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Replication study of susceptibility variants associated with allergic rhinitis and allergy in Han Chinese

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Abstract

Background: Allergic rhinitis (AR) is believed to be a complex genetic disease. The last decade has been marked by the publication of more than 20 genome-wide association studies (GWASs) of AR and associated allergic phenotypes and allergic diseases, which have shown allergic diseases and traits to share a large number of genetic susceptibility loci. The aim of present study was therefore to investigate the highly replicated allergy related genes and variants as candidates for AR in Han Chinese subjects.

Methods: A total of 762 AR patients and 760 control subjects were recruited, and a total of 58 susceptible variants previously reported to be associated with allergic traits were choose for replication.

Results: Logistic regression analyses revealed that in the co-dominant-effect model as assessed by the AIC, compared with wild-type carriers, significant AR risk were associated with rs9865818 in LPP ($P = 0.029$, OR = 1.469 for GG vs. AA); rs6554809 in DNAH5 ($P = 0.000$, OR = 1.597 for TC vs. CC); rs1438673 in WDR36-CAMK4 loci ($P = 0.037$, OR = 1.396 for CC vs. TT), rs7775228 in HLA region ($P = 0.000$, OR = 1.589 for TC vs. TT), rs7203459 in CLEC16A ($P = 0.025$, OR = 0.731 for TC vs. TT).

Conclusion: We replicated Han Chinese AR-specific susceptibility loci in LPP, DNAH5, HLA, CLEC16A and WDR36-CAMK4. Further understanding the molecular mechanisms underlying these associations may provide new insights into the etiology of allergic disease.

Keywords: Allergic rhinitis, Single nucleotide polymorphism, Replication

Background

Allergic rhinitis (AR) is a major chronic respiratory disease induced by an immunoglobulin E (IgE)-mediated reaction in allergen-sensitized subjects. It is believed that AR and the comorbid conditions including allergic asthma, eczema, or any other allergic disease, are complex genetic diseases resulting from the effect of

both multiple genetic and interacting environmental factors on their pathophysiology. Moreover, Barnes [1] has proposed that as allergic diseases such as asthma, AR, and atopic dermatitis share common systemic characteristics [e.g. high total and/or specific IgE (sIgE)], then it was reasonable that a number of susceptibility genes could contribute to the allergic process regardless of the specific clinical phenotype.

The last decade has been marked by the publication of more than 20 genome-wide association studies (GWASs) of AR, atopy and the associated allergic phenotypes and allergic diseases and traits [2–7]. Andiappan et al. [8] first carried out GWAS strategy to investigate the associations of novel genetic variants with AR in a cohort of ethnic

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Chinese in Singapore. We previously performed the independent replication of these AR susceptibility genes in a Han Chinese population [9]. Recently, four large-scale, meta-based GWASs were of interest to identify the genetic variants that affect the susceptibility to AR and allergy. Ramasamy et al. [10] reported 3 genome-wide and 12 suggestively significant loci using existing GWAS data as well as 3 loci using a candidate gene approach associated with self-reported seasonal AR and grass sensitization in four large European cohorts. Bønnelykke [11] and colleagues found 10 genome-wide associated loci for allergic sensitization in a GWAS meta-analysis of subjects of European descent. Hinds et al. [12] identified 16 genome-wide and 6 suggestively significant loci in a GWAS meta-analysis for shared effects across pollen, dust mite and cat allergies also in European cohorts. Recently a study integrated GWAS, coexpression network and expression SNP analysis and reported 5 genome-wide significant loci for AR in ethnically diverse North American individuals [13]. Besides, a genome-wide linkage scan in 295 families of French Epidemiological Study showed strong evidence of linkage of NFIA locus to the combined asthma plus AR phenotype [14]. Bønnelykke et al. [15] reported 5 genome-wide significant loci in a GWAS of early childhood asthma with severe exacerbations. Of these, 4 loci (GSDMB, IL33, RAD50 and IL1R1) were hot spot as allergic traits susceptibility loci with larger effect sizes, as well as CDHR3 was a new susceptibility gene for asthma, which also was found to be associated with chronic rhinosinusitis [15], suggesting their potential relevance to allergic rhinitis. Together 6 studies reported a set of 58 genome-wide significant or suggestively significant SNPs in 55 loci as associated with AR and allergy phenotypes. The functional implications of above mentioned loci were evaluated using multi-gene-list meta-analysis at the level of pathways or protein complexes with Metascape website. Cytokines and immunity related pathways, such as cytokine production, Th17 cell differential and regulation of cytokine secretion, were significantly enriched and shared among GWASs (Additional file 1: Figure S1). However, few of studies have been convincingly and highly replicated especially in Chinese [5, 16]. The aim of the present study is to investigate the highly replicated allergy related genes and variants as candidates for AR in Han Chinese.

Materials and methods

Study subjects

The study protocol was approved by the Ethics Committee of Beijing TongRen Hospital and performed in accordance with the guidelines of the World Medical Association's Declaration of Helsinki. All subjects involved were adults (≥ 18 years) of Han Chinese ethnic

origin from the Beijing region, China, and provided written informed consent prior to entry in the study.

