Published in final edited form as:

J Allergy Clin Immunol. 2010 June ; 125(6): 1403–1407.e4. doi:10.1016/j.jaci.2010.03.016.

Genetic Variants in *TSLP* are Associated with Atopic Dermatitis and Eczema Herpeticum

Pei-Song Gao, MD, PhD¹, Nicholas M Rafaels, MS¹, Deguang Mu, MD, PhD^{1,8}, Tracey Hand, MS¹, Tanda Murray, MHS², Mark Boguniewicz, MD³, Tissa Hata, MD⁴, Lynda Schneider, MD⁵, Jon M Hanifin, MD⁶, Richard L Gallo, MD⁴, Li Gao, MD, PhD¹, Terri H Beaty, PhD², Lisa A Beck, MD⁷, Donald YM Leung, MD³, and Kathleen C Barnes, PhD^{1,*}

¹Johns Hopkins Asthma & Allergy Center, Johns Hopkins University School of Medicine, Baltimore, MD 21224

²Department of Epidemiology, Bloomberg School of Public Health, JHU, Baltimore, MD 21287

³Department of Pediatrics, National Jewish Health, Denver, CO 80206

⁴Division of Dermatology, University of California San Diego, San Diego, CA 92093

⁵Division of Immunology, Children's Hospital Boston, Boston, MA 02115

⁶Department of Dermatology, Oregon Health & Science University, Portland, OR 97239

⁷Department of Dermatology, University of Rochester Medical Center, Rochester, NY 14642

⁸Department of Respiratory Medicine, Tangdu Hospital, The Fourth Military Medical University, Xi'an, 710038, PR China

Keywords

Atopic dermatitis; eczema herpeticum; thymic stromal lymphopoietin (*TSLP*); interleukin (IL) 7-like cytokine (*IL7R*); thymic stromal lymphopoietin receptor (*TSLPR*); single nucleotide polymorphism (SNP); gene-gene interaction

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 (ADEH). We recently reported subjects with ADEH have more severe Th2-polarized disease with greater allergen sensitization, and more commonly, have a history of food allergy, asthma, or both¹. Only ~5% of AD patients with HSV seropositivity (e.g. evidence of exposure) develop eczema herpeticum². This observation, coupled with the evidence that susceptibility to EH can be familial and that most subjects report recurrent EH episodes, collectively suggest that genetic susceptibility may be important.

Address correspondence to: Kathleen C. Barnes PhD, Johns Hopkins Asthma & Allergy Center, 5501 Hopkins Bayview Circle, Room 3A.62, Baltimore, MD 21224, USA; 410-550-2071 (Tel); 410-550-2130 (FAX); kbarnes@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7-like cytokine that triggers dendritic cells (DCs) to induce differentiation of naïve T cells into Th2 cells, and is implicated in the pathogenesis of allergic diseases³. *TSLP* exerts its biological activities by binding to a heterodimeric receptor consisting of the IL7 receptor α -chain (IL7R α) and the TSLP receptor chain (TSLPR) to initiate signal transducer and activator of transcription (STAT) 3 and STAT5 phosphorylation⁴. Recent studies have demonstrated that polymorphisms of the *TSLP* gene appear to contribute to Th2-polarized immunity through higher TSLP production by bronchial epithelial cells in response to viral respiratory infections⁵. In this study, we hypothesized variants in *TSLP* and its receptors were associated with the risk of AD, ADEH, and related sub-phenotypes. To test the hypothesis, we conducted genetic association studies in two independent and racially diverse groups of patients participating in the multicenter Atopic Dermatitis Vaccinia Network (ADV N)¹. Detailed information on the participants in the ADV N has been previously described⁶. Local IRBs and clinics approved the study, and written informed consent was obtained from all study participants.

A total of 29 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), two SNPs (rs1898671 and rs10062929) within the initially identified region of *TSLP*, and three newly validated *TSLPR* dbSNPs. Details for each SNP and minor allele frequencies (MAF) are presented in Table EI in the Online Repository at www.jacionline.org. Of these, 20 SNPs were genotyped using a custom-designed Illumina (San Diego, CA) oligonucleotide pool assay (OPA) for the BeadXpress Reader System, and nine SNPs were genotyped using ABI TaqMan (Applied Biosystems, Foster City, CA). Quality controls were performed as described previously⁶. The Cochran–Armitage trend test was used to test for association between each individual SNP and disease status using PLINK⁷. A linear regression analysis was performed to test for associations between individual genetic markers and log-adjusted tIgE and log-adjusted EASI score, and a multiple logistic regression model was used to test for SNP-SNP interaction among cases only between *TSLP* and its receptors using SAS v9.1.

As graphically summarized in Fig 1, A and Table I, among the primary European American group, a significant association was observed for *TSLP* SNP rs11466749 and AD ($P=0.028$). Significant associations were also observed for ADEH and two additional *TSLP* SNPs (rs1898671, $P=0.001$; rs2416259, $P=0.021$) and 4 *IL7R* SNPs (rs12516866, $P=0.044$; rs10213865, $P=0.027$; rs1389832, $P=0.011$; rs10058453, $P=0.022$). When replication was sought in the smaller African American sample, modest associations were observed for AD and two *TSLP* SNPs (rs10043985, $P=0.034$; rs2289276, $P=0.018$) and 2 *IL7R* SNPs (rs12516866, $P=0.033$; rs1053496, $P=0.011$). The number of ADEH patients 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 EII. Associations with total serum IgE concentrations (tIgE) were observed for six *TSLP* SNPs, including three SNPs in the European American sample (rs10062929, $P=0.031$; rs11466750, $P=0.048$; rs2416259, $P=0.034$) and three SNPs in the African American sample (rs991035, $P=0.012$; rs17551370, $P=0.023$; rs11466749, $P=0.015$), and one *IL7R* SNP (rs7737000, $P=0.024$) in African American sample. Two *TSLP* SNPs associated with tIgE were also significantly associated with the Eczema Area and Severity Index (EASI), including rs2416259 in the European American sample ($P=0.011$) and rs991035 in African American sample ($P=0.013$). To identify functional mutations for *TSLPR*, we sequenced the *TSLPR* coding regions and validated three dbSNPs with allele frequencies $>10\%$: rs36139698 (P196L), rs36177645 (X201W), and rs36133495 (A238V). All three SNPs were modestly associated with AD in the European American sample (rs36139698, $P=0.026$; rs36177645, $P=0.039$;

and rs36133495, $P = 0.041$, Table I) but this did not replicate in the African American sample. When AD subjects were limited to male, the most significant association was observed for the SNP rs36133495 ($P=0.0005$, data not shown). Given the biological relationship between *TSLP* and its receptors, we tested for epistatic effects (*i.e.*, gene-gene interaction) and observed significant interactive effects for AD and ADEH between *TSLP* and *IL7R* and *TSLPR* (Table EIII). The strongest interaction was observed for AD between *TSLP* SNP rs2289276 and *IL7R* SNP rs12516866 (OR, 1.86, $P = 0.007$) among European Americans, and between *TSLP* SNP rs1898671 and *IL7R* SNP rs10213865 (OR, 5.12, $P = 0.005$) among African Americans.

