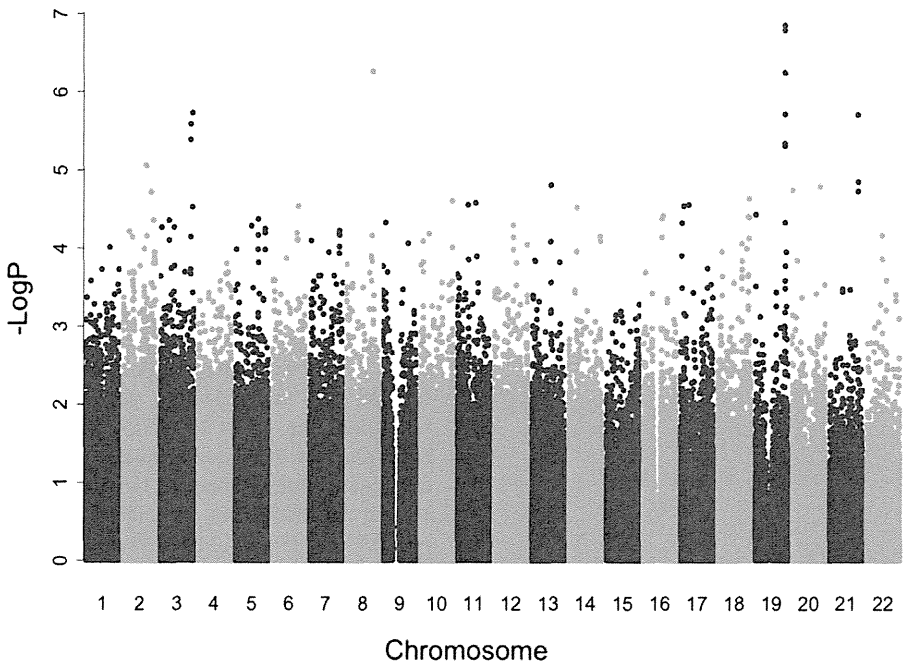

FIG 1. Manhattan plot of −log10 (*P* value) for the analysis in CAMP, demonstrating that multiple SNPs in chromosome 19 may be associated with BDR and modulated by ICS treatment.

TABLE II. Results of gene by environment analysis, summarizing the results of testing and replication in CAMP and LOCCS by using the additive model sorted by combined population-based *P* value

CHR	SNP	β estimate for SNP	SE	β estimate for ICS exposure	SE2	MAF	HWE *P* value	A1	A2	CAMP P_GXE	LOCCS P_GXE	Combined P_GXE
19	rs10411428	0.042	0.0099	−0.027	0.0087	0.41	.17	T	C	1.41×10^{-7}	0.00036	1.24×10^{-9}
19	rs11666341	0.042	0.0098	−0.027	0.0087	0.45	.17	A	G	1.62×10^{-7}	0.00036	1.42×10^{-9}
19	rs12460587*	0.035	0.0097	−0.029	0.0085	0.33	.22	G	T	5.69×10^{-7}	0.00018	2.43×10^{-9}
19	rs3450	0.031	0.0099	−0.030	0.0082	0.18	.72	C	T	1.93×10^{-6}	0.00017	7.56×10^{-9}
19	rs3752120†	0.032	0.0107	−0.031	0.0090	0.15	.21	T	C	4.58×10^{-6}	0.00050	4.81×10^{-8}
19	rs2288884	0.033	0.0107	−0.031	0.0090	0.34	.40	T	C	4.91×10^{-6}	0.00050	5.14×10^{-8}
8	rs6469488	0.072	0.0170	−0.032	0.0127	0.07	.79	G	A	5.54×10^{-7}	0.77	6.70×10^{-6}
21	rs4919929	0.062	0.0153	−0.031	0.0122	0.07	.42	T	C	1.96×10^{-6}	0.79	2.22×10^{-5}
3	rs9868563	0.099	0.0190	−0.012	0.0138	0.06	.10	T	C	2.62×10^{-6}	0.60	2.25×10^{-5}
3	rs4686399	0.041	0.0094	−0.015	0.0068	0.29	.41	G	A	1.87×10^{-6}	0.87	2.33×10^{-5}
3	rs1889261	0.084	0.0180	−0.018	0.0134	0.06	.76	A	G	4.14×10^{-6}	0.45	2.66×10^{-5}
2	rs4233808	0.041	0.0100	−0.019	0.0089	0.16	.69	G	A	8.84×10^{-6}	0.72	8.20×10^{-5}

CHR, Chromosome; *HWE*, Hardy-Weiberg equilibrium; *MAF*, minor allele frequency.
*Located in *ZNF841*.
†Located in *ZNF432*.

preprocessed expression level of a gene probe from dexamethasone-treated cell lines. For each gene probe, we considered all SNPs within 50 KB of both ends of the gene in the cis-eQTL analysis.

RESULTS

Descriptive statistics

Baseline demographic characteristics measured in our study population are provided in Table I. Our initial study population included a total of 808 white subjects, including 581 white subjects from CAMP. The replication population included 227 white subjects from LOCCS who had available genotype information. The mean (SD) age was 8.87 ± 2.14 years in CAMP and 40.8 ± 14.1 years in LOCCS. Sixty percent of the CAMP population were male subjects, whereas 37% of the LOCCS population were male subjects. The mean (SD) BDR while on ICS was 8.7% ± 8.1% in CAMP and 6.5% ± 6.8% in LOCCS.

Gene by environment analysis

The corresponding quantile-quantile plot (see Fig E1 in the Online Repository at www.jacionline.org) demonstrates that the SNPs with the lowest *P* values deviate from what is expected for a null distribution, which suggests that some may reflect true associations with BDRs that are modulated by exposure to ICS. The genomic inflation factor was 1.03, which demonstrates minimal population stratification. The Manhattan plot (Fig 1) for this analysis shows that the regions of SNPs that are most significantly associated with BDR while accounting for ICS

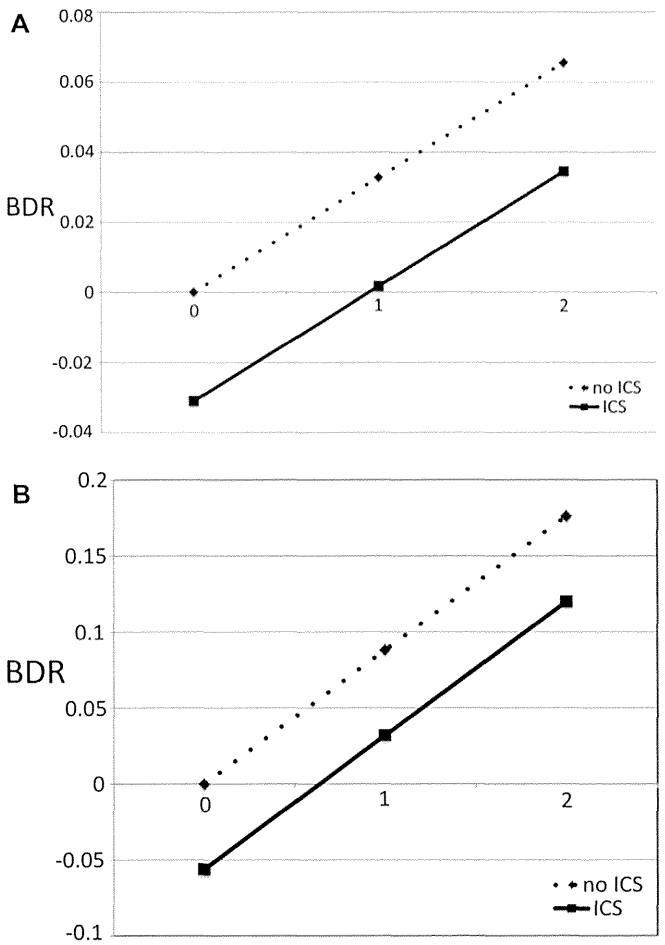

FIG 2. Depiction of the effect of treatment with ICS vs no ICS and genotype on the outcome of BDR for rs3752120. The *x-axis* shows the number of genotypes. There are 3 possibilities for the number of copies of rs3752120: 0 copy, 1 copy, or 2 copies. The *y-axis* shows the BDR. This figure demonstrates that having 2 copies of the mutant allele and not being treated with ICS produces a higher BDR than having 2 mutant alleles and being treated with ICS. **A**, CAMP population. **B**, LOCCS population.

treatment are in chromosomes 19 and 8. The top SNPs (gene by environment interaction $P < 1 \times 10^{-5}$) in CAMP and LOCCS are shown in Table II. We attempted to replicate these 12 SNPs in the LOCCS population. The strongest associations are on chromosome 19. One combined P value for the CAMP and LOCCS populations reached genome-wide significance: 4.81×10^{-8} for rs3752120, which was in linkage disequilibrium with rs3450 ($R^2 = 0.82$ in CAMP and 0.75 in LOCCS, combined $P = 7.56 \times 10^{-9}$) and rs12460587 ($R^2 = 0.84$ in CAMP and 0.84 in LOCCS, combined $P = 2.43 \times 10^{-9}$). These 3 SNPs are in or near the *ZNF432* gene.

The effect of treatment with ICS versus no ICS and genotype on the outcome of BDR for rs3752120 is depicted in Fig 2. The x-axis shows the number of genotypes. There are 3 possibilities for the number of copies of rs3752120: 0 copy, 1 copy, or 2 copies. The y-axis shows the BDR. This figure demonstrates that having 2 copies of the mutant allele and not being treated with ICS produces a higher BDR than having 2 mutant alleles and being treated with ICS. In addition, rs2288884 is located near a second gene on chromosome 19, *ZNF614* (combined $P = 5.14 \times 10^{-8}$) and rs11666341 is located near *ZNF841* (combined $P = 1.42 \times 10^{-9}$). A plot of the regional association results from our genome-wide association study on chromosome 19, in the region of rs10411428, is depicted in Fig 3. The plot demonstrates the magnitude of association of SNPs in this region in addition to the pairwise linkage disequilibrium patterns associated with rs10411428. Multiple SNPs in this region are in linkage disequilibrium and are associated with BDR while modulated by ICS.

We also conducted individual regression models for BDR as an outcome for the ICS group alone and for the placebo group alone, while adjusting for age and sex. Our results are depicted in Table III and show that the β estimates are in opposite directions for the ICS and placebo groups, which suggests that ICS modulates the effect of SNPs on BDR in a direction distinct from placebo. Analysis of microarray data from lymphoblastoid cell lines from a subset of CAMP subjects determined that the variant rs11666341 is associated with variable gene expression of *ZNF432* ($P = .046$). Results are presented in Table E1 (in the Online Repository available at www.jacionline.org). Cells from subjects who were homozygous for the major allele, rs11666341, had lower expression levels under dexamethasone-treated conditions (Fig E2 in the Online Repository available at www.jacionline.org).

DISCUSSION

Our study has several key findings. Treatment with ICS appears to modify the effect of SNPs on BDR. Our analysis was conducted with a pediatric population and was replicated in an adult population, which suggests that our results are generalizable across age groups. We have identified a region of association on chromosome 19 that contains multiple zinc finger protein genes. Variation in this region could influence the locus of CAMP. Finally, synergistic effects observed between ICS and β_2-agonists were caused by specific genes.

Results of previous studies have suggested that the anti-inflammatory effects of corticosteroids increase airway response to β_2-agonists by upregulating β_2 adrenergic receptor expression and by increasing cyclic AMP production by airway epithelial cells.[2,16,17] This leads to the synergistic effect that corticosteroids have on BDR to β_2-agonists.[2,16,17] A study by Jin et al[18] assessed for effect modification by use of ICS and examined whether SNPs in the dual-specificity phosphatase 1, *DUSP1* gene, which are associated with BDR, are modified by concurrent use of ICS medication. The researchers found that the *DUSP1* polymorphisms did modulate the effect between ICS use and BDR.[18] Thus, our study is consistent with previous studies in suggesting that genetic factors could mediate the relationship between ICS use and BDR. The clinical implications of a variant that predicts BDR while on ICS are unclear. Patients with a variant that leads to higher BDR while on ICS may benefit by preserving or restoring smooth-muscle function; however, patients with the variant may need to avoid ICS because a higher BDR while on ICS may signify that the ICS is not working. Further *in vitro* and *in vivo* studies are necessary to elucidate the clinical implications of our results.

We have identified a region on chromosome 19 that appears to influence the effect of ICS on asthma. We focused on the gene that coincides with the peak, the zinc finger protein gene, *ZNF432*, which is located on chromosome 19. The

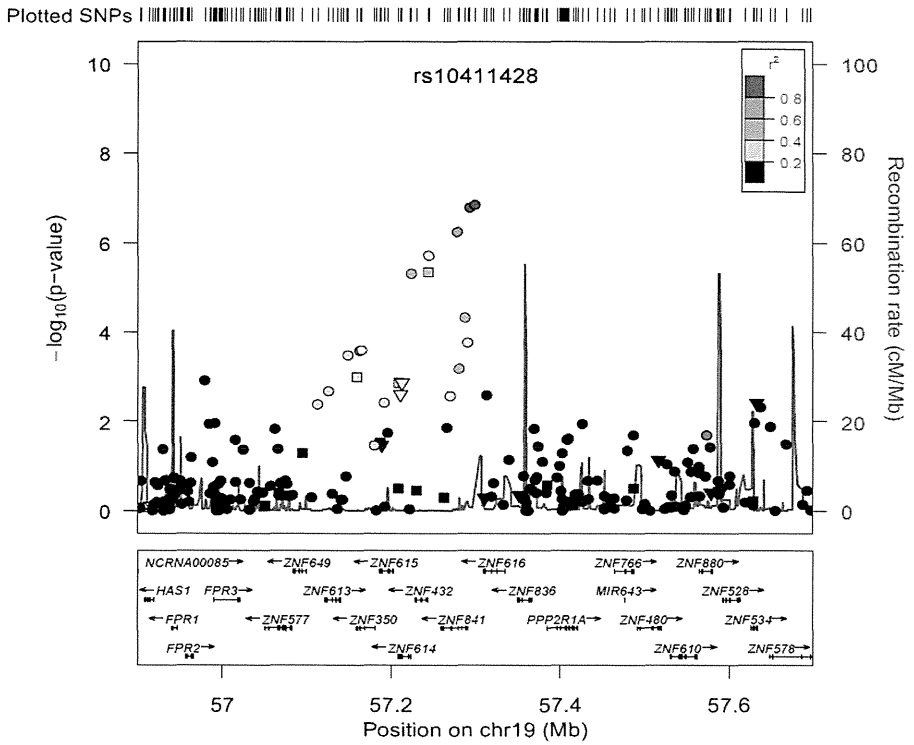

FIG 3. The region of association near rs10411428 to BDR while modulated by treatment with ICS. The *x-axis* denotes the position along the chromosome 19. The *y-axis* denotes −log10 *(P)*, which corresponds to 1000GP imputed data *P* values between the SNP, rs10411428, to each SNP in the plot is denoted in colors and was computed according to 1000GP June 2010 CEU data. The plot was created by using LocusZoom (see Pruim et al[22]).

TABLE III. Individual regression models for bronchodilator as an outcome stratified by ICS or placebo groups in CAMP

	ICS group (n = 172)		Placebo group (n = 409)	
SNP	**β estimate**	***P* value**	**β estimate**	***P* value**
rs4233808	0.041	8.25×10^{-5}	−0.020	.026
rs9868563	0.099	7.13×10^{-7}	−0.012	.37
rs1889261	0.084	5.98×10^{-6}	−0.018	.19
rs4686399	0.04	3.28×10^{-5}	−0.014	.040
rs6469488	0.072	2.71×10^{-10}	−0.032	.012
rs2288884	0.032	.0032	−0.030	.00096
rs3752120	0.033	.0030	−0.030	.00096
rs3450	0.031	.0019	−0.029	.00047
rs12460587	0.035	.00041	−0.028	.0012
rs11666341	0.042	3.86×10^{-5}	−0.025	.0042
rs10411428	0.042	4.12×10^{-5}	−0.026	.0034
rs4919929	0.061	.00011	−0.032	.0094

results from the eQTL analysis suggested that the variant rs11666341 is associated with variable gene expression of *ZNF432*, which further supports the finding that *ZNF432* modulates the effect of ICS in adults and children with asthma. Cells from the subjects who were homozygous for the major allele had lower expression levels under dexamethasone-treated conditions, which supports our hypothesis that this variant modulates BDR. Although the function of *ZNF432* is unknown, other zinc fingers have been found to play a role in asthma. For example, Zfp35 *(ZNF271)* appears to influence the pathogenesis of airway inflammation and hyperresponsiveness in asthma by controlling Th2 cell generation and Th2 cytokine expression.[19] Many zinc finger proteins have been demonstrated to be involved in transcription.[20] *ZNF432* has been found to be associated with inflammatory bowel disease, at $P = 8.3 \times 10^{-5}$.[21] Thus, further study of these zinc finger proteins may provide insight on the mechanisms by which ICS influence BDR.

Despite the strengths of our study, a few limitations deserve mention. First, our study was relatively small, with only 808 subjects in total. However, we did not have access to data for larger populations because few pharmacogenetic trials have genetic data on subjects who are taking both ICS and have short-acting BDR measurements. We relied on patient reports of ICS use in our replication population. It is possible that subjects who are randomized to an ICS group in a clinical trial such as CAMP may be more likely to be taking ICS than subjects who report taking ICS that are prescribed by their physicians during the run-in period of a trial, such as LOCCS. In neither trial is ICS use directly monitored. Furthermore, we were unable to conduct functional studies to study whether corticosteroids modulate the zinc finger proteins that we identified; nevertheless the eQTL analyses do support our findings.

In conclusion, ICS appear to influence the effect of genetic information on BDR. A zinc finger protein, *ZNF432*, appears to modulate the effect on ICS on BDR in adults and children with asthma.

We thank all the subjects for their ongoing participation in this study. We thank the CAMP investigators and research team, supported by the National Heart, Lung, and Blood Institute, National Institutes of Health, for collection of CAMP Genetic Ancillary Study data, and the American Lung Association Asthma Clinical Research Centers (ALA-ACRC) investigators and research team for data from the LOCCS trial.

Clinical implications: Clinicians who treat patients with asthma and with ICSs should be aware that the patient's genetic makeup likely influences the response as measured in lung function.

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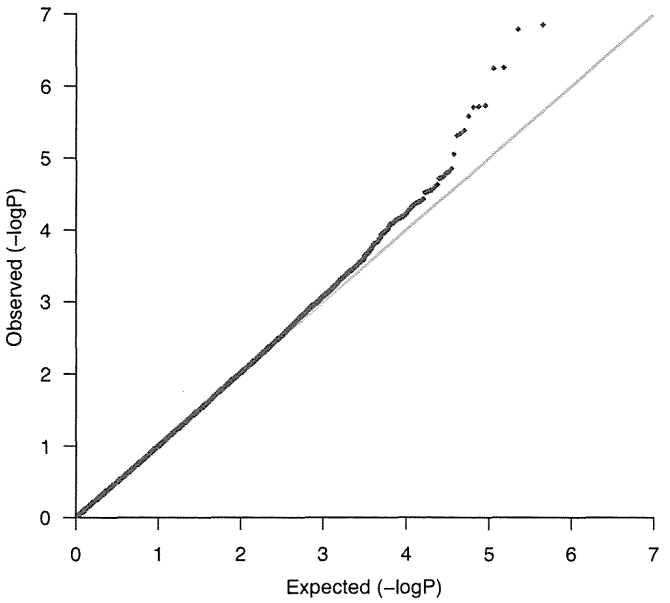

FIG E1. Quantile-quantile plot in CAMP, demonstrating that the SNPs with the lowest *P* values deviate from what is expected for a null distribution, which suggests that some may reflect true associations with BDR that are modulated by exposure to ICS.

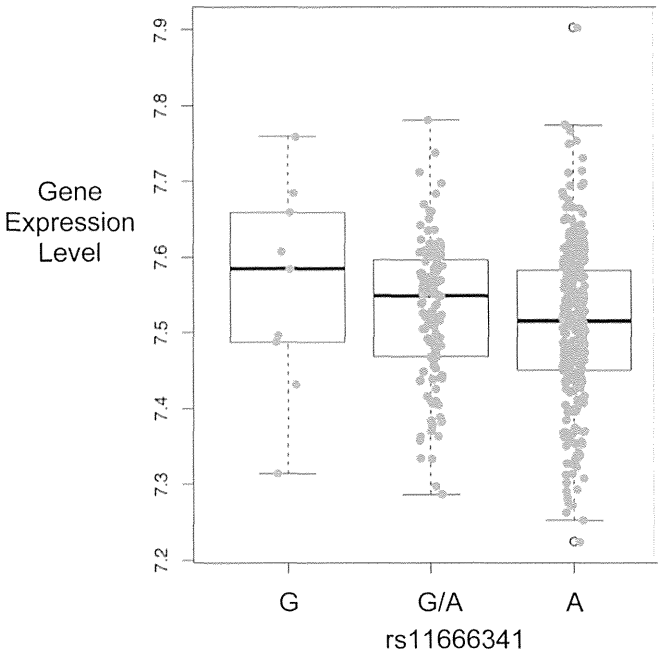

FIG E2. Box plot of genotype vs expression levels for rs11666341. Cells from subjects homozygous for the major allele rs11666341 had lower expression levels under dexamethasone-treated conditions, $P = .046$. *A*, Major allele; *G*, minor allele.