Two rhinology specialists (Y. Z. and L. Z.) were responsible for the screening the study subjects in outpatient clinic of Allergy department at Beijing TongRen Hospital, during the study period from February 2010 to February 2011. Finally, a total of 762 consecutive adult subjects with physician-diagnosed clinical AR were recruited. The flow diagram of recruitment was shown in Additional file 1: Figure S2. The diagnosis of AR fulfilled all criteria of the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines [17], including (i) presence of persistent or discontinuous symptoms of anterior rhinorrhoea, continuous sneezing, nasal obstruction and itching, (ii) demonstration of a pale and edematous nasal mucosa, nasal discharge and swollen inferior turbinate by nasal endoscopy, and (iii) positive serum antigen sIgE, measured by the ImmunoCAP 100 system (Pharmacia, Uppsala, Sweden). A diagnosis of AR was further confirmed by the presence of symptoms induced by exposure to allergen shown to produce positive serum allergen sIgE response. The tested allergens included house dust mite (HDM) (*Der f* and *Der p*); seasonal grass pollens (Giant Ragweed; Mugwort; Lamb's quarters; Humulus; Chenopodium album); animal hair (dog and cat); molds (indoor and outdoor mustiness or floricultural environment) and cockroach. Subjects were also considered to be sensitized to allergens when the serum sIgE was greater than 0.35 kU/l. AR subjects with (i) co-morbid asthma, eczema, or any other allergic disease; (ii) hypertension, diabetes or other chronic diseases; or (iii) tumor in the nasal cavity or any other inflammatory nasal disease were excluded. The diagnosis of asthma was confirmed by a physician according to Global Initiative for Asthma (GINA) guidelines [18].

A total of 760 adult healthy control volunteers were also recruited during the study period. For matching with AR groups, health control group had similar age distribution, gender ratio, and also Han Chinese ethnic origin from the Beijing region for an ethnically similar local population to determine background population allele frequencies. None of the control subjects had a history of allergic or any nasal disease, nor demonstrated any abnormal clinical features in the nasal cavity or positive serum sIgE by Phadiatop (Pharmacia, Uppsala, Sweden) testing.

Extraction of genomic DNA

A 2-ml volume of venous blood samples from each participant was taken in citrate-anticoagulated glass tubes, and were frozen at -40°C . Total genomic DNA of the leucocyte was extracted from 1 ml of peripheral blood using the Whole Blood DNA Extraction Kit

(Tiangen Biotech, Co., Ltd, Beijing, China), according to the manufacturer's instructions. Genomic DNA extracted was dissolved in 0.1× TE buffer (10 mM Tris-1 mM EDTA, pH 8.0) and stored at −20 °C.

Replication SNP selection

58 susceptible single nucleotide polymorphisms (SNPs) in 55 gene/regions previously reported as highly associated with allergic traits [11–13, 15] were chosen for replication. The detailed information of the selected candidate SNPs were summarized in Additional file 1: Table S1.

SNP genotyping assays

SNPs were typed using iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS, named as MassARRAY system, manufactured by Sequenom, Inc.) [19]. In brief, (i) Multiplex PCR amplification: PCR reactions were carried out in standard 384-well plates in 5 µl per reaction with 10 ng of genomic DNA, 0.5 units of Taq polymerase (HotStarTaq, Qiagen), 500 µmol of each deoxynucleotide triphosphate (dNTP), and 100 nmol of each PCR primer. PCR thermal cycling was carried out in an ABI-9700 instrument for 15 min at 94 °C, followed by 45 cycles of 20 s at 94 °C, 30 s at 56 °C, and 60 s at 72 °C. PCR products were electrophoresed on 2.0% agarose. (ii) Removing residual primers and dNTPs: After PCR reaction, 2 µl containing 0.3 units of Shrimp Alkaline Phosphatase was added, and the reaction was incubated at 37 °C for 20 min followed by inactivation for 5 min at 85 °C. (iii) PCR with single base extension: After adjusting the concentrations of extension primers to equilibrate signal-to-noise ratios, the post-PCR primer extension reaction of the iPLEX Gold Kits (Sequenom, Inc.) assay was done in a final 9 µl volume extension reaction containing 0.2 µl (100 µmol) of termination mix, 0.04 µl containing 0.05 units of DNA polymerase (Sequenom, Inc.), and 625 to 1250 nmol/l extension primers. A 200-short-cycle program was used for the iPLEX reaction: initial denaturation was for 30 s at 94 °C followed by 5 s at 94 °C and five cycles of 5 s at 52 °C and 5 s at 80 °C. An additional 40 annealing and extension cycles were then looped back to 5 s at 94 °C, five cycles of 5 s at 52 °C and 5 s at 80 °C. The final extension was carried out at 72 °C for 3 min and the sample was cooled to 4 °C. (iv) Analyses of purified extension reaