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Genome-wide association study for pollinosis identified two novel loci in *interleukin (IL)-1B* in a Japanese population

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ABSTRACT

The number of pollinosis patients in Japan has significantly increased over the past 20 years. The majority of genome-wide association studies (GWAS) on pollinosis have been conducted in subjects of European descent, with few studies in Japanese populations. The aim of our GWAS was to identify genetic loci associated with self-reported pollinosis in a Japanese population and to understand its molecular background using a combination of single nucleotide polymorphisms (SNPs) and gene- and pathway-based analyses. A total of 731 and 560 individuals who were recruited as participants of the Japan Multi-Institutional Collaborative Cohort Study participated in the discovery and replication phases, respectively. The phenotype of pollinosis was based on the information from a self-administered questionnaire. In the single-SNP analysis, four SNPs (rs11975199, rs11979076, rs11979422, and rs12669708) reached suggestive significance level ($P < 1 \times 10^{-4}$) and had effects in the same direction in both phases of the study. The pathway-based analysis identified two suggestive pathways (nucleotide-binding oligomerization domain -like receptor and tumor necrosis factor signaling pathways). Both rs1143633 and rs3917368 in the interleukin-1B gene showed associations in the retrace (from pathway to gene and SNP) analysis. We performed single-SNP, gene, and pathway analysis and shed light on the molecular mechanisms underlying pollinosis in a Japanese population.

Keywords: genome-wide association study, pollinosis, cedar pollinosis, cypress pollinosis, allergic rhinitis

INTRODUCTION

Allergic rhinitis (AR) is a common disease worldwide, and its prevalence has increased in industrialized countries over the last few decades[1-5]. AR is generally defined as an immunoglobulin E (IgE)-mediated inflammatory response of the nasal mucous membrane. This reaction is induced by exposure to specific allergens such as pollen, dust mites, molds, and pets. Pollen is suspected as a major cause of seasonal AR, whereas other allergens are associated with perennial AR. The major allergens for seasonal AR in Japan are pollen from Japanese cedar (*Cryptomeria japonica*; sugi) and Japanese cypress (*Chamaecyparis obtusa*; hinoki)[6]. The dispersion of Japanese cedar pollen generally peaks between February and April[7], and Japanese cypress pollen is dispersed from April to May. Moreover, 70% of cedar pollinosis patients also experience seasonal AR as a result of cypress pollinosis; thus, the allergic symptoms caused by these types of pollinosis can continue for up to 4 months[8]. Therefore, pollinosis is considered a common health issue in Japan[9-11].

From an economic standpoint, the medical expenses associated with AR management are considerable[12]. From the patient's perspective, AR adversely influences quality of life because of widespread symptoms such as sneezing, watery rhinorrhea, nasal congestion, and/or itchy nose. Previous studies reported that these symptoms affect daytime activity, work productivity, and sleep quality in patients[8,13,14]. In children, seasonal AR impacts on learning performance and impairs behavior and attention[15,16].

To better understand the genetic factors underlying AR, genome-wide association studies (GWAS) have recently been conducted. A genome-wide meta-analysis by Ramasamy et al. identified three single nucleotide polymorphisms (SNPs) with genome-wide significance and 12 loci with suggestive associations of AR and grass sensitization[17]. Hinds et al. found 16 shared loci associated with self-reported cat, dust mite, and pollen allergy[18]. Further, Bønnelykke et al. reported that 10 loci are significantly associated with allergic sensitization in Caucasian individuals[19]. Combining the findings from previous GWAS together, only four loci (*toll-like receptor (TLR)6-TLR1*, *HLA-DQA2-HLA-DQA1*, *interleukin (IL)2-ADA1*, and *LRRC32-C11orf30*) of all 47 SNPs overlap. Although considerable work has been performed to identify genetic variants associated with allergic traits such as AR, prior studies have focused almost exclusively on subjects of European descent[17-22]. However, recent work suggests that GWAS in non-European populations are needed to elucidate ethnically different variants[23]. In Japan, although there have been some candidate gene studies on pollinosis[24-29], no GWAS has been conducted. The aim of our study was to conduct a genome-wide agnostic screening for genetic loci associated with self-reported pollinosis, a major cause of seasonal AR, in a Japanese genome cohort study. Furthermore, to augment our single-SNP screen, we also utilized gene- and pathway-based analyses to expand our knowledge on the molecular factors underlying pollinosis.