TABLE E1. Results of eQTL analysis

	SNP	χ^2	*P* value	Gene
19	rs11666341	4	.046	*ZNF432*
19	rs2288884	3.15	.076	*ZNF432*
19	rs3752120	3.15	.076	*ZNF432*
19	rs3450	2.74	.098	*ZNF432*
19	rs12460587	2.72	.099	*ZNF432*
19	rs12460587	2.6	.107	*ZNF841*
19	rs11666341	2.41	.121	*ZNF841*
19	rs2288884	1.93	.165	*ZNF841*
19	rs3752120	1.93	.165	*ZNF841*
19	rs2288884	1.9	.168	*ZNF350*
19	rs3450	0.86	.354	*ZNF614*
19	rs3450	0.78	.377	*ZNF841*
19	rs2288884	0.71	.399	*ZNF614*
19	rs3752120	0.71	.399	*ZNF614*
19	rs3450	0.31	.578	*ZNF615*
19	rs2288884	0.15	.699	*ZNF615*
19	rs3752120	0.15	.699	*ZNF615*
19	rs11666341	0.13	.718	*ZNF616*

Allergology International. 2014;63:27-35
DOI: 10.2332/allergolint.13-OA-0579

ORIGINAL ARTICLE

Lung Functions of Japanese Patients with Chronic Rhinosinusitis Who Underwent Endoscopic Sinus Surgery

Shota Tanaka[1,2], Tomomitsu Hirota[1], Atsushi Kamijo[3], Hiroki Ishii[2], Kyosuke Hatsushika[2], Shigeharu Fujieda[4], Junichi Ishitoya[5], Keisuke Masuyama[2] and Mayumi Tamari[1]

ABSTRACT

Background: Chronic rhinosinusitis (CRS), which is clinically classified into CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP), shows considerable geographic differences and heterogeneity. Eosinophilic (E) CRS with nasal polyps (ECRSwNP) has a higher degree of disease severity and higher frequency of comorbid asthma. Epidemiologic studies in different ethnic populations have improved understanding of the pathophysiology of the disease. Here we report the clinical characteristics of Japanese patients with medically refractory CRS undergoing endoscopic sinus surgery (ESS).

Methods: We recruited a total of 210 CRS patients and assessed them by nasal endoscopy, the Lund-Mackay score using computed tomography (CT), peripheral eosinophilia and smoking status. We also examined the comorbidity of asthma, effects of age and lung functions in the patients.

Results: In this study, 13% of CRSwNP patients and 20% of CRSwNP patients with peripheral blood eosinophilia exhibited obstructive lung dysfunction (FEV_1/FVC <70%) despite the absence of an asthma diagnosis. Among elderly nonsmoker patients (≥ 60 years) who had never been diagnosed with asthma, 50% of CRSwNP patients with peripheral blood eosinophilia showed decreased FEV_1/FVC <70%.

Conclusions: Our findings suggest that asthma is under-diagnosed in CRS patients who undergo ESS, especially the elderly. Although the association between CRS and asthma has been recognized, increased attention to the comorbidity of obstructive airway diseases such as asthma is still needed for management of medically refractory CRS.

KEY WORDS

asthma, chronic rhinosinusitis, eosinophils, lung functions, nasal polyps

INTRODUCTION

Chronic rhinosinusitis (CRS), a common disease associated with persistent inflammation of the nasal and paranasal sinuses, is a public health problem resulting in a socioeconomic burden throughout the world.[1,2] CRS is commonly classified into two groups, CRSsNP and CRSwNP. Considerable heterogeneity within CRSwNP has been recognized and there are geographic differences in the condition.[3,4] Tissue from Caucasian patients with CRSwNP is characterized by eosinophilic inflammation, whereas samples from Asian patients are biased toward neutrophilic inflammation.[3,4] Eosinophilic (E) CRSwNP has been found in 65-90% of subjects with CRSwNP in Caucasians and in 50% of them in East Asian populations.[1-3] ECRSwNP represents a higher degree of disease severity, with an impaired sense of smell, higher recurrence rate after surgery and higher frequency of comorbid asthma.[2] The association of ECRSwNP and asthma is well recognized in western countries; [5,6] however, asthma comorbidity in CRSwNP patients in

[1]Laboratory for Respiratory and Allergic Diseases, Center for Integrative Medical Sciences, The Institute of Physical and Chemical Research (RIKEN), [5]Department of Otorhinolaryngology, Yokohama City University Medical Center, Kanagawa, [2]Department of Otolaryngology-Head and Neck Surgery, University of Yamanashi, Faculty of Medicine, Yamanashi, [3]Department of Otorhinolaryngology, Allergy Center, Saitama Medical University, Saitama and [4]Department of Otorhinolaryngology-Head and Neck Surgery, University of Fukui, Faculty of Medicine, Fukui, Japan.

Conflict of interest: No potential conflict of interest was disclosed.
Correspondence: Mayumi Tamari, MD, PhD, Laboratory for Respiratory and Allergic Diseases, Center for Integrative Medical Sciences, The Institute of Physical and Chemical Research (RIKEN), 1-7-22 Suehiro, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan.
Email: tamari@src.riken.jp
Received 17 May 2013. Accepted for publication 25 July 2013.

Table 1 Clinical characteristics of patients with chronic rhinosinusitis

	CRSsNP $n = 40$	CRSwNP $n = 170$	*P*-value
Age (median, range)	57 (35-64)	54.5 (44-63)	NS
Sex (male/female) (female %)	31/9 (23)	109/61 (36)	NS
Smoker, no. (%)	21 (53)	56 (33)	<0.05
Current smoker, no. (%)	21 (53)	46 (27)	<0.01
Brinkman index of smokers	430 (290-728)	560 (300-854)	NS
Asthma, no. (%)	2 (5)	44 (26)	<0.01
Aspirin-induced asthma, no. (%)	0 (0)	10 (6)	NS
Allergic rhinitis, no. (%)	8 (20)	48 (28)	NS
Rhinorrhea, no. (%)	34 (85)	124 (73)	NS
Nasal congestion, no. (%)	23 (58)	138 (81)	<0.01
Headache, no. (%)	17 (43)	38 (22)	<0.05
Olfactory dysfunction, no. (%)	11 (28)	112 (66)	<0.0001

P values were determined using the Mann-Whitney U test, or Fisher's exact test as appropriate. CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; NS, not significant.

Asian countries has not been fully studied. Nor are the clinical features of CRS such as sinus scores evaluated by CT scanning and lung functions in Asian populations well investigated.

Epidemiologic studies have shown that asthma and rhinitis often coexist in the same patients and suggest the 'united airways' concept.[4,7-11] Underdiagnosis and undertreatment of asthma is a significant public problem all over the world, especially in the elderly.[12] The awareness of asthma is frequent in those with comorbid rhinitis,[13] and it has also been suggested that symptoms may predominate in one organ and be unrecognized in other organs even though they exist.[8] A higher CT score and more nasal polyp formation are observed in elderly patients with CRS.[14] However, the influence of aging on the clinical features of CRS in Japanese patients has not been examined.

ESS is the treatment choice for medically refractory CRS with or without nasal polyposis.[15] To clarify the clinical features of refractory CRS in Japanese patients, we conducted a cross-sectional study with a total of 210 CRS patients who underwent ESS. We assessed their clinical phenotypes through the use of nasal endoscopy, considering peripheral eosinophilia, CT scores based on the Lund-Mackay system, smoking status, comorbidity of asthma, effects of age and lung functions.

METHODS

SUBJECTS

CRS was defined as a condition with at least two of the following symptoms: anterior and/or posterior rhinorrhea, nasal obstruction, decreased sense of smell, and nasal pressure existing for 12 weeks despite medical management.[1,2] We recruited all patients with CRS who underwent ESS at the University of Yamanashi Hospital from January 2002 to October 2011. All individuals were Japanese, and we excluded patients with autoimmune disease, cancer, papilloma, fungal infection, postoperative maxillary cysts, choanal polyps, and nasal foreign bodies before enrollment. We excluded patients with allergic fungal rhinosinusitis based on CT and/or histopathological findings from the start. We included patients with unilateral CRS and those with nasal polyps. The presence or absence of nasal polyps was confirmed by endoscopy. Finally, a total of 210 subjects were analyzed (Table 1). CRS patients were classified into CRSwNP and CRSsNP groups based on the criteria of the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force.[1] Comorbidity of asthma was determined from the patients' medical histories based on doctors' interviews. We defined individuals who had asthma diagnosed by a doctor at any point in their lifetime as having bronchial asthma. This study was approved by the ethics committee of the University of Yamanashi Hospital. We informed patients that any clinical data would be used for research analyses by placing a notice on walls in the medical examination room and hospital lobby in the University of Yamanashi Hospital. We individually responded to patients who dissented from such use. In this study, we recruited subjects who did not dissent from use of the clinical data. This process of obtaining informed consent was approved by the ethics committee of the University of Yamanashi Hospital.

PERIPHERAL EOSINOPHIL COUNT

A total of 19 patients (9%) were treated with systemic corticosteroids in the two months before the operation, and 91 patients (43%) were treated with intranasal steroids in the month before the operation. Since the eosinophil count is sensitive to steroid treatment, we recorded the highest peripheral blood eosinophil percentage among all the blood tests done before operation.

LUNG FUNCTION AND COMPUTED TOMOGRAPHY

Spirometry was performed for all patients using a DISCOM 21 FXII spirometer (CHEST MI., Inc., Tokyo, Japan). Forced expired volume in one second (FEV_1)/forced vital capacity (FVC) (FEV_1%) was calculated. Maximum expiratory flow rates at 50% and 25% of the FVC (V50 and V25) were obtained from flow/volume curves. FEF25-75 and lung functions after application of a bronchodilator were not available for this study. CT scans of the sinuses were available for 54 patients with CRSwNP and were graded based on the Lund-Mackay staging system.[16] We used the average of the right and left side scores in the following analyses.

HISTOLOGICAL ANALYSIS

It is well known that infiltration of eosinophils in nasal polyps is a characteristic of ECRSwNP. However, it remains unclear whether eosinophilic infiltration exists in nasal mucosal tissue of CRS without nasal polyp involvement. To assess eosinophil infiltration in sinonasal mucosal tissues without polyps, we examined the maxillary mucosa histologically. Among the 210 CRS patients enrolled in this study, non-polyp mucosal tissues from the maxillary sinuses were obtained from 46 CRSwNP patients. Paraffin-embedded sections were stained with hematoxylin and eosin, and the number of infiltrating eosinophils were counted in four randomly selected high power (×400) magnification fields.

STATISTICAL ANALYSIS

Demographic data, lung functions, and CT scores were compared using the Mann-Whitney U test or Fisher's exact test. The correlational validity of the peripheral blood eosinophil percentage with the mucosal eosinophil count was assessed using Spearman rank correlations. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using R (R Development Core Team, http://www.r-project.org/).

RESULTS

SUBJECTS' CHARACTERISTICS

Of the 210 CRS patients who underwent ESS, 40 (19%) had CRSsNP and 170 (81%) had CRSwNP. Clinical features of each group are shown in Table 1. Current smokers were more frequent in the CRSsNP group (53%) than in the CRSwNP group (27%). The comorbidity of asthma was significantly higher for CRSwNP (26%) than for CRSsNP (5%). Among subjective symptoms, olfactory dysfunction was more frequent in CRSwNP (66%) than in CRSsNP (28%).

Recent studies have shown that the blood eosinophil percentage is the most accurate clinical factor to distinguish the eosinophilic type of CRSwNP from the non-eosinophilic type;[17,18] however, precise clinical diagnostic criteria including the peripheral eosinophil percentage for ECRSwNP have not been determined. In this study, we divided the patients with CRSwNP into two groups to clarify the clinical characteristics of each group. Since the median value of the eosinophil percentage of the total leukocyte count in subjects with CRSwNP was 5%, we defined subjects with an eosinophil percentage <5% as having CRSwNP without peripheral blood eosinophilia and subjects with ≥5% as having CRSwNP with peripheral blood eosinophilia. A total of 87 subjects (51%) were classified into the CRSwNP group without peripheral blood eosinophilia, and 83 subjects (49%) were classified into the group having CRSwNP with peripheral blood eosinophilia (Table 2). The comorbidity of asthma was significantly higher for CRSwNP with peripheral blood eosinophilia (43%) than for CRSwNP without peripheral blood eosinophilia (9%), and the comorbidity of aspirin-induced asthma (AIA) or allergic rhinitis was significantly higher for CRSwNP with peripheral blood eosinophilia than for the CRSwNP without peripheral blood eosinophilia (Table 2). Olfactory dysfunction was also frequently observed in the group having CRSwNP with peripheral blood eosinophilia (Table 2).

CT SCORES IN PATIENTS WITH CRSwNP

The Lund-Mackay score is commonly used for assessment of the stage and severity of CRS.[16] We examined the severity of the disease in subjects with CRSwNP using CT scores based on the Lund-Mackay system. The total score for CRSwNP with peripheral blood eosinophilia had a tendency to be higher than that for CRSwNP without peripheral blood eosinophilia (Fig. 1) but the difference was not statistically significant ($P = 0.051$). There was no significant difference between CRSwNP without peripheral blood eosinophilia and CRSwNP with peripheral blood eosinophilia for the maxillary sinus score, sphenoid sinus score, frontal sinus score, and ostiomeatal complex score (data not shown). However, the anterior ethmoid (AE) sinus score and posterior ethmoid (PE) sinus score of CRSwNP with peripheral blood eosinophilia were significantly higher those for CRSwNP without peripheral blood eosinophilia ($P < 0.05$ and < 0.001, respectively) (Fig. 1).

CORRELATION BETWEEN EOSINOPHIL INFILTRATION IN SINONASAL MUCOSA AND PERIPHERAL BLOOD EOSINOPHIL COUNT IN PATIENTS WITH CRSwNP

Infiltration of eosinophils in nasal polyps is a characteristic of ECRSwNP. Since whether eosinophilic infiltration exists in nasal mucosal tissue of CRS without nasal polyp involvement remains unknown, we investigated the correlation between blood and mucosal eosinophilia in a total of 46 patients with CRSwNP. The count of mucosal eosinophils strongly correlated

Table 2 Clinical characteristics of patients with CRSwNP

	CRSwNP (n = 170)		P-value
	without peripheral blood eosinophilia n = 87	with peripheral blood eosinophilia n = 83	
Age (median, range)	58 (45-66)	52 (44-61)	NS
Sex (male/female) (female %)	54/33 (38)	55/28 (34)	NS
Smoker, no. (%)	31 (36)	25 (31)	NS
Current smoker, no. (%)	25 (29)	21 (26)	NS
Brinkman Index of smokers (median, range)	730 (330-900)	460 (293-620)	NS
Asthma, no. (%)	8 (9)	36 (43)	<0.000001
Aspirin-intolerant asthma, no. (%)	1 (1)	9 (11)	<0.01
Allergic rhinitis, no. (%)	16 (18)	32 (39)	<0.01
Rhinorrhea, no. (%)	64 (74)	60 (72)	NS
Nasal congestion, no. (%)	70 (81)	68 (82)	NS
Headache, no. (%)	23 (26)	15 (18)	NS
Olfactory dysfunction, no. (%)	49 (56)	63 (76)	<0.01

P values were determined using the Mann-Whitney U test or Fisher's exact test as appropriate. NS, not significant.

with the peripheral blood eosinophil percentage (R = 0.67, $P < 0.000001$) (Fig. 2).

LUNG FUNCTIONS OF THE CRS PATIENTS

The close relationship between asthma and CRS is widely recognized. We therefore assessed lung functions of the CRS patients. We first excluded nine patients whose smoking histories were unknown from the analysis. FEV_1/FVC was significantly lower in subjects with CRSwNP than in subjects with CRSsNP, and was also lower in the group having CRSwNP with peripheral blood eosinophilia than in the group having CRSwNP without peripheral blood eosinophilia (Fig. 3a, Table 3). There were 18 patients being treated with corticosteroids among the subjects whose lung functions were assessed. One patient with CRSsNP (2.6%), two patients with CRSwNP without peripheral blood eosinophilia (2.4%) and 15 patients who had CRSwNP with peripheral blood eosinophilia (19%) had received systemic corticosteroid treatment prior to surgery. Although there were more patients receiving corticosteroids in the group having CRSwNP with peripheral blood eosinophilia than in the groups having CRSsNP and CRSwNP without peripheral blood eosinophilia, FEV_1/FVC was significantly lower in the subjects who had CRSwNP with peripheral blood eosinophilia than in the subjects of the other two CRS groups. Furthermore, %V25 was lower in the CRSwNP group than in the CRSsNP group, and both %V25 and %V50 were lower for CRSwNP with peripheral blood eosinophilia than for CRSwNP without peripheral blood eosinophilia (Table 3).

Decreased FEV_1/FVC is a clinical feature of obstructive lung diseases such as bronchial asthma and chronic obstructive pulmonary disease (COPD). Since smoking is the leading cause of COPD, we next examined the influence of smoking status on lung functions of subjects with CRSsNP and CRSwNP. There was no significant difference in lung functions (FEV_1/FVC, %V25, and %V50) between current smokers, former smokers (smokers) and never smokers (nonsmokers) among the entire group with CRS (Fig. 3b, Table 3). After excluding current and former smokers, FEV_1/FVC was lower in the CRSwNP group than in the CRSsNP group, and was lower in the CRSwNP group with peripheral blood eosinophilia than in the CRSwNP group without peripheral blood eosinophilia (Fig. 3c). Both %V25 and %V50 were lower in patients who had CRSwNP with peripheral blood eosinophilia than in those who had CRSwNP without peripheral blood eosinophilia (Table 3).

We next stratified the CRS patients who had never been diagnosed with asthma prior to ESS by the comorbidity of asthma, and focused on their lung functions. Since obstruction has been traditionally defined by an FEV_1/FVC ratio of less than a certain percentage, usually 70 to 75%,[19] we divided subjects into three classes according to their lung function: FEV_1/FVC <70%, 70%≤ FEV_1/FVC <75% and FEV_1/FVC ≥75% (Fig. 4). In 13% of patients with CRSwNP who had never been diagnosed as having asthma, FEV_1/FVC was less than 70% (Fig. 4a). Furthermore, 20% of patients having CRSwNP with peripheral blood eosinophilia had FEV_1/FVC of less than 70% despite the absence of a previous asthma diagnosis (Fig. 4a). The same tendency was observed even after excluding current and former smokers, and smoking status did not influence the obstructive lung dysfunction in subjects who had CRSwNP and CRSwNP with peripheral blood eosinophilia (Fig. 4b).

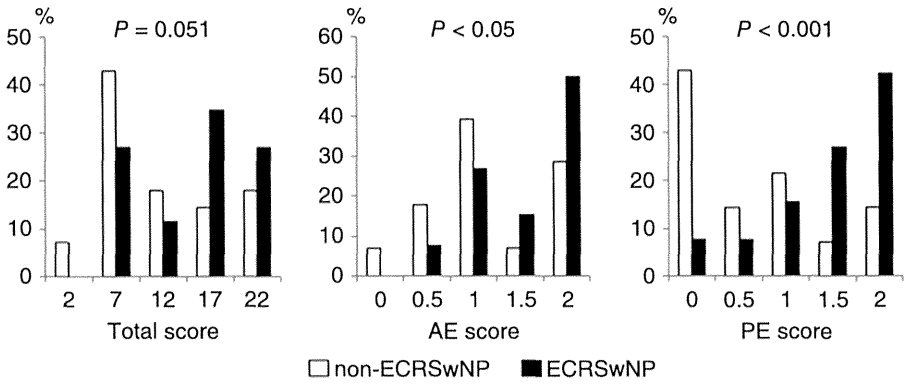

Fig. 1 Comparison of CT scores for CRSwNP with and without peripheral blood eosinophilia. CT scores based on the Lund-Mackay staging system were compared between subjects who had CRSwNP without peripheral blood eosinophilia (n = 28) and CRSwNP with peripheral blood eosinophilia P (n = 26). P values were determined using the Mann-Whitney U test. AE score, anterior ethmoid sinus score; PE score, posterior ethmoid sinus score.

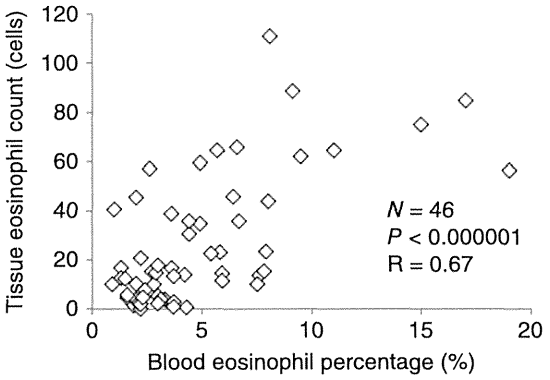

Fig. 2 Correlation between percentage of eosinophils in peripheral blood and the tissue eosinophil count in the mucosa of the maxillary sinus. The tissue eosinophils of patients with CRSwNP (n = 46) were counted at high power maginification (×400). Correlation validity was assessed by using Spearman rank correlations.

Underdiagnosis of asthma is an important problem, especially in the elderly.[12] Therefore we further examined whether there were age-related differences in the prevalence of each subgroup or in FEV_1/FVC. We divided nonsmoker patients who had never been diagnosed with asthma into nonelderly (<60 years) and elderly groups (≥60 years). In the elderly group, obstructive lung dysfunction (FEV_1/FVC <70%) was more frequently seen in those with CRSwNP (28%) than in those with CRSsNP (0%), and for CRSwNP with peripheral blood eosinophilia (50%) than for CRSwNP without peripheral blood eosinophilia (18%) (Fig. 4c).

DISCUSSION

There is considerable heterogeneity within the CRSwNP subgroup,[3,4] and importance of understanding CRS within the context of racial and ethnic populations has been suggested.[20] This study revealed the clinical characteristics of Japanese patients with medically refractory CRS who underwent ESS. Epidemiologic studies have reported that rhinitis and asthma often coexist in the same patients.[1,5,6,8] The 'united airways' concept implies that there is a link between upper and lower airway inflammation.[8,9,21] A recent study reported the asthma comorbidity in patients with CRSwNP who underwent ESS (n = 19) to be 32% in Japanese patients.[22] In the United States, the frequencies of comorbidity of asthma were reported to be 11% in those with CRSsNP and 44% in those with CRSwNP.[23] In this study, the frequency was 26% for CRSwNP, and it was significantly higher for CRSwNP with peripheral blood eosinophilia (43%) than for CRSwNP without peripheral blood eosinophilia (9%). The frequency of comorbidity of asthma observed in the group having CRSwNP with peripheral blood eosinophilia (43%) in our study was similar to that in the CRSwNP group (44%) in the United States. Furthermore, it has been reported that ECRSwNP has a higher degree of disease severity in western countries.[1] AIA represents a severe phenotype of asthma and we found significantly higher comorbidity of AIA for CRSwNP with peripheral blood eosinophilia than for the CRSwNP without peripheral blood eosinophilia in this study.

A recent report has shown that CRS patients with anosmia have a higher density of eosinophils infiltrating the olfactory epithelium, and exhibit more abnormalities on CT and endoscopic examination, including being more likely to exhibit nasal polyposis than other CRS patients.[24] We observed olfactory dysfunction with high frequency in subjects with CRSwNP (66%), especially in those who had CRSwNP with peripheral blood eosinophilia (76%). Our findings support previous research results. CT scanning is useful

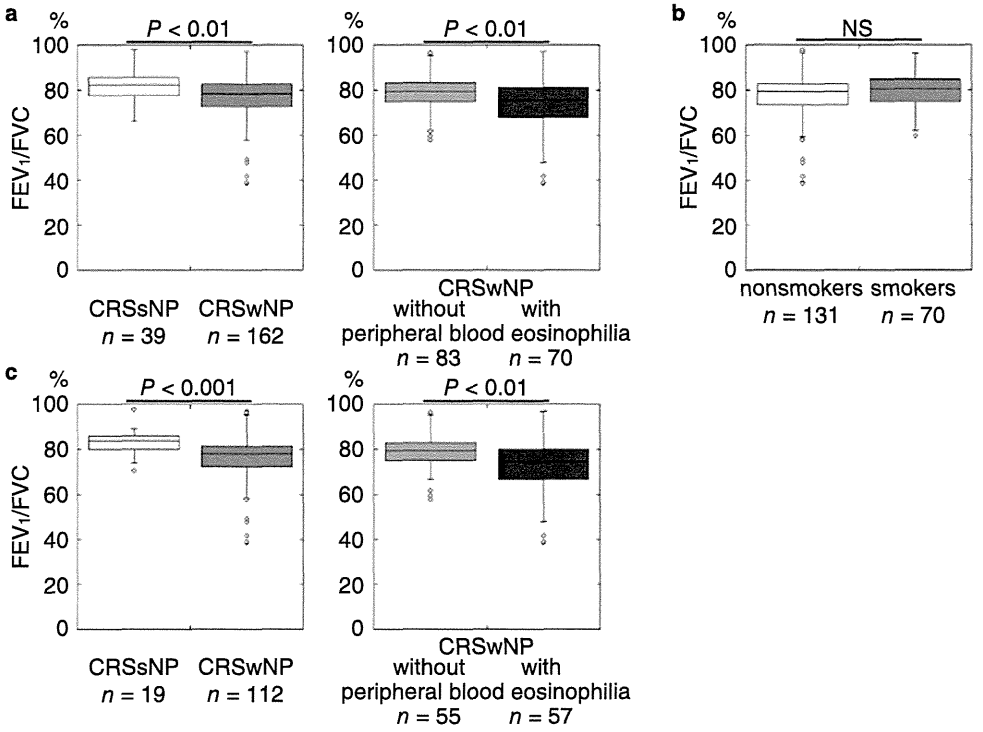

Fig. 3 Comparison of FEV_1/FVC ratios among subgroups of CRS. **a**) Comparison between CRSsNP and CRSwNP, and between CRSwNP with and without peripheral blood eosinophilia. **b**) Comparison between nonsmokers and (current and former) smokers among the entire group with CRS. **c**) Comparison between CRSsNP and CRSwNP, and between CRSwNP with and without peripheral blood eosinophilia after excluding current and former smokers. Rectangles include the range from the 25th to 75th percentiles, horizontal lines indicate the median and vertical lines indicate the 10th to 90th percentiles. *P* values were determined using the Mann-Whitney U test. FVC, forced vital capacity; FEV_1, forced expired volume in one second.

Table 3 Lung functions of subgroups of CRS

	CRS		*P*-value	Nonsmokers with CRS		*P*-value
	CRSsNP (*n* = 39)	CRSwNP (*n* = 162)		CRSsNP (*n* = 19)	CRSwNP (*n* = 112)	
%FVC	105.0 (93.5-111.8)	106.7 (95.2-118.3)	NS	102.0 (88.2-110.9)	107.8 (95.3-118.5)	<0.05
$\%FEV_1$	102.5 (95.7-109.7)	101.5 (89.6-110.8)	NS	106.5 (94.0-111.2)	103.3 (88.5-112.1)	NS
FEV_1/FVC	82.3 (77.4-85.6)	78.4 (72.5-82.6)	<0.01	83.9 (80.2-86.3)	78.1 (72.4-81.4)	<0.001
%V50	68.5 (61.4-85.7)	67.3 (45.7-83.9)	NS	68.4 (60.7-76.1)	64 (43.8-81.9)	NS
%V25	58.8 (44.2-66.2)	42.7 (29.3-55.3)	<0.001	60.8 (52.7-66.2)	42.7 (27.7-54.5)	<0.001
	CRSwNP		*P*-value	Nonsmokers with CRSwNP		*P*-value
	without peripheral blood eosinophilia P (*n* = 83)	with peripheral blood eosinophilia (*n* = 79)		without peripheral blood eosinophilia (*n* = 55)	with peripheral blood eosinophilia (*n* = 57)	
%FVC	106.4 (94.7-113.2)	108.2 (97.5-121.0)	NS	106.7 (95.1-113.2)	110.3 (97.6-121.6)	NS
$\%FEV_1$	101.8 (91.9-112.9)	101.5 (80.1-108.5)	NS	105.1 (92.6-114.4)	98.2 (78.1-108.8)	NS
FEV_1/FVC	79.5 (74.7-83.3)	75.3 (67.8-81.2)	<0.01	79.4 (75.1-83.2)	74.4 (66.7-80.1)	<0.01
%V50	69.6 (55.2-85.7)	58.5 (37.7-81.7)	<0.01	68.3 (57.3-84.7)	53.5 (36.8-74.0)	<0.01
%V25	45.6 (36.8-59.5)	40.9 (25.8-51.4)	<0.01	45.1 (37.5-56.3)	38.7 (22.4-48.4)	<0.01

Lung functions are expressed as percentages of predicted values and are presented as medians (interquartile ranges). *P* values were determined using the Mann-Whitney U test. FVC, forced vital capacity; FEV_1, forced expired volume in one second; V50 and V25 of FVC, maximum expiratory flow rates at 50% and 25%; $FEV_1\%$, FEV_1/FVC %.

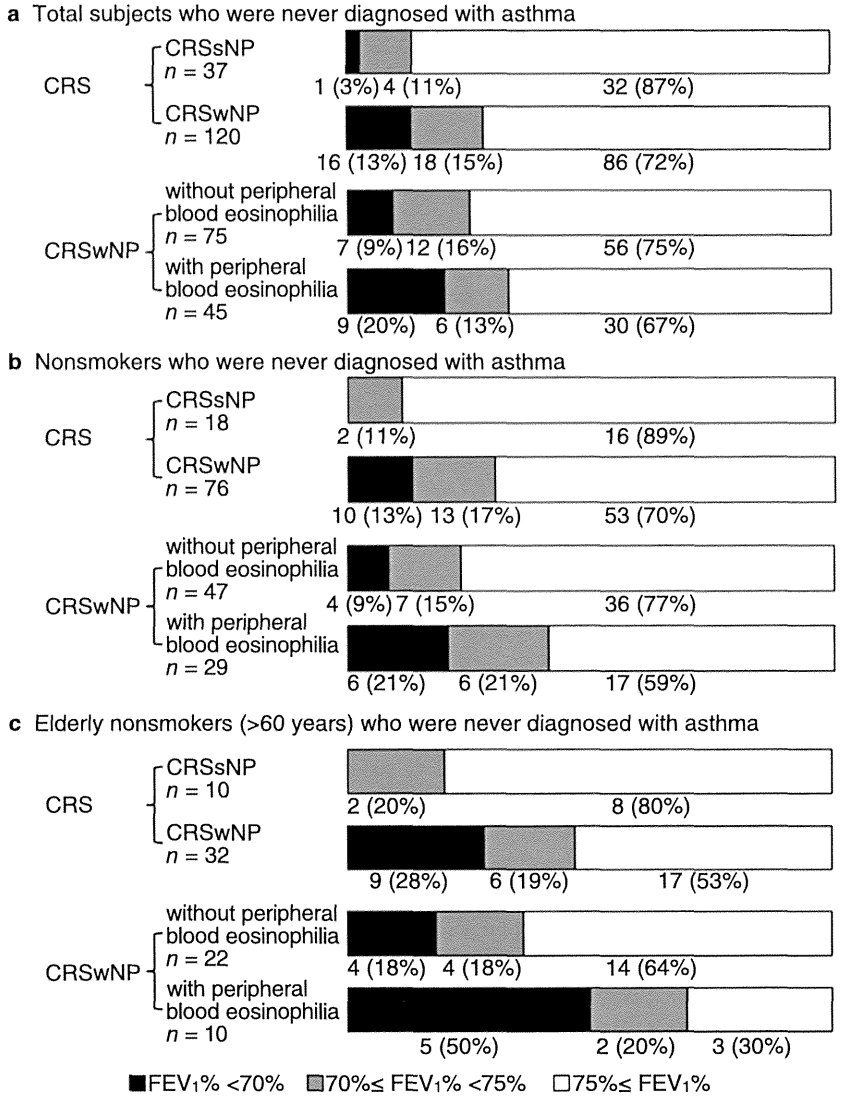

Fig. 4 Obstructive lung dysfunctions of CRS patients who had never been diagnosed with asthma prior to ESS. **a-c**) Comparison between CRSsNP and CRSwNP, and between CRSwNP with and without peripheral blood eosinophilia. **a**) In total subjects. **b**) In subjects excluding current and former smokers. **c**) In elderly nonsmoker subjects (>60 years) who had never been diagnosed with asthma.

for the visualization of disease and enables us to determine the extent of rhinosinusitis accurately. The Lund-Mackay score evaluated on CT scans is widely used in assessment of CRS.[16] A recent study has shown that an increased blood eosinophil percentage and the CT image scores for the PE sinus and the olfactory cleft are good predictors of ECRSwNP.[18] In this study, although the total score for CRSwNP with peripheral blood eosinophilia did not significantly differ from that for CRSwNP without peripheral blood eosinophilia, the AE sinus and PE sinus scores of CRSwNP with peripheral blood eosinophilia were significantly higher than those for CRSwNP without peripheral blood eosinophilia. Thus, our results support previous findings on involvement of the posterior ethmoid sinus in ECRSwNP.

Infiltration and activation of eosinophils in nasal polyps is a characteristic of eosinophilic CRSwNP, and it has been reported that the peripheral blood eosinophil percentage is positively correlated with the number of eosinophils in nasal polyps.[25] In this study, we found eosinophilic infiltration in nasal mucosal tissue not involving nasal polyps and a positive correlation between blood and mucosal eosinophilia in patients with CRSwNP. Thus, it is necessary to be aware of the possibility of eosinophilic inflammation not only in nasal polyps but also in nasal mucosa for the treatment of CRSwNP patients with high blood

eosinophil levels.

A recent report showed a high prevalence of asymptomatic lower airway dysfunctions in patients with CRSwNP in the United Kingdom.[26] In this study, 28% of CRSwNP patients exhibited FEV_1/FVC of less than 75%, and 20% of patients who had CRSwNP with peripheral blood eosinophilia had FEV_1/FVC of less than 70% despite the absence of an asthma diagnosis. Decreased FEV_1/FVC (<70%) was more frequently observed in patients having CRSwNP with peripheral blood eosinophilia (21%) than in those having CRSwNP without peripheral blood eosinophilia (9%), even after excluding current and former smokers. Never smokers comprise a substantial proportion of patients with COPD; however, asymptomatic decreased lung function suggestive of an asthmatic phenotype was frequently observed in CRSwNP, especially in CRSwNP with peripheral blood eosinophilia. Most patients with asthma have symptoms of rhinitis, but in many cases symptoms may predominate in one organ and be unrecognized in other organs even though they exist.[8] Our findings also suggest the necessity of paying increased attention to the possible comorbidity of obstructive airway diseases such as asthma for management of refractory CRS.

The underdiagnosis and undertreatment of asthma are serious problems throughout the world,[13,27-30] especially in the elderly.[29] About half of elderly people with asthma have not been diagnosed, and the underuse of objective testing such as spirometry has been considered to be one reason.[30] In this study, after excluding patients who had ever been diagnosed with asthma and current or former smokers, decreased FEV_1/FVC (<70%) tended to be more prevalent in elderly patients in the CRSwNP and CRSwNP groups with peripheral blood eosinophilia. Although FEV_1/FVC normally decreases with age and FEV_1/FVC lower than 70% might be a normal finding, an FEV_1/FVC ratio of less than 70% increases the probability of asthma in elderly patients with asthma symptoms.[30] Careful assessment of asthma by means of systemic inquiries about respiratory symptoms and objective testing by spirometry seems to be necessary in subjects with refractory CRS, especially elderly patients. Early diagnosis and good asthma control are important to reduce morbidity and healthcare costs as well as minimize the development of chronic illnesses,[13] and appropriate diagnosis and management of asthma would contribute to mitigating the severity of their CRS.

In conclusion, we found that 19% of subjects with CRS who underwent ESS had CRSsNP, and 81% CRSwNP. We confirmed that both the AE and PE sinus CT scores of the Lund-Mackay staging system were helpful for identifying CRSwNP with peripheral blood eosinophilia. Obstructive lung dysfunctions are frequently observed in CRSwNP with peripheral blood eosinophilia, especially in elderly persons, despite the absence of an asthma diagnosis. Although further studies are needed, our findings will contribute to better understanding of the pathophysiology of CRS.

ACKNOWLEDGEMENTS

We thank all the patients for participating in the study as well as the collaborating physicians for collecting samples. We would like to thank Chizuru Nagagata and Yuko Hiraoka for data collection. We thank K. Barrymore for proofreading this document.

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Letter to the Editor

An association study of 36 psoriasis susceptibility loci for psoriasis vulgaris and atopic dermatitis in a Japanese population

Keywords:
Genetic polymorphism; Genome-wide association study; Atopic dermatitis; Psoriasis vulgaris; Japanese population

To the Editor,

Psoriasis is a chronic inflammatory skin disease caused by interplay between genetic and environmental factors, and psoriasis vulgaris (PsV) is the most common form of the disease [1]. Recent genome-wide association studies (GWASs) and meta-analysis of GWAS of psoriasis in European individuals have identified a total of 36 susceptibility loci at a genome-wide level of significance ($P < 5 \times 10^{-8}$) [2]. Conditional analysis of the 36 loci was also conducted and further identified five additional SNPs, rs2111485, rs2910686, rs4379175, rs13437088 and rs12720356 with genome-wide significance. We conducted a validation study of these 41 SNPs among the 36 loci in Japanese patients with PsV. Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease, and recent linkage and association studies have identified overlapping susceptibility loci of psoriasis and AD [3,4]. Since identification of shared genetic components helps to highlight the key molecular pathways involved in chronic inflammatory skin diseases, we also explored the association of these 41 SNPs in Japanese patients with AD.

We recruited a total of 259 patients with PsV diagnosed by clinical and histopathological findings (median age 53, 11–85 years, male:female ratio = 2.4:1.0) and a total of 999 patients with AD diagnosed according to the criteria of Hanifin and Rajka (median age 29, 3–77 years, male:female ratio = 1.3:1.0). As controls, 938 individuals who had never been diagnosed with AD or PsV were recruited from Fukui University and Miyatake Clinic (median age 50, 20–75 years, male:female ratio = 2.0:1.0). All individuals were unrelated Japanese and gave written informed consent to participate in the study. The study was approved by the ethical committees of the hospitals involved, the University of Tokyo, the Jikei University School of Medicine, Fukui University, Miyatake Clinic and the Institute of Physical and Chemical Research (RIKEN). Genomic DNA was prepared in accordance with standard protocols. We selected a total of 41 SNPs achieving genome-wide significance in a previous meta-analysis of GWASs [2]. SNPs were genotyped by using the multiplex PCR-based Invader assay (Third Wave Japan). We calculated allele frequencies and tested agreement with Hardy–Weinberg equilibrium using a χ^2 goodness of fit test as described [5], and no SNP was excluded from the analysis. Among 41 SNPs, four (rs9988642, rs12188300,

Table 1
Summary of allele frequencies of 37 previously reported loci for psoriasis.