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 Americans ($P = 0.001$), which remained significant after a Bonferroni correction for multiple testing ($P = 0.015$). Although the function of this variant is unknown, two SNPs (rs3806933 and rs2289276) in the promoter region of *TSLP* are in strong LD with rs1898671, and all three markers are localized in a single LD block (Fig 1, C). Elsewhere, these markers have been associated with an increase in promoter activity and enhanced AP-1 binding to the regulatory element of *TSLP*⁵. SNP rs2416259 was also associated with risk of ADEH, as well as associated phenotypes, high tIgE and EASI. Of interest, SNP rs2289276, a SNP previously associated with specific IgE levels to cockroach antigen and total IgE⁸, was associated with AD in our study. Three validated coding dbSNPs in *TSLPR* were significantly associated with AD in European Americans, one of which is a non-sense SNP (rs36177645 X201W), which may lead to premature termination of peptides; investigation into the effects on gene expression are underway. Potential epistatic effects between SNPs in *TSLP* and its two 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 and EASI) 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 and EASI in the African Americans were not for the same SNPs as those associated with these three traits in the European Americans. Failure to observe SNP-for-SNP replication in ethnically diverse populations is not uncommon⁹, 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 using identical protocols at multiple centers¹. Previously we evaluated the potential effect of admixture on the case-control design using Ancestry Informative Markers (AIMs)⁶, but 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 two populations, highlighting considerable genetic heterogeneity between the two ethnic groups, and the LD structure differed considerably in these two 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(s). That said, it is well accepted that African Americans are more genetically diverse than European ancestry populations, and have shorter LD blocks, and it is therefore not surprising that different sets of tagging SNPs may be associated with the trait of interest compared to whites. We contend that by capturing all of the potential risk-conferring variants, a gene-based approach - rather than a SNP-for-SNP - may provide evidence for genetic analysis at the functional level¹⁰.

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 population¹. In the ADVN study, 93% of the ADEH group was seropositive for HSV-1, compared to seropositivity of just over 50% for AD patients without ADEH (ADEH-, 52%) and control individuals (54%)¹. 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 controls, none of which showed enhanced associations compared to the associations with ADEH (data not shown). Because there were no statistical differences in HSV infection between ADEH- and control individuals¹, we also tested for association between the associated SNPs and ADEH- 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, due largely to the rarity of the trait of interest, ADEH, which occurs only 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 risk of developing ADEH and/or AD, 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*⁵, 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 risk of AD and its most serious complication, ADEH, in a multicenter case-control study. Our findings suggest *TSLP* may be an important candidate for AD, AD severity and ADEH. This is the first study to implicate *TSLP* as potential causal gene for ADEH. A clearer understanding of these risk factors may improve our ability to identify patients at greatest risk for ADEH, which may ultimately lead to early intervention and improved surveillance in this vulnerable population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge several groups without whom this study would not have been possible: ADVN coordinators (Patricia Taylor NP, Trista Berry BS, Susan Tofte RN, Shahana Baig-Lewis, Peter Brown BS, Lisa Heughan BA, CCRC, Meggie Nguyen BS, Doru Alexandrescu MD, Lorianne Stubbs RC, CCRC, Reena Vaid MD, Diana Lee MD), ADVN regulatory advisors (Judy Lairsmith RN and Lisa Leventhal, MSS, CIM, CIP), biological sample tracking (Jessica Scarpola, Muralidhar Bopparaju, Mary Bolognino MS, Lisa Latchney MS), NIAID-DAIT support (Marshall Plaut MD and Joy Laurienzo Panza RN, BSN, CCRC), DACT Laboratory (Robert Hamilton PhD) and all of the patients who participated in this study. A special thanks to Dan Zaccaro MS, Jamie Reese BS and Susan Lieff PhD, and Gloria David, PhD at Rho, Inc. for coordination of the study, and Patricia Oldewurtel for technical assistance.

Funding: This research was supported by The Atopic Dermatitis and Vaccinia Network NIH/NIAID contracts HHSN266200400029C and HHSN266200400033C. KCB was supported in part by the Mary Beryl Patch Turnbull Scholar Program.

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. [PubMed: 19541356]
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. [PubMed: 10697251]
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. [PubMed: 17129180]
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. [PubMed: 10881176]
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. [PubMed: 18787178]
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. [PubMed: 19733298]
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. [PubMed: 17701901]
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. [PubMed: 18244952]
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. [PubMed: 18458106]
10. Neale BM, Sham PC. The future of association studies: gene-based analysis and replication. *Am J Hum Genet* 2004;75:353–62. [PubMed: 15272419]

Abbreviations

AD	Atopic dermatitis
ADEH	Atopic dermatitis eczema herpeticum
ADVNI	Atopic Dermatitis and Vaccinia Network
EASI	Eczema Area and Severity Index
EH	Eczema Herpeticum
HSV	Herpes simplex virus
MAF	minor allele frequency
SNP	single nucleotide polymorphism
TSLP	Thymic stromal lymphopoietin
TSLPR	Thymic stromal lymphopoietin receptor

C.