MATERIALS AND METHODS

The present study was nested within the broader Japan Multi-Institutional Collaborative Cohort (J-MICC) Study, a large-scale cohort study performed in Japan. The aim of the J-MICC Study is to examine and detect gene–environmental interactions associated with lifestyle-related diseases, primarily cancer. This cohort study was launched in 2005, and the recruitment of 100,000 participants was completed in March 2014. Participants in the J-MICC Study are individuals aged 35 to 69 years who enrolled by responding to study announcements in 14 different areas throughout Japan. The J-MICC Study has been well-described previously[30] and on its website

(URL: http://www.jmicc.com/). In our current study, we performed a two-stage GWAS using information from two independent sites. The first stage was for screening (discovery phase) and the second stage for confirming the findings from the first stage (replication phase). In both phases, all research procedures complied with the Ethical Guidelines for Human Genome and Genetic Sequencing Research in Japan.

Discovery phase (Daiko Study)

The discovery phase analysis was carried out using data from the participants of the Daiko Study, one of the study sites of the J-MICC Study. A total of 766 individuals (231 men and 535 women) with information on pollinosis history were selected for this analysis. At this site, we inquired about three different types of pollinosis (the presence of any pollinosis, Japanese cedar pollinosis, and Japanese cypress pollinosis) using a self-administered questionnaire. Written informed consent was obtained from all participants. The protocol of this study was approved by the Ethics Review Committee of the Nagoya University Graduate School of Medicine (No. 2015-0274).

Replication phase (Shizuoka–Sakuragaoka Study)

We used data from the baseline survey of the Shizuoka–Sakuragaoka Study, another site of the J-MICC Study, to validate the findings from the discovery phase. In this phase, 581 participants (360 men and 221 women) were included. Participants at this site were asked only whether they had a pollen allergy or not. We obtained written informed consent from all participants. The protocol of this study was approved by the Ethics Committee of the University of Shizuoka (No. 22-39).

Genotyping and quality control

DNA samples were automatically extracted from all buffy-coat fractions using the BioRobot M48 Workstation (QIAGEN Group, Tokyo, Japan). For both the discovery and replication phases, 964,193 SNPs were genotyped using the Illumina HumanOmniExpressExome ver1.2 platform (Illumina, San Diego, CA) at the RIKEN Center for Integrative Medical Sciences (Yokohama, Japan). At the quality control (QC) phase, we excluded SNPs matching any one of the following five criteria: (1) call rate < 98%, (2) Hardy–Weinberg equilibrium $P < 1 \times 10^{-6}$, (3) minor allele frequency < 0.01, (4) insertion and deletion, and (5) non-autosomal SNP (X, Y, mitochondrial chromosome) and linkage disequilibrium (LD) pruning. After the QC for SNPs, participants who had a proportion of identity by descent > 0.1875 and outliers in a principal component analysis were excluded. Following QC, a total of 570,398 SNPs were finally available for the analysis of 731 and 560 individuals in the discovery and replication phases, respectively.

Single-SNP analysis

All statistical analyses were performed using software R ver3.3.1 and PLINK ver1.07. Principal components (PCs) were calculated by EIGENSOFT ver6.1.4 to remove the influence of population stratification[31]. We carried out a logistic regression to screen the associations between pollinosis and each individual SNP. SNPs were coded under an additive genetic model, and a logistic regression analysis with adjustment for age, sex, and the top five PCs was conducted. *P*-values $< 5 \times 10^{-8}$ and $< 1 \times 10^{-4}$ were considered to signify genome-wide significance and suggestive significance, respectively. A combined *P*-value in both phases was calculated using Fisher's method[32].

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Gene- and pathway-based analyses

To augment our single-SNP analysis, we then performed gene- and pathway-based analyses to identify particular genes and pathways associated with pollinosis. We used the sequence kernel association test (SKAT)[33,34], which allows us to test for associations between a group of genetic variants in a gene or pathway and outcomes, with adjustment for covariates. Briefly, the approach works by comparing pairwise similarity in phenotypes to pairwise similarity in genotypes as measured through a kernel function. Different kernel functions capture different underlying models. Gene-based SNP sets for each study were created by matching SNPs to gene locations, which were determined using the UCSC Genome Browser (GRCh37) database. We analyzed a total of 24,756 genes (447,224 SNPs) in the discovery phase and 24,717 genes (427,203 SNPs) in the replication phase. Pathway-based SNP sets were subsequently generated by aggregating the SNPs within genes comprising pathways. Gene–pathway membership was confirmed using the Kyoto Encyclopedia of Genes and Genomes Pathway Database. In the pathway-based approach, we selected 10 biologically plausible pathways associated with pollinosis: (1) asthma, (2) B cell receptor signaling, (3) cytokine receptors, (4) Fc gamma R-mediated phagocytosis, (5) nuclear factor-kappa B signaling, (6) natural killer cell-mediated cytotoxicity, (7) nucleotide-binding oligomerization domain-like receptor (NLR) signaling, (8) T cell receptor signaling, (9) TLR signaling, and (10) tumor necrosis factor (TNF) signaling. *P*-values in the discovery phase were corrected for the false discovery rate (FDR) using the Benjamini–Hochberg method[35]. The significance threshold after FDR correction was set at $P < 0.25$ in the present study. The genes and pathways passing the threshold in the discovery phase were analyzed in the replication phase. All *P*-values in the replication phase were reported.

