A genetic variant near TSLP is associated with chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease in Japanese populations

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Letter to the Editor

A genetic variant near *TSLP* is associated with chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease in Japanese populations

Dear Editor,

Chronic rhinosinusitis with nasal polyps (CRSwNP), is a heterogenous disease associated with asthma, high degrees of disease severity, and a poor quality of life.[1] Some patients with CRSwNP have a specific condition called aspirin-exacerbated respiratory disease (AERD) which is characterized by a triad of symptoms including CRSwNP, asthma, and sensitivity to non-steroidal anti-inflammatory drugs (NSAIDs).[1,2]

Genome-wide association studies (GWASs) have found significant associations between the *thymic stromal lymphopoietin* (*TSLP*) locus and asthma, allergic rhinitis, eosinophilic esophagitis, and nasal polyps (Supplementary Table 1).[3] TSLP is produced by barrier epithelial cells and plays a central role in activating type 2-related inflammatory cells including T_H2 lymphocytes, type 2 innate lymphoid cells, eosinophils, basophils, and mast cells.[2,4] A recent study examined the presence of TSLP, IL-25 and IL-33 in nasal polyps from patients with CRSwNP and AERD and found significant elevations of *TSLP* mRNA in nasal polyps from CRSwNP and AERD compared to controls.[5] Another study has shown that TSLP activates eosinophils and induces the expression of mast cell-derived PGD2 via mast cells, which plays a crucial role in the pathophysiology of AERD.[2] To improve our understanding of the molecular mechanisms of the CRSwNP and AERD, we performed an association study of CRSwNP and AERD with genetic variants in the *TSLP* locus (Supplementary Table 2).

We recruited a total of 499 patients with CRSwNP who received their diagnoses by otolaryngologists. We also recruited a total of 194 patients with AERD. We searched the NHGRI-EBI catalog of published genome-wide association studies for *TSLP* (https://www.ebi.ac.uk/gwas/search). A total of 11 GWASs have reported an association of the *TSLP* locus with allergy-related phenotypes (Supplementary Table 1). We assessed the eight variants that showed the most significant associations at the *TSLP* locus among GWASs (Supplementary Table 3, Supplementary Fig. 1). The study populations and methods are described in detail in the Supplementary Methods.

We first conducted an association study of CRSwNP in the 303 cases and 904 controls for the primary set. We found significant associations between CRSwNP and rs1837253, rs3806932 and rs3806933, with the most significant association being observed at rs1837253 ($P = 1.27 \times 10^{-6}$; odds ratio, 1.60; 95% CI, 1.32–1.94), which is located 5.7 kb upstream of the *TSLP* gene (Supplementary Table 3). We next conducted conditional logistic regression analysis for rs1837253, but no additional independent association signals were observed (Supplementary Table 3). We then performed a validation study for rs1837253 using an independent set of 196 CRSwNP cases and 1004 controls and observed a significant association with CRSwNP after combining the results using fixed-effects meta-analysis ($P_{combined} = 1.01 \times 10^{-10}$; odds ratio, 1.61; 95% CI, 1.40–1.86) (Table 1). Interestingly, a recent GWAS on nasal polyps in individuals of European descent reported a significant association at rs1837253 ($P = 3.7 \times 10^{-29}$; odds ratio, 1.35; 95% CI, 1.28–1.43), and the effect of allele was in the same direction.[3]

We stratified 499 patients with CRSwNP by comorbidity of AERD, and a total of 68 subjects among the 499 patients had AERD (Supplementary Table 2). We conducted an association study of AERD and rs1837253 in the 68 cases and 900 controls for the primary set and observed a significant association ($P = 1.16 \times 10^{-3}$; odds ratio, 1.78; 95% CI, 1.26–2.52). We further performed a replication study using an independent set of 126 AERD cases and 1004 controls for the validation set, and observed a significant association ($P_{combined} = 4.84 \times 10^{-6}$; odds ratio, 1.64; 95% CI, 1.33–2.04) (Table 1, Supplementary Table 2).