dbSNP	Gene	Control $n=938$	PsV $n=259$	AD $n=999$	*P* value	PsV	*P* value	AD
		MAF	MAF	MAF		OR (95% CI)		OR (95% CI)
rs11121129	*SLC45A1, TNFRSF9*	0.222	0.230	0.227	7.19E−01	1.04 (0.83–1.32)	7.16E−01	1.03 (0.88–1.20)
rs7552167	*IL28RA*	0.192	0.141	0.196	8.12E−03	0.69 (0.53–0.91)	7.86E−01	1.02 (0.87–1.20)
rs7536201	*RUNX3*	0.388	0.344	0.358	6.55E−02	0.83 (0.67–1.01)	5.40E−02	0.88 (0.77–1.00)
rs6677595	*LCE3B, LCE3D*	0.412	0.363	0.417	4.52E−02	0.81 (0.67–1.00)	7.33E−01	1.02 (0.90–1.16)
rs62149416	*FLJ16341, REL*	0.039	0.043	0.038	6.70E−01	1.11 (0.68–1.81)	8.73E−01	0.97 (0.70–1.36)
rs10865331	*B3GNT2*	0.315	0.347	0.311	1.67E−01	1.16 (0.94–1.42)	7.97E−01	0.98 (0.86–1.13)
rs2111485	*IFIH1*	0.173	0.181	0.174	6.72E−01	1.06 (0.82–1.36)	9.03E−01	1.01 (0.85–1.19)
rs17716942	*KCNH7, IFIH1*	0.000	0.000	0.001	–	–	3.30E−01	–
rs27432	*ERAP1*	0.406	0.373	0.382	1.73E−01	0.87 (0.71–1.06)	1.30E−01	0.90 (0.79–1.03)
rs2910686	*ERAP2*	0.484	0.516	0.510	2.10E−01	1.13 (0.93–1.38)	1.19E−01	1.11 (0.97–1.26)
rs1295685	*IL13, IL4*	0.302	0.274	0.346	2.18E−01	0.87 (0.70–1.08)	3.54E−03	1.22 (1.07–1.40)
rs2233278	*TNIP1*	0.089	0.174	0.086	3.50E−08	2.15 (1.63–2.84)	7.34E−01	0.96 (0.77–1.20)
rs4379175	*IL12B*	0.430	0.311	0.450	1.25E−06	0.60 (0.49–0.74)	2.02E−01	1.09 (0.96–1.23)
rs9504361	*EXOC2, IRF4*	0.217	0.234	0.213	4.00E−01	1.10 (0.88–1.39)	7.51E−01	0.98 (0.84–1.14)
rs4406273	*HLA-B, HLA-C*	0.011	0.045	0.010	5.86E−07	4.12 (2.26–7.51)	6.13E−01	0.85 (0.46–1.59)
rs13437088	*MICA*	0.290	0.278	0.269	5.94E−01	0.94 (0.76–1.17)	1.38E−01	0.90 (0.78–1.04)
rs33980500	*TRAF3IP2*	0.017	0.050	0.021	9.39E−06	3.13 (1.84–5.32)	2.96E−01	1.28 (0.80–2.05)
rs582757	*TNFAIP3*	0.056	0.060	0.063	7.20E−01	1.08 (0.71–1.63)	3.70E−01	1.13 (0.86–1.48)
rs2451258	*TAGAP*	0.029	0.021	0.021	3.14E−01	0.72 (0.37–1.38)	1.05E−01	0.72 (0.48–1.07)
rs2700987	*ELMO1*	0.094	0.109	0.094	2.87E−01	1.19 (0.86–1.63)	9.86E−01	1.00 (0.81–1.25)
rs11795343	*DDX58*	0.224	0.241	0.257	4.23E−01	1.10 (0.87–1.38)	1.92E−02	1.19 (1.03–1.39)
rs10979182	*KLF4*	0.399	0.442	0.416	8.14E−02	1.19 (0.98–1.45)	2.89E−01	1.07 (0.94–1.22)
rs1250546	*ZMIZ1*	0.473	0.417	0.422	2.43E−02	0.80 (0.66–0.97)	1.65E−03	0.82 (0.72–0.93)
rs645078	*RPS6KA4, PRDX5*	0.239	0.234	0.239	8.33E−01	0.98 (0.78–1.23)	9.94E−01	1.00 (0.86–1.16)
rs4561177	*ZC3H12C*	0.365	0.371	0.382	8.24E−01	1.02 (0.84–1.25)	2.95E−01	1.07 (0.94–1.22)
rs3802826	*ETS1*	0.269	0.301	0.292	1.45E−01	1.17 (0.95–1.45)	1.12E−01	1.12 (0.97–1.29)
rs2066819	*STAT2, IL23A*	0.046	0.027	0.037	6.15E−02	0.58 (0.33–1.03)	2.01E−01	0.81 (0.59–1.12)
rs8016947	*NFKBIA*	0.479	0.454	0.471	3.15E−01	0.90 (0.74–1.10)	6.23E−01	0.97 (0.85–1.10)
rs367569	*PRM3, SOCS1*	0.094	0.081	0.099	3.59E−01	0.85 (0.60–1.21)	5.87E−01	1.06 (0.86–1.31)
rs12445568	*PRSS53, FBXL19*	0.085	0.093	0.066	5.77E−01	1.10 (0.78–1.54)	2.60E−02	0.76 (0.60–0.97)
rs28998802	*NOS2*	0.009	0.010	0.009	8.09E−01	1.13 (0.41–3.11)	9.97E−01	1.00 (0.50–1.99)
rs963986	*PTRF, STAT3, STAT5A/B*	0.372	0.442	0.377	4.18E−03	1.33 (1.09–1.63)	7.89E−01	1.02 (0.89–1.16)
rs11652075	*CARD14*	0.409	0.440	0.413	2.17E−01	1.13 (0.93–1.38)	7.98E−01	1.02 (0.89–1.16)
rs545979	*POL1, STARD6, MBD2*	0.024	0.029	0.022	5.31E−01	1.21 (0.67–2.19)	6.97E−01	0.92 (0.60–1.40)
rs892085	*ILF3, CARM1*	0.275	0.285	0.260	6.54E−01	1.05 (0.85–1.30)	2.92E−01	0.93 (0.80–1.07)
rs1056198	*RNF114*	0.315	0.308	0.341	7.51E−01	0.97 (0.78–1.19)	8.67E−02	1.13 (0.98–1.29)
rs4821124	*UBE2L3*	0.357	0.375	0.371	4.40E−01	1.08 (0.88–1.33)	3.80E−01	1.06 (0.93–1.21)

rs34536443 and rs12720356) were found to be monomorphic in this study. We compared differences in the allele frequencies of the 37 polymorphisms between case and control subjects by using a contingency χ^2 test or Fisher's exact test, and calculated odds ratios (ORs) with 95 percent confidence intervals (95% CI). We then applied Bonferroni corrections, the multiplication of P values by the number SNPs assessed ($n = 37$). Statistical significance was set at $P < 0.05$.

All genotype frequencies and statistical results are shown in Table 1 and Supplementary Tables S1 and S2. We found significant associations with PsV in four SNPs, *TNIP1* (rs2233278, $P = 3.5 \times 10^{-8}$), *IL12B* (rs4379175, $P = 1.2 \times 10^{-6}$), the MHC class I region (rs4406273, $P = 5.9 \times 10^{-7}$) and *TRAF3IP2* (rs33980500, $P = 9.4 \times 10^{-6}$), after Bonferroni correction for 37 tests with $P < 1.4 \times 10^{-3}$ (0.05/37) (Table 1). The direction of associations of susceptibility to PsV was similar to that in the recent study [2], and confirmed previous Japanese studies that have shown significant associations with *IL12B* [6], *TRAF3IP2* [7] and the MHC class I region [8]. The strongest association was observed in Japanese patients with PsV for the first time at *TNIP* for SNP rs2233278, and *TNIP* encodes ABIN-1, an A20-binding protein that links A20 to NEMO/IKKγ and results in inhibition of NF-κB by facilitation of A20-mediated deubiquitination of NEMO/IKKγ [9].

A recent GWAS of childhood-onset AD showed the genetic relationship between AD and psoriasis [4]. The study examined SNPs previously shown to associate with psoriasis by a GWAS, and approximately two-thirds of those associated variants exhibited opposite risk profiles for AD versus psoriasis. The most significant association with AD in that study was observed at SNP rs1295685 in the *IL13* locus at a genome-wide level that exhibited opposing effects in AD and psoriasis. In this study, we observed marginal associations between AD and the two susceptibility SNPs for psoriasis, *IL13* (rs1295685, $P = 3.5 \times 10^{-3}$) and *ZMIZ1* (rs1250546, $P = 1.7 \times 10^{-3}$), in the opposite and same direction, respectively. *ZMIZ1* encodes a member of the PIAS (protein inhibitor of activated STAT) family of proteins that regulates the activity of several transcription factors [10]. The *IL13* and *ZMIZ1* loci might contain common genetic factors shared by these two common skin diseases.

Our data strongly suggests the importance of the *TNIP*, *IL12B*, *TRAF3IP2* loci and the MHC class I region in the susceptibility to PsV in the Japanese population. However, there are two limitations in our study. First, the sample size was relatively small. Second, we conducted the validation study using only SNPs reported in previous GWASs of psoriasis. Therefore, the associations of the other SNPs within the susceptibility loci remain unclear and further studies are necessary to achieve better understanding the genetic components of these chronic inflammatory skin diseases.

Acknowledgments

We thank all the individuals who participated in the study. We also thank M.T. Shimizu, H. Sekiguchi, A.I. Jodo, N. Kawaraichi and the technical staff of the Center for Genomic Medicine for providing technical assistance and K. Barrymore for proofreading this manuscript. This work was supported by Health Science Research Grants from the Ministry of Health, Welfare and Labor of Japan and the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2014.08.005.

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Mayumi Tamari[a,1], Hidehisa Saeki[b,1,*], Mitsuha Hayashi[c], Yoshinori Umezawa[c], Toshihiro Ito[c], Osamu Fukuchi[c], Yoshimasa Nobeyama[c], Koichi Yanaba[c], Hidemi Nakagawa[c], Yuichiro Tsunemi[d], Toyoaki Kato[e], Sayaka Shibata[e], Makoto Sugaya[e], Shinichi Sato[e], Yayoi Tada[f], Satoru Doi[g], Akihiko Miyatake[h], Kouji Ebe[i], Emiko Noguchi[j], Shigeharu Fujieda[k], Tamotsu Ebihara[l], Masayuki Amagai[l], Hitokazu Esaki[m], Satoshi Takeuchi[m], Masutaka Furue[m], Tomomitsu Hirota[a]

[a]*Laboratory for Respiratory and Allergic Diseases, Center for Integrative Medical Sciences, RIKEN, Japan*; [b]*Department of Dermatology, Nippon Medical School, Japan*; [c]*Department of Dermatology, The Jikei University School of Medicine, Japan*; [d]*Department of Dermatology, Tokyo Women's Medical University, Japan*; [e]*Department of Dermatology, Faculty of Medicine, University of Tokyo, Japan*; [f]*Department of Dermatology, Teikyo University School of Medicine, Japan*; [g]*Osaka Prefectual Medical Center for Respiratory and Allergic Diseases, Japan*; [h]*Miyatake Asthma Clinic, Japan*; [i]*Takao Hospital, Japan*; [j]*Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan*; [k]*Department of Otorhinolaryngology-Head and Neck Surgery, School of Medicine, University of Fukui, Japan*; [l]*Department of Dermatology, Keio University School of Medicine, Japan*; [m]*Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Japan*

*Corresponding author at: Department of Dermatology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan.
Tel.: +81 3 3822 2131; fax: +81 3 3823 6731

E-mail address: h-saeki@nms.ac.jp (H. Saeki).

[1]These authors contributed equally to this article.