RESULTS

Single-SNP analysis

The demographic data of the participants in the discovery and replication phases are summarized in Table 1. We identified no SNPs meeting the genome-wide significance level ($P < 5 \times 10^{-8}$) when comparing those with each of the allergen-specific types of pollinosis (cedar and cypress pollinosis) and those without. However, 58 SNPs showed associations with both types of pollinosis at the suggestive significance level ($P < 1 \times 10^{-4}$). The GWAS for cedar and cypress pollinosis identified 42 and 54 SNPs that reached the suggestive threshold, respectively. Consequently, 123 unique SNPs passed into the replication phase in the 560 unrelated samples. Our replication study identified six significant SNPs out of these 123 loci. However, when comparing the results of the discovery phase with those of the replication phase, two of the six significant SNPs were inconsistent in their direction of effects across the phases (Table 2). The four remaining SNPs at rs11975199 between *LOC100631260* and *MARK2P7* (OR = 0.76, *P* = 0.024), rs11979076 between *LOC100631260* and *MARK2P7* (OR = 0.75, *P* = 0.021), rs11979422 between *LOC100631260* and *MARK2P7* (OR = 0.76, *P* = 0.024), and rs12669708 in *MARK2P7* (OR = 0.77, *P* = 0.034) showed consistent effects in the two phases. When we combined the *P*-values in the discovery and replication phases, no SNPs reached genome-wide significance levels.

Table 1 The characteristics of the participants in the discovery and replication phases

	Discovery phase			Replication phase
	All pollinosis	Cedar	Cypress	All pollinosis
N	731	649	554	560
Age (Mean, SD)	53.1 (10.6)	53.2 (10.5)	53.3 (10.7)	50.2 (9.4)
Sex (Male)	222 (30.4%)	195 (30.0%)	164 (29.6%)	344 (61.4%)
Pollinosis (Yes)	311 (42.5%)	229 (35.3%)	134 (24.2%)	277 (49.5%)

Table 2 Summary of results in the single-SNP association test

SNP	CHR	Major allele	MAF	Discovery phase			Replication phase	
				OR (95% CI)	P	Pollinosis	OR (95% CI)	P
rs11617981	13	C	0.45	1.60 (1.26–2.02)	9.83E-05	Cedar	0.77 (0.61–0.98)	0.036
rs540607	19	T	0.21	1.98 (1.42–2.76)	5.55E-05	Cypress	0.73 (0.54–0.99)	0.045
rs11975199	7	A	0.43	0.54 (0.40–0.73)	7.30E-05	Cypress	0.76 (0.59–0.96)	0.024
rs11979076	7	T	0.43	0.54 (0.40–0.73)	7.30E-05	Cypress	0.75 (0.59–0.96)	0.021
rs11979422	7	A	0.43	0.54 (0.40–0.73)	7.30E-05	Cypress	0.76 (0.59–0.96)	0.024
rs12669708	7	G	0.44	0.55 (0.40–0.74)	8.41E-05	Cypress	0.77 (0.60–0.98)	0.034

CHR, chromosome; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval.

Gene-based analysis

In the discovery phase, the gene-based analysis identified no significant or suggestive genes for the risk of all pollinosis and cedar pollinosis. For cypress pollinosis, we identified two suggestive genes that passed the criteria into the replication phase: *PHF13* (chromosome 1, Q_{linear} = 0.244) and *KLHL21* (chromosome 1, Q_{linear} = 0.244) (Table 3). In the replication phase, however, neither *PHF13* nor *KLHL21* was significantly associated with pollinosis (P_{linear} = 0.489 and 0.459, respectively). Integrated analysis also showed no significant genes.

Table 3 The results of the gene-based analysis in the discovery and replication phases (in linear kernel function)

Gene name	Discovery phase[a]			Replication phase[b]
	All pollinosis	Cedar	Cypress	All pollinosis
PHF13	0.945	0.814	0.244	0.489
KLHL21	0.945	0.814	0.244	0.459

[a]Q-values (Benjamini-Hochberg method-corrected P-values) are shown.
[b]P-values were not corrected in the replication phase.

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Pathway-based analysis

In the discovery phase, pathway-based analysis identified one significant and one suggestive pathway related to pollinosis (Table 4). The NLR signaling pathway showed a significant relationship with cypress pollinosis when we applied linear and identity-by-state (IBS) kernel function (Q_{linear} = 0.025 and Q_{IBS} = 0.045, respectively). The TNF signaling pathway reached a suggestive significance level of association with cedar pollinosis (Q = 0.231). The SKAT results in the replication phase are shown in Table 5. The NLR and TNF signaling pathways reached a suggestive significance level (P = 0.094 and 0.095 in linear kernel function, respectively). Thus, the NLR and TNF signaling pathways seem to be candidate pathways for association with pollinosis.

Table 4 The results of the pathway-based analysis in the discovery phase (*Q*-value)

Pathway	All pollinosis		Cedar		Cypress	
	Linear/ W.linear	IBS/ W.IBS	Linear/ W.linear	IBS/ W.IBS	Linear/ W.linear	IBS/ W.IBS
Asthma	0.861/ 0.741	0.808/ 0.808	0.888/ 0.549	0.873/ 0.487	0.642/ 0.859	0.582/ 0.885
B cell receptor signaling	0.861/ 0.741	0.808/ 0.808	0.888/ 0.904	0.873/ 0.905	0.730/ 0.877	0.582/ 0.885
Cytokine receptors	0.888/ 0.808	0.912/ 0.822	0.888/ 0.549	0.873/ 0.487	0.783/ 0.859	0.582/ 0.885
Fc gamma R-mediated phagocytosis	0.861/ 0.808	0.808/ 0.822	0.888/ 0.904	0.873/ 0.905	0.959/ 0.877	0.915/ 0.885
Nuclear factor (NF)-kappa B signaling	0.861/ 0.741	0.808/ 0.808	0.888/ 0.549	0.873/ 0.529	0.642/ 0.877	0.582/ 0.885
Natural killer cell-mediated cytotoxicity	0.861/ 0.808	0.808/ 0.838	0.801/ 0.904	0.873/ 0.905	0.619/ 0.859	0.582/ 0.885
NOD-like receptor signaling	0.861/ 0.808	0.808/ 0.822	0.888/ 0.549	0.873/ 0.487	0.025**/ 0.859	0.045**/ 0.885
T cell receptor signaling	0.861/ 0.741	0.808/ 0.808	0.888/ 0.498	0.873/ 0.487	0.866/ 0.859	0.760/ 0.885
Toll-like receptor signaling	0.888/ 0.741	0.916/ 0.822	0.888/ 0.549	0.873/ 0.487	0.647/ 0.859	0.582/ 0.885
Tumor necrosis factor (TNF) signaling	0.861/ 0.741	0.808/ 0.808	0.888/ 0.231*	0.873/ 0.408	0.959/ 0.877	0.930/ 0.885

Linear, linear kernel; W.linear, weighted linear kernel; IBS, IBS kernel; W.IBS, weighted IBS kernel.
**: *Q*-value < 0.1, *: *Q*-value < 0.25.

Table 5 The results of the pathway-based analysis in the replication phase (*P*-value)

	Linear	W.linear	IBS	W.IBS
NOD-like receptor (NLR) signaling	0.094*	0.427	0.223	0.269
Tumor necrosis factor (TNF) signaling	0.095*	0.987	0.171	0.961

Linear: linear kernel; W.linear: weighted linear kernel; IBS: IBS kernel; W.IBS: weighted IBS kernel. *: *P*-value < 0.1.

Retrace (pathway–gene–SNP) analysis

We again performed gene-based tests by focusing on genes in the TNF and NLR signaling pathways that reached suggestive significance levels in the pathway analysis. No significant genes were observed in the TNF signaling pathway, although six genes in the NLR signaling pathway passed the threshold of Q-value < 0.25 in the discovery phase (Table 6). Of these genes, only the *IL-1B* gene was found to be associated with pollinosis in the replication phase (P_{linear} = 0.022 and P_{IBS} = 0.027) (Table 7). Thus, we traced back the results of the single-SNP test to find unique loci associated with pollinosis. As shown in Table 8 and 9, two SNPs (rs1143633 and rs3917368) in *IL-1B* were significantly associated with pollinosis in both phases without correction for multiple comparison.

Table 6 The results of the gene-based analysis in the NOD-like receptor (NLR) signaling pathway in the discovery phase (Q-value)

	All pollinosis		Cedar		Cypress	
	Linear/ W.linear	IBS/ W.IBS	Linear/ W.linear	IBS/ W.IBS	Linear/ W.linear	IBS/ W.IBS
TAB2	0.464/ 0.513	0.635/ 0.557	0.447/ 0.388	0.611/ 0.524	0.065**/ 0.070**	0.130*/ 0.139*
MAPK9	0.715/ 0.681	0.635/ 0.557	0.447/ 0.428	0.113*/ 0.158*	0.533/ 0.541	0.626/ 0.669
CARD9	0.464/ 0.513	0.661/ 0.557	0.441/ 0.305	0.611/ 0.524	0.056**/ 0.070**	0.626/ 0.698
CARD6	0.715/ 0.642	0.635/ 0.557	0.511/ 0.388	0.203*/ 0.267	0.540/ 0.507	0.626/ 0.669
CARD8	0.949/ 0.910	0.950/ 0.838	0.867/ 0.869	0.878/ 0.557	0.204*/ 0.128*	0.626/ 0.669
IL1B	0.464/ 0.513	0.961/ 1.000	0.441/ 0.198*	0.878/ 0.801	0.540/ 0.507	0.874/ 0.892

Linear, linear kernel; W.linear, weighted linear kernel; IBS, IBS kernel; W.IBS, weighted IBS kernel.
**: Q-value < 0.1, *: Q-value < 0.25.

Table 7 The results of the gene-based analysis in the NOD-like receptor (NLR) signaling pathway in the replication phase (P-value)

	Linear	W.linear	IBS	W.IBS
TAB2	0.503	0.503	0.496	0.496
MAPK9	0.525	0.339	0.665	0.206
CARD9	0.445	0.18	0.602	0.228
CARD6	0.586	0.196	0.736	0.241
CARD8	0.284	0.326	0.391	0.417
IL1B	0.022*	0.179	0.027*	0.155

Linear: linear kernel; W.linear: weighted linear kernel; IBS: IBS kernel; W.IBS: weighted IBS kernel. *: P-value < 0.05.

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Table 8 Results of the single-SNP association test in the *IL1B* region in the discovery phase (no correction for multiple comparison)

SNP [Major allele]	All pollinosis		Cedar		Cypress	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
exm2265188 [G]	1.23 (0.99–1.52)	0.062	1.22 (0.96–1.55)	0.102	1.24 (0.93–1.66)	0.144
rs10169916 [T]	1.24 (0.99–1.53)	0.053	1.22 (0.96–1.55)	0.101	1.24 (0.93–1.66)	0.144
rs1071676 [G]	0.97 (0.58–1.62)	0.912	1.22 (0.72–2.06)	0.465	1.13 (0.60–2.12)	0.716
rs1143633 [C]	1.29 (1.04–1.60)	0.018*	1.35 (1.07–1.72)	0.012*	1.32 (0.99–1.75)	0.056
rs1143634 [A]	0.97 (0.58–1.62)	0.912	1.22 (0.72–2.06)	0.465	1.13 (0.60–2.12)	0.716
rs12621220 [T]	1.17 (0.93–1.48)	0.170	1.18 (0.92–1.52)	0.195	1.07 (0.78–1.48)	0.660
rs16944 [A]	1.24 (0.99–1.53)	0.051	1.23 (0.97–1.56)	0.089	1.26 (0.94–1.69)	0.116
rs2853550 [A]	1.02 (0.75–1.39)	0.892	0.94 (0.66–1.33)	0.722	1.12 (0.75–1.67)	0.574
rs3136558 [G]	0.86 (0.69–1.06)	0.153	0.87 (0.69–1.10)	0.241	0.88 (0.67–1.17)	0.377
rs3917366 [A]	0.97 (0.58–1.62)	0.912	1.22 (0.72–2.06)	0.465	1.13 (0.60–2.12)	0.716
rs3917368 [C]	1.26 (1.02–1.55)	0.034*	1.32 (1.04–1.68)	0.020*	1.33 (1.00–1.76)	0.049*
rs7596684 [C]	1.05 (0.71–1.55)	0.806	1.13 (0.74–1.72)	0.565	1.16 (0.71–1.90)	0.561

SNP: single nucleotide polymorphism; OR: odds ratio; 95% CI: 95% confidence interval.
*: $P < 0.05$.

Table 9 Results of the single-SNP association test in the *IL1B* region in the replication phase (no correction for multiple comparison)

SNP [Major allele]	All pollinosis	
	OR (95% CI)	P
exm2265188 [G]	1.05 (0.82–1.35)	0.697
rs10169916 [T]	1.04 (0.81–1.33)	0.762
rs1071676 [G]	1.44 (0.82–2.51)	0.204
rs1143633 [C]	1.34 (1.04–1.72)	0.023*
rs1143634 [A]	1.44 (0.82–2.51)	0.204
rs12621220 [T]	1.04 (0.81–1.35)	0.739
rs16944 [A]	1.06 (0.83–1.36)	0.641
rs2853550 [A]	0.82 (0.55–1.25)	0.360
rs3136558 [G]	1.20 (0.94–1.52)	0.145
rs3917366 [A]	1.44 (0.82–2.51)	0.204
rs3917368 [C]	1.35 (1.05–1.73)	0.018*
rs7596684 [C]	1.32 (0.87–2.02)	0.194

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval. *: $P < 0.05$.

DISCUSSION

We performed a GWAS with single-SNP as well as gene- and pathway-based approaches to investigate the genetic etiology of pollinosis, including cedar and cypress pollinosis in Japan. We identified two possible loci associated with self-reported pollinosis in two independent studies, that is, the discovery phase (311 cases and 420 controls) and the replication phase (277 cases and 283 controls).

The present GWAS has three distinctive features. First, we believe that this is the first GWAS on pollinosis in a Japanese population. Earlier studies utilized candidate gene or pathway approaches. As for Asian populations, to the best of our knowledge, the only GWAS on allergic traits was conducted in a Chinese population from Singapore[36,37]. Second, our results focused specifically on cedar and cypress pollen, which are major allergens of seasonal AR in Japan. This enabled us to gain an insight into allergen-specific mechanisms and enhance the study power, even with small sample sizes. Third, we attempted to use powerful gene- and pathway-based approaches to augment the standard GWAS analysis. Although little has been reported on the genes and pathways associated with pollinosis, a recent review by Bønnelykke et al. suggested the importance of grouped analysis[23]. In the present study, we used SKAT for the gene and pathway analysis and found some plausible variants after identifying suggestive genes and pathways.

Two SNPs (rs1143633 and rs3917368) in the *IL1B* region on chromosome 2q14 were potentially associated with pollinosis in both the discovery and replication phases. IL-1 is a well-known pro-inflammatory cytokine involved in host defense and autoimmune disease[38]. Additionally, a previous *in vitro* study demonstrated that IL releases histamine from basophils and mast cells, which implies an association between IL and human allergy reaction[39]. In fact, genetic variants in *IL-1B*, a subtype of IL, have been related to the severity of chronic inflammation and autoimmune diseases such as asthma and atopic dermatitis[40-42]. Regarding rs1143633, a previous study has suggested an association between rs1143634 in *IL1B*, a SNP located 77 kb upstream of rs11343633, with AR[43]. Furthermore, rs1143634 is reportedly linked to increased IL1B secretion[44]. Additionally, rs1143634 has strong LD with rs1143627, a variant that alters transcription efficacy of *IL1B*[45], although we could not confirm the LD between rs1143633 and rs1143627. Taken together, we can presume that rs1143634, a nearby SNP of rs1143633, could potentially change the amount of IL-1B secretion. Another inference is that the association between rs1143627 and rs1143634 may modify *IL-1B* transcription. Moreover, the results of a previous study on a Chinese population in Taiwan suggested an association between rs3917368 in *IL1B* and Graves' ophthalmopathy, the most common symptom of Graves' disease[46]. In a study on hand osteoarthritis, Moxley et al. observed strong LD of rs3917368 with rs16944, a well-known SNP in *IL-1B* that can alter *IL-1B* transcription[47]. Indeed, we confirmed moderate LD of rs3917368 and rs16944 in both phases (r^2 = 0.435 and 0.361, respectively). In consideration of these findings, because rs3917368 has strong LD with rs16944, the former may be associated with immune systems through regulation of *IL-1B* gene expression.

Our study has several limitations. First, the phenotype definition of pollinosis was based on self-reported history, not on a blood test (*e.g.* IgE level) or skin prick test, which are routinely used for clinical diagnosis. However, consistency of the results between questionnaire-based and diagnosis-based pollinosis was shown in a previous study of AR[24]. Second, the sample sizes in both phases (731 and 560, respectively) were smaller than those in typical GWAS, which might have made it difficult to find significant associations of SNPs found in previous studies on pollinosis. Nevertheless, we believe that our approaches provide a useful scientific basis for future studies on the genetic etiology underlying pollinosis.

In conclusion, we identified two variants in *IL-1B* associated with pollinosis in a Japanese population. Our gene- and pathway-based analyses may shed light on the molecular mechanisms of pollinosis. Further studies are expected to identify functionally related variants and shared loci for several allergic phenotypes.

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ADDITIONAL INFORMATION

Competing financial interests
The authors declare no competing financial interests.

Data availability
The datasets used in the current study are not publicly available due to ethical restrictions, but are available from the corresponding author upon reasonable request.