We next assessed whether the number of eosinophils in mucosal tissues of patients with CRSwNP correlated with the rs1837253 genotype. We examined correlations between genotypes and the eosinophil numbers in nasal polyps by linear regression analysis. Interestingly, a significant positive correlation between the risk allele and the number of eosinophils was observed ($P = .017$) (Fig. 1). A recent study has shown the influence of the rs1837253 genotype on TSLP secretion in response to double-stranded RNA in human nasal epithelium.[6] The study showed decreased TSLP secretion in subjects with the TT and CT genotypes, and suggested that rs1837253 might be directly involved in the regulation of TSLP secretion.[6]

To investigate the impact of the rs1837253 variant on the binding affinities of transcription factors, we next conducted electrophoretic mobility shift assay. Functional annotations using the HaploReg v4.1 tool indicated that rs1837253 mapped to enhancer histone marks H3K4me1 and H3K27ac in a fetal lung fibroblast cell line. *TSLP* mRNA and protein are expressed

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Table 1
Association results of rs1837253 in CRSwNP and AERD.

Stage	Subject	Genotype			Total	RAF	*P* value[†]	OR (95% CI)[†]	*P* het[‡]
		TT	TC	CC					
CRSwNP									
Primary	Case	93	146	64	303	0.45	1.27×10^{-6}	1.60 (1.32–1.94)	
	Control	396	395	109	900	0.34			
Validation	Case	61	92	43	196	0.45	1.78×10^{-5}	1.63 (1.30–2.04)	
	Control	449	431	122	1002	0.34			
Combined							1.01×10^{-10}	1.61 (1.40–1.86)	0.900
AERD									
Primary	Case[§]	25	20	23	68	0.49	1.16×10^{-3}	1.78 (1.26–2.52)	
	Control	396	395	109	900	0.34			
Validation	Case	35	67	23	125	0.45	1.08×10^{-3}	1.57 (1.20–2.05)	
	Control	449	431	122	1002	0.34			
Combined							4.86×10^{-6}	1.64 (1.33–2.04)	0.575

RAF, risk allele frequency; OR, odds ratio; CI, confidence interval.
[†] *P* values and ORs of the logistic regression test with adjustment for sex and age as covariates. Results of combined analyses were calculated using fixed-effects meta-analysis.
[‡] *P* values of Cochran's heterogeneity statistics.
[§] Among a total of 499 patients with CRSwNP (303 cases in primary set and 196 cases in validation set), a total of 68 subjects had AERD.

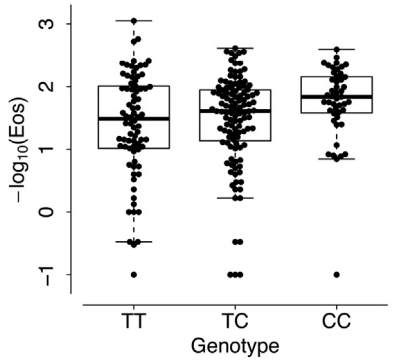

Fig. 1. Relationship between rs1837253 genotype and the number of eosinophils in CRSwNP ($n = 240$). The *P* value was calculated by linear regression analysis ($P = .017$). Log-transformed individual eosinophil numbers are used in the figure. Zero was replaced by 0.1 before log transformation.

in epithelial cells, fibroblasts, and endothelial cells in nasal polyps.[7] We investigated the influence of the rs1837253 variant on the binding affinities of transcription factors using nuclear extracts from nasal fibroblasts. The signal intensity of the DNA–protein complex derived from the susceptible C allele of rs1837253 was higher than that from the T allele in the presence of the nuclear extract (Supplementary Fig. 2). We next examined putative transcription factor binding sites in the DNA sequences, and rs1837253 was found to potentially alter the affinities of two transcription factors, upstream stimulatory factor (USF) 1 and USF2. The findings of a super-shift binding assay using anti-USF1 and -USF2 antibodies suggested that rs1837253 altered nuclear-factor recruitment to the site (Supplementary Fig. 2). Interestingly, USF1-regulated genes are associated with immune response following viral and bacterial infections,[8] and USF1 and USF2 are indispensable for the transcription of RORγT, which regulates the development of Th17 cells and the expression of IL-17.[9]

In conclusion, we identified significant associations between rs1837253 near *TSLP* and susceptibility to CRSwNP and AERD, and also found a positive correlation between the risk allele and the number of eosinophils in mucosal tissues of patients with CRSwNP. Our functional study suggests an allele-specific influence of rs1837253 on affinity for USF1 and USF2 in nasal fibroblasts. Since type 2 immune responses driven by TSLP is known to contribute to both of the pathophysiology of AERD and CRSwNP,[1,2] the shared genetic risk might influence dysregulation of the innate system. Further studies are warranted to investigate the roles of the variants near *TSLP* in the pathogenesis of those chronic inflammatory airway diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2019.06.007.

Conflict of interest
The authors have no conflict of interest to declare.

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