Gunderson KL, Bibikova M, Yeakley JM, Chen J, Wickham Garcia E, et al. Illumina universal bead arrays. *Methods Enzymol* 2006;410:57-73.

**TABLE E1.** Chromosomal location, base change, and MAFs of *TSLP*, *IL7R*, and *TSLPR* SNPs in both European American and African American subjects

| Gene         | Location    | dbSNP reference | Base change | Role       | MAF                                     |                                        |                  |                  |
|--------------|-------------|-----------------|-------------|------------|-----------------------------------------|----------------------------------------|------------------|------------------|
|              |             |                 |             |            | European American subjects <sup>†</sup> | African American subjects <sup>†</sup> | CEU <sup>‡</sup> | YRI <sup>‡</sup> |
| <i>TSLP</i>  | 110,426,932 | rs991035        | A/G         | Promoter   | 0.445                                   | 0.095                                  | 0.478            | 0.018*           |
| <i>TSLP</i>  | 110,428,090 | rs764916        | G/C         | Promoter   | 0.064                                   | 0.227                                  | 0.050            | 0.183*           |
| <i>TSLP</i>  | 110,429,367 | rs10043985      | A/C         | Promoter   | 0.017                                   | 0.125                                  | 0.008            | 0.150*           |
| <i>TSLP</i>  | 110,429,771 | rs1837253       | C/T         | Promoter   | 0.245                                   | 0.253                                  | 0.283            | 0.323            |
| <i>TSLP</i>  | 110,432,084 | rs17551370      | G/A         | Promoter   | 0.139                                   | 0.138                                  | 0.150            | 0.150            |
| <i>TSLP</i>  | 110,434,641 | rs3806933       | C/T         | Promoter   | 0.410                                   | 0.223                                  | 0.438            | 0.252*           |
| <i>TSLP</i>  | 110,435,406 | rs2289276       | C/T         | 5'-UTR     | 0.288                                   | 0.131                                  | 0.296            | 0.106*           |
| <i>TSLP</i>  | 110,435,901 | rs1898671       | C/T         | Intron     | 0.338                                   | 0.116                                  | 0.317            | 0.104*           |
| <i>TSLP</i>  | 110,436,078 | rs10062929      | C/A         | Intron     | 0.147                                   | 0.129                                  | 0.133            | 0.083            |
| <i>TSLP</i>  | 110,436,604 | rs11466741      | C/T         | Intron     | 0.287                                   | 0.252                                  | 0.305            | 0.310            |
| <i>TSLP</i>  | 110,436,966 | rs2289277       | G/C         | Intron     | 0.419                                   | 0.381                                  | 0.431            | 0.449            |
| <i>TSLP</i>  | 110,438,763 | rs10035870      | A/G         | Intron     | 0.032                                   | 0.070                                  | 0.000            | 0.198*           |
| <i>TSLP</i>  | 110,440,484 | rs11466749      | A/G         | 3'-UTR     | 0.188                                   | 0.141                                  | 0.173            | 0.150            |
| <i>TSLP</i>  | 110,440,793 | rs11466750      | G/A         | 3'-UTR     | 0.166                                   | 0.187                                  | 0.142            | 0.270            |
| <i>TSLP</i>  | 110,447,641 | rs2416259       | T/C         | Downstream | 0.167                                   | 0.043                                  | 0.186            | 0.000*           |
| <i>IL7R</i>  | 35,887,018  | rs12516866      | G/T         | Promoter   | 0.481                                   | 0.463                                  | 0.379            | 0.289            |
| <i>IL7R</i>  | 35,892,964  | rs1353251       | G/A         | Intron     | 0.202                                   | 0.056                                  | 0.186            | 0.000*           |
| <i>IL7R</i>  | 35,893,607  | rs10213865      | A/C         | Intron     | 0.299                                   | 0.250                                  | 0.224            | 0.125            |
| <i>IL7R</i>  | 35,894,478  | rs1389832       | C/T         | Intron     | 0.313                                   | 0.145                                  | 0.258            | 0.033*           |
| <i>IL7R</i>  | 35,898,742  | rs11567714      | C/T         | Intron     | 0.106                                   | 0.023                                  | 0.075            | 0.000*           |
| <i>IL7R</i>  | 35,907,030  | rs7737000       | C/T (H165H) | Exon       | 0.138                                   | 0.151                                  | 0.152            | 0.125            |
| <i>IL7R</i>  | 35,908,958  | rs11567762      | G/A         | Intron     | 0.449                                   | 0.063                                  | 0.107            | 0.058*           |
| <i>IL7R</i>  | 35,913,262  | rs10063294      | A/G         | Downstream | 0.215                                   | 0.268                                  | 0.438            | 0.181            |
| <i>IL7R</i>  | 35,915,186  | rs1053496       | C/T         | Downstream | 0.327                                   | 0.359                                  | 0.350            | 0.392            |
| <i>IL7R</i>  | 35,916,577  | rs10058453      | T/C         | Downstream | 0.353                                   | 0.161                                  | 0.173            | 0.058*           |
| <i>IL7R</i>  | 35,918,933  | rs11957503      | G/T         | Downstream | 0.481                                   | 0.469                                  | 0.505            | 0.479            |
| <i>TSLPR</i> | 741,621     | rs36139698      | C/T (P196L) | Exon       | 0.344                                   | 0.261                                  | NA               | NA               |
| <i>TSLPR</i> | 741,636     | rs36177645      | A/G (X210W) | Exon       | 0.318                                   | 0.366                                  | NA               | NA               |
| <i>TSLPR</i> | 741,747     | rs36133495      | C/T (A238V) | Exon       | 0.500                                   | 0.486                                  | NA               | NA               |