Fig 1.

The $-\log_{10}(P \text{ value})$ for associations of *TSLP* and *IL-7R* SNPs with (A) AD and ADEH, and (B) tIgE and EASI. The x-axis represents individual SNP. The grey line represents $P = 0.05$. (C) Gene structure and patterns of LD (D'). Shading represents significance of LD with a red to white reflecting higher to lower LD. Square without a number corresponds to $D' = 1$.

TABLE I

Association tests between *TSLP* and *IL7R* and *TSLPR* single nucleotide polymorphisms (SNPs) and AD and ADEH in European American (N= 444) and African American subjects (N = 339). Odds ratios (*ORs*) and *P*-values were calculated under the dominant model. Bold *ORs* and *p*-values are statistically significant. *ADEH+ plus ADEH-.

Gene	Marker	Base change	Role	European American				African American			
				AD* vs. Healthy		ADEH+ vs. ADEH-		AD* vs. Healthy		ADEH+ vs. ADEH-	
				OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
TSLP	rs10043985	A/C	Promoter	NA	NA	NA	NA	0.5 (0.3-1.0)	0.034	1.8 (1.1-3.1)	0.018
	rs2289276	C/T	5'-UTR	1.3 (0.8-1.9)	0.270	1.2 (0.7-2.1)	0.480	1.8 (1.1-3.1)	0.018	1.3 (0.8-2.1)	0.306
	rs1898671	C/T	Intron	0.9 (0.7-1.3)	0.619	0.6 (0.4-0.9)	0.001	1.3 (0.8-2.1)	0.306	0.7 (0.5-1.3)	0.321
	rs11466749	A/G	3'-UTR	0.6 (0.4-1.0)	0.028	0.7 (0.3-1.3)	0.213	1.0 (0.4-2.1)	0.906	0.8 (0.5-1.2)	0.033
	rs2416259	T/C	Downstream	0.8 (0.5-1.3)	0.356	2.0 (1.1-3.6)	0.021	1.2 (0.7-2.0)	0.464	1.8 (1.1-2.9)	0.011
IL7R	rs12516866	G/T	Promoter	0.7 (0.4-1.1)	0.108	0.6 (0.3-1.0)	0.044	1.2 (0.7-2.1)	0.453	1.2 (0.7-1.8)	0.555
	rs10213865	A/C	Intron	0.7 (0.5-1.1)	0.114	0.6 (0.3-1.0)	0.027	1.2 (0.7-2.1)	0.453	1.2 (0.7-1.8)	0.555
	rs1389832	C/T	Intron	1.1 (0.7-1.7)	0.508	2.0 (1.1-3.7)	0.011	1.2 (0.7-2.1)	0.453	1.2 (0.7-1.8)	0.555
	rs1053496	C/T	Downstream	1.1 (0.7-1.7)	0.561	1.2 (0.7-2.1)	0.453	1.2 (0.7-2.1)	0.453	1.2 (0.7-1.8)	0.555
	rs10058453	T/C	Downstream	0.9 (0.6-1.4)	0.674	1.9 (1.1-3.4)	0.022	1.2 (0.7-2.1)	0.453	1.2 (0.7-1.8)	0.555
TSLPR	rs36139698	C/T (P196L)	Exon	0.6 (0.4-1.0)	0.026	0.9 (0.5-1.5)	0.064	1.1 (0.7-1.8)	0.584	1.0 (0.6-1.6)	0.916
	rs36177645	A/G (X210W)	Exon	1.5 (1.0-2.3)	0.039	1.6 (0.9-2.7)	0.096	1.0 (0.6-1.6)	0.916	0.9 (0.5-1.4)	0.547
	rs36133495	C/T (A238V)	Exon	1.6 (1.0-2.7)	0.041	1.2 (0.6-2.5)	0.553	0.9 (0.5-1.4)	0.547		