Received 5 June 2014

http://dx.doi.org/10.1016/j.jdermsci.2014.08.005

nature COMMUNICATIONS

ARTICLE

Received 10 Jul 2014 | Accepted 20 Sep 2014 | Published 15 Dec 2014

DOI: 10.1038/ncomms6340 OPEN

The structural basis for receptor recognition of human interleukin-18

Naotaka Tsutsumi[1,*], Takeshi Kimura[2,*], Kyohei Arita[3], Mariko Ariyoshi[1,4], Hidenori Ohnishi[2], Takahiro Yamamoto[2], Xiaobing Zuo[5], Katsumi Maenaka[6], Enoch Y. Park[7], Naomi Kondo[2,8], Masahiro Shirakawa[1,9], Hidehito Tochio[10] & Zenichiro Kato[2,11]

Interleukin (IL)-18 is a proinflammatory cytokine that belongs to the IL-1 family and plays an important role in inflammation. The uncontrolled release of this cytokine is associated with severe chronic inflammatory disease. IL-18 forms a signalling complex with the IL-18 receptor α (Rα) and β (Rβ) chains at the plasma membrane, which induces multiple inflammatory cytokines. Here, we present a crystal structure of human IL-18 bound to the two receptor extracellular domains. Generally, the receptors' recognition mode for IL-18 is similar to IL-1β; however, certain notable differences were observed. The architecture of the IL-18 receptor second domain (D2) is unique among the other IL-1R family members, which presumably distinguishes them from the IL-1 receptors that exhibit a more promiscuous ligand recognition mode. The structures and associated biochemical and cellular data should aid in developing novel drugs to neutralize IL-18 activity.

[1] Department of Molecular Engineering, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan. [2] Department of Pediatrics, Graduate School of Medicine, Gifu University, Yanagido 1-1, Gifu 501-1194, Japan. [3] Graduate School of Nanobioscience, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama Kanagawa 230-0045, Japan. [4] Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8501, Japan. [5] X-Ray Science Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439, USA. [6] Laboratory of Biomolecular Science and Center for Research and Education on Drug Discovery, Faculty of Pharmaceutical Sciences, Hokkaido University, , Kita-12, Nishi-6, Kita-ki, Sapporo 060-0812, Japan. [7] Research Institute of Green Science and Technology, Department of Bioscience, Graduate school of Science and Technology, Shizuoka University, 836 Ohya Suruga-ku, Shizuoka 422-8529, Japan. [8] Heisei College of Health Sciences, 180 Kurono, Gifu 501-1131, Japan. [9] Core Research of Evolution Science (CREST), Japan Sciences and Technology Agency, Tokyo 102-0076, Japan. [10] Department of Biophysics, Graduate School of Science, Kyoto University, Kitashirakawa-oiwake, Sakyo-ku, Kyoto 606-8502, Japan. [11] Biomedical Informatics, Medical Information Sciences Division, The United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Gifu 501-1194, Japan. * These authors contributed equally to this work. Correspondence and requests for materials should be addressed to H.O. (email: ohnishih@gifu-u.ac.jp) or to H.T. (email: tochio@mb.biophys.kyoto-u.ac.jp).

Interleukin (IL)-18 belongs to the IL-1 superfamily and was first discovered as an interferon gamma (IFN-γ)-inducing factor in sera from mice with hepatitis stimulated with *Propionibacterium* acnes and lipopolysaccharide[1]. This proinflammatory cytokine is secreted by various types of cells and strongly augments IFN-γ production in type-1 helper T (Th1) cells and natural killer (NK) cells following activation of NK-cell cytotoxicity; thus, it plays a critical role in inflammation and the host defense against microbes. In addition to IL-1β[2,3], IL-18 is synthesized as a biologically inactive precursor (proIL-18) on activation of a certain class of receptors, such as Toll-like receptors and proinflammatory cytokine receptors, and then stored in the cytosol. Once it matures via caspase-1 (ref. 4), which is regulated by a large protein complex referred to as the inflammasome[5], IL-18 is extracellularly secreted and binds IL-18 receptor α (Rα) as well as IL-18 receptor β (Rβ) at the immunocyte plasma membrane in a stepwise manner. IL-18/IL-18Rα/IL-18Rβ ternary complex formation juxtaposes the intracellular Toll-Interleukin-1 receptor domains of IL-18Rα and IL-18Rβ, to which the adaptor molecule myeloid differentiation factor 88 (MyD88) is recruited presumably with the aid of TRAM[6]. MyD88 further interacts with IL-1 receptor associating kinase (IRAK) 4 and IRAK1/2 to form the large molecular assembly referred to as Myddosome, which subsequently activates IKK via TRAF6. Finally, the signal activates the NF-κB and mitogen-activated protein kinase pathways[7], which upregulate the expression of various inflammatory cytokines.

Of the IL-1 family cytokines, IL-18 and IL-1β have garnered much attention because they are causal cytokines that lead to severe chronic inflammatory syndrome. IL-1β is associated with immunological disorders, such as autoinflammatory syndromes[8,9]. The central pathogenic feature of autoinflammatory syndromes is excess production of mature IL-1β derived from abnormal inflammasome activation due to certain gene mutations. IL-1β-related autoinflammatory diseases are treated through neutralizing IL-1β by anti-IL-1β (canakinumab and gevokizumab), engineered soluble receptors (rilonacept) or the receptor antagonist IL-1Ra (anakinra), which is remarkably effective; thus, these treatments are currently in clinical use[10]. Similar to IL-1β, IL-18 overproduction likely leads to severe autoimmune, autoinflammatory, allergic, neurological and metabolic disease, which might be associated with IL-18 or IL-18 receptor genetic polymorphisms[11–14]. Two recent papers have revealed that constitutive activation of the inflammasome caused by single point mutations in NLRC4 is associated with a novel autoinflammatory disorder, and the patient with NLRC4-mediated macrophage activation syndrome showed ultra-high circulation levels of IL-18 even after IL-1 blockade[15,16]. Consistent with these observations, therapeutic approaches that block IL-18 activity have been effective in inflammatory disease models[17,18]. Therefore, developing drugs that impede binding between IL-18 and the receptors is clinically important. Generally, the atomic structures of targeted proteins and their complexes play vital roles in drug design. Thus far, despite the reported structures for free IL-18 and its related complexes[19–22], a structure for the genuine complex between IL-18 and its receptors has not yet been determined.

Previously, we reported a solution structure for IL-18 and identified the functional residues for which mutation markedly decreased its binding affinity for IL-18Rα[19]. The results suggest that the binary complex between IL-18 and IL-18Rα exhibits an essentially identical binding mode to the complex between IL-1β and its receptors (IL-1RI or IL-1RII). However, the binding mode for IL-18Rβ, which is the IL-18 co-receptor, to IL-18/IL-18Rα remained ambiguous. Recent structural studies on the ternary complex between IL-1β and its receptors' ectodomains[23,24] demonstrate that IL-1RAcP, which is the commonly used co-receptor for IL-1α, IL-1β, IL-33 and IL-36s, adopted a 'left' binding mode. In this mode, IL-1RAcP binds the IL-1β/IL-1RI or IL-1β/IL-1RII binary complexes from the left side as seen from the concave IL-1β recognition surface of IL-1RI or IL-1RII. Furthermore, the other IL-1 superfamily molecule, IL-33/ST2/IL-1RAcP, was also suggested to adopt the 'left' binding mode based on the model structure from the small angle X-ray scattering (SAXS) profiles[25]. Thus, left binding seems common in complexes that employ IL-1RAcP. In contrast to other IL-1 family cytokines, IL-18 is unique due to its pair of specialized receptors (IL-18Rα and IL-18Rβ); hence, the recognition details are not sufficiently understood based only on homology to the IL-1β and IL-33 system.

Here, we performed X-ray crystallography using human IL-18 and its complexes with the receptors' extracellular domains. The structures demonstrate that the co-receptor (IL-18Rβ) binding mode is generally identical to IL-1β; however, substantial differences were observed in the subdomain orientations and interaction details throughout the complex. Intriguingly, the second domain (D2) of the two IL-18 receptors lacked one β-strand, d2, which is conserved among other IL-1-related receptors, and was previously shown to contribute to the inter-receptor interaction. In addition, N-linked glycans played a role in bridging the two receptors, which was observed in the signalling IL-1β receptor complex but was absent in its decoy complex. We further show that other IL-18Rα N-linked glycans proximal to IL-18 in the complexes contributed to the binding affinity. With the associated biochemical and cell biological data, the structures comprehensively clarify the IL-18 receptor recognition mode, which will facilitate rational drug development to neutralize IL-18 activity, the uncontrolled release of which has been shown to cause severe chronic inflammatory diseases.

Results

Structural comparison between the IL-18 and IL-1β complexes. We determined the crystal structures of IL-18 (Fig. 1a), the IL-18/IL-18Rα binary complex (Fig. 1b) and the IL-18/IL-18Rα/IL-18Rβ signalling ternary complex (Fig. 1c,d) at the resolutions 2.33, 3.10 and 3.10 Å, respectively. The crystallographic statistics are provided in Table 1.

IL-18Rα curls around IL-18, and IL-18Rβ contacts the lateral portion of the IL-18/IL-18Rα binary complex in a similar manner as the IL-1β/IL-1RI(RII)/IL-1RAcP complex[23,24]. The IL-18 structure essentially does not change on complex formation, maintaining the β-trefoil fold that comprises 12 β-strands (β1-β12) and 2 α-helices (α1-α2) (Supplementary Fig. 1a), as previously reported[19]. The IL-18Rα ectodomain folds into three immunoglobulin (Ig)-like domains, which are referred to as D1, D2 and D3, in the same manner as the IL-1 receptors[23,24,26]. Each domain comprises a two-layer sandwich of six to nine β-strands and contains at least one intra-domain disulfide bond (Supplementary Fig. 1b). Within IL-18Rα, D1 extensively contacts D2, whereas D3 is distant and is connected by the long D2-D3 linker (Fig. 1d middle), which implies that D1 and D2 behave as a single module, similar to IL-1-related primary receptors. In the Ig superfamily, including the IL-1 receptor family (Fig. 2a), the core cysteine residues on the b and f strands are highly conserved (Fig. 2b). However, for IL-18Rα-D1, the f strand cysteine is replaced with phenylalanine (Fig. 2b), which yields two unexpected surface disulfide bonds (Fig. 2c). In addition, the D2 domain lacks one β-strand (d2 in Fig. 2b,d) that is structurally conserved among most IL-1 receptor family members, IL-1RI, IL-1RII and ST2 as well as IL-1RAcP.

On ternary complex formation, the IL-18/IL-18Rα binary complex structure essentially does not change; the root mean