UTR, Untranslated region.

\* $P < .01$  (European American vs African American).<sup>†</sup>MAFs from healthy control subjects participating in the ADVN study.<sup>‡</sup>Allele frequencies from HapMap (<http://www.hapmap.org>) for 60 Yoruba (YRI) and 60 Utah European American (CEU) subjects.

**TABLE E2.** Summary of data confirming associations of *TSLP* and *IL7R* SNPs with total serum levels of IgE (in kIU/L) and EASI scores in European American (n = 414) and African American (n = 328) populations

| Gene        | SNP ID     | Allele | Geometric mean total IgE*  |                |                           |                | Geometric mean EASI*       |                |                           |                |
|-------------|------------|--------|----------------------------|----------------|---------------------------|----------------|----------------------------|----------------|---------------------------|----------------|
|             |            |        | European American subjects |                | African American subjects |                | European American subjects |                | African American subjects |                |
|             |            |        | $\beta$                    | <i>P</i> value | $\beta$                   | <i>P</i> value | $\beta$                    | <i>P</i> value | $\beta$                   | <i>P</i> value |
| <i>TSLP</i> | rs991035   | A      | 0.068                      | .409           | −0.366                    | <b>.012</b>    | 0.094                      | <b>.013</b>    | −0.030                    | .771           |
| <i>TSLP</i> | rs17551370 | A      | −0.238                     | .064           | 0.308                     | <b>.023</b>    | 0.032                      | .592           | 0.153                     | .106           |
| <i>TSLP</i> | rs10062929 | A      | −0.300                     | <b>.031</b>    | 0.120                     | .492           | −0.030                     | .705           | 0.020                     | .857           |
| <i>TSLP</i> | rs11466749 | C      | −0.217                     | .064           | 0.330                     | <b>.015</b>    | 0.024                      | .664           | 0.159                     | .097           |
| <i>TSLP</i> | rs11466750 | A      | −0.234                     | <b>.048</b>    | 0.151                     | .140           | −0.004                     | .968           | 0.068                     | .350           |
| <i>TSLP</i> | rs2416259  | A      | 0.271                      | <b>.034</b>    | −0.219                    | .327           | 0.168                      | <b>.011</b>    | 0.014                     | .931           |
| <i>IL7R</i> | rs7737000  | T      | 0.133                      | .245           | −0.317                    | <b>.024</b>    | 0.014                      | .809           | −0.052                    | .606           |

Boldfaced *P* values are statistically significant.

$\beta$ , Regression coefficient.

\*Total IgE concentrations and EASI scores were adjusted for age and sex.

**TABLE E3.** Interaction tests for risk of AD and ADEH between *TSLP* and its receptors, *IL7R* and *TSLPR*, in both European American and African American populations

| <b>TSLP</b>                          | <b>IL7R</b> | <b>TSLPR</b> | <b>OR_INT</b> | <b>P value</b> |
|--------------------------------------|-------------|--------------|---------------|----------------|
| European American subjects with AD   |             |              |               |                |
| rs2289276                            | rs12516866  |              | 1.86          | .007           |
| rs1837253                            | rs11957503  |              | 1.79          | .018           |
| rs2289277                            | rs1389832   |              | 0.56          | .019           |
| European American subjects with ADEH |             |              |               |                |
| rs10062929                           | rs11957503  |              | 4.59          | .014           |
| rs11466749                           | rs11957503  |              | 3.06          | .020           |
| rs1898671                            |             | rs36139698   | 2.32          | .011           |
| African American AD                  |             |              |               |                |
| rs1898671                            | rs10213865  |              | 5.12          | .005           |
| rs2289277                            | rs10213865  |              | 0.36          | .006           |
| rs991035                             | rs12516866  |              | 3.45          | .011           |
| rs10043985                           |             | rs36177645   | 0.21          | .010           |

*OR\_INT*, Odds ratio for interaction.

\*Interaction tests at  $P < .02$  are listed.