REFERENCES

1) Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*, 2008; 63: 8–160.
2) Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir*, 2004; 24: 758–764.
3) Bachert C, van Cauwenberge P, Olbercht J, van Schoor J. Prevalence, classification and perception of allergic and non allergic rhinitis in Belgium. *Allergy*, 2006; 61: 693–698.
4) Katelaris CH, Lee BW, Potter PC, Maspero JF, Cingi C, Lopatin A, et al. Prevalence and diversity of allergic rhinitis in regions of the world beyond Europe and North America. *Clin Exp Allergy*, 2012; 42: 186–207.
5) Zhang Y, Zhang L. Prevalence of allergic rhinitis in China. *Allergy Asthma Immunol Res*, 2014; 6: 105–113.
6) Okamoto Y, Horiguchi S, Yamamoto H, Yonekura S, Hanazawa T. Present situation of cedar pollinosis in Japan and its immune responses. *Allergol Int*, 2009; 58: 155–162.
7) Yamada T, Saito H, Fujieda S. Present state of Japanese cedar pollinosis: the national affliction. *J Allergy Clin Immunol*, 2014; 133: 632–639.
8) Kanzaki S, Ogawa H, Ikeda M, Masuda T, Ogawa K. Quality of life of Japanese seasonal allergic rhinitis patients is related to timing of pollen dispersal-multicenter analysis. *Acta Otolaryngol*, 2011; 131: 290–297.
9) Baba K, Nakae K. National epidemiological survey of nasal allergy 2008 (compared with 1998) in otolaryngologists and their family members. *Prog Med*, 2012 (in Japanese); 28: 2001–2012.
10) Okubo K, Kurono Y, Fujieda S, Ogino S, Uchio E, Odajima H, et al. Japanese guideline for allergic rhinitis 2014. *Allergol Int*, 2014; 63: 357–375.
11) Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A. Increasing prevalence of Japanese cedar

pollinosis: a meta-regression analysis. *Int Arch Allergy Immunol*, 2005; 136: 365–371.

12) Blaiss MS. Allergic rhinitis: Direct and indirect costs. *Allergy Asthma Proc*, 2010; 31: 375–380.

13) Nathan RA. The burden of allergic rhinitis. *Allergy Asthma Proc*, 2007; 28: 3–9.

14) Lamb CE, Ratner PH, Johnson CE, Ambegaonkar AJ, Joshi AV, Day D, et al. Economic impact of workplace productivity losses due to allergic rhinitis compared with select medical conditions in the United States from an employer perspective. *Cur Med Res Opin*, 2006; 22: 1203–1210.

15) Jáuregui I, Mullol J, Dávila I, Ferrer M, Batra J, del Cuvillo A, et al. Allergic rhinitis and school performance. *J Investig Allergol Clin Immunol*, 2009; 19(Suppl 1): 32–39.

16) Mir E, Panjabi C, Shah A. Impact of allergic rhinitis on school going children. *Asia Pac. Allergy*, 2012; 2: 93–100.

17) Ramasamy A, Curjuric I, Coin LJ, Kumar A, McArdle WL, Imboden M, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol*, 2011; 128: 996–1005.

18) Hinds DA, McMahon G, Kiefer AK, Do CB, Eriksson N, Evans DM, et al. A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. *Nat Genet*, 2013; 45: 907–911.

19) Bønnelykke K, Matheson MC, Pers TH, Granell R, Strachan DP, Alves AC, et al. Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. *Nat Genet*, 2013; 45: 902–906.

20) Nilsson D, Henmyr V, Haldén C, Säll T, Kull I, Wickman M, et al. Replication of genomewide associations with allergic sensitization and allergic rhinitis. *Allergy*, 2014; 69: 1506–1514.

21) Ferreira MA, Matheson MC, Tang CS, Granell R, Ang W, Hui J, et al. Genome-wide association analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *J Allergy Clin Immunol*, 2014; 133: 1564–1571.

22) Šaulienė I, Greičiuvienė J, Šukienė L, Juškevičiūtė N, Benner C, Zinkevičiené A, et al. Genetic loci associated with allergic sensitization in Lithuanians. *PLoS ONE*, 2015; 10: e0134188.

23) Bønnelykke K, Sparks R, Waage J, Milner JD. Genetics of allergy and allergic sensitization: common variants, rare mutations. *Curr Opin Immunol*, 2015; 36: 115–126.

24) Tomita K, Sakashita M, Hirota T, Tanaka S, Masuyama K, Yamada T, et al. Variants in the 17q21 asthma susceptibility locus are associated with allergic rhinitis in the Japanese population. *Allergy*, 2013; 68: 92–100.

25) Inoue H, Mashimo Y, Funamizu M, Yonekura S, Horiguchi S, Shimojo N, et al. Association of the MMP9 gene with childhood cedar pollen sensitization and pollinosis. *J Hum Genet*, 2012; 57: 176–183.

26) Tanaka T, Hitomi Y, Kambayashi Y, Hibino Y, Fukutomi Y, Shibata A, et al. The difference in the involvements of loci of promoter region and Ile50Val in interleukin-4 receptor α chain gene between atopic dermatitis and Japanese cedar pollinosis. *Allergol Int*, 2012; 61: 57–63.

27) Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy*, 2008; 38: 1875–1881.

28) Kawai T, Takeshita S, Imoto Y, Matsumoto Y, Sakashita M, Suzuki D, et al. Associations between decay-accelerating factor polymorphisms and allergic respiratory diseases. *Clin Exp Allergy*, 2009; 39: 1508–1514.

29) Matsumoto Y, Noguchi E, Imoto Y, Nanatsue K, Takeshita K, Shibasaki M, et al. Upregulation of IL17RB during natural allergen exposure in patients with seasonal allergic rhinitis. *Allergol Int*, 2011; 60: 87–92.

30) The J-MICC Study Group. The Japan Multi-institutional Collaborative Cohort Study (J-MICC Study) to detect gene-environment interactions for cancer. *Asian Pac J Cancer Prev*, 2007; 8: 317–323.

31) Price AL, Patterson NJ, Plenge RM, Weinbiatt ME, Shadiak NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*, 2006; 38: 904–909.

32) Fisher RA. Statistical methods for research workers. pp. 99–101, 1950, Oliver & Boyd, London.

33) Wu MC, Kraft P, Epstein MP, Taylor DM, Chanock SJ, Hunter DJ, et al. Powerful SNP-set analysis for case-control genome-wide association study. *Am J Hum Genet*, 2010; 86: 929–942.

34) Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet*, 2011; 89: 82–93.

35) Benjamini Y, Hochenberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc*, 1995; 57: 289–300.

36) Andiappan AK, Wang de Y, Anantharaman R, Parate PN, Suri BK, Low HQ, et al. Genome-wide association study for atopy and allergic rhinitis in a Singapore Chinese population. *PLoS ONE*, 2011; 6: e19719.

37) Andiappan AK, Wang de Y, Anantharaman R, Suri BK, Lee BT, Rotzschke O, et al. Replication of genome-wide association study loci for allergic rhinitis and house dust mite sensitization in an Asian population of ethnic Chinese in Singapore. *J Allergy Clin Immunol*, 2013; 131: 1431–1433.

38) Nambu A, Nakae S. IL-1 and allergy. *Allergol Int*, 2010; 59: 125–135.

39) Subramanian N, Bray MA. Interleukin 1 releases histamine from human basophils and mast cells in vitro. *J Immunol*, 1987; 138: 271–275.

40) Karjalainen J, Nieminen MM, Aromaa A, Klaukka T, Hurme M. The IL-1B genotype carries asthma susceptibility only in men. *J Allergy Clin Immunol*, 2002; 109: 514–516.

41) Joki-Erkkilä VP, Karjalainen J, Hulkkonen J, Pessi T, Nieminen MM, Aromaa A, et al. Allergic rhinitis and polymorphisms of the interleukin 1 gene complex. *Ann Allergy Asthma Immunol*, 2003; 91: 275–279.

42) Zeyrek D, Esen D, Asude A, Ferda O, Figen G, Remziye T. Association of interleukin-1 beta and inter-leukin-1 receptor antagonist gene polymorphisms in Turkish children with atopic asthma. *Allergy Asthma Proc*, 2008; 29: 468–474.

43) Nasiri R, Amirzargar AA, Movahedi M, Hirbod-Mobarakeh A, Farhadi E, Behniafard N, et al. Single-Nucleotide polymorphisms of TNFA and IL1 in allergic rhinitis. *J Investig Allergol Clin Immunol*, 2013; 23: 455–461.

44) Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest*, 1992; 22: 396–402.

45) Melzer D, Perry JR, Hernandez D, Corsi AM, Stevens K, Rafferty I, et al. A genome-wide association study identifies protein qualitative trait loci (PQTLS). *PLoS Genet*, 2008; 4: e100072.

46) Liu YH, Chen RH, Wu HH, Liao WL, Chen WC, Tsai Y, et al. Association of *interleukin-1B* (*IL1B*) polymorphisms with Graves' ophthalmopathy in Taiwan Chinese patients. *Invest Ophthalmol Vis Sci*, 2010; 51: 6238–6246.

47) Moxley G, Han J, Stern AG, Riley BP. Potential influence of *IL1B* haplotype and *IL1A-IL1B-IL1RN* extended haplotype on hand osteoarthritis risk. *Osteoarthritis Cartilage*, 2007; 15: 1106–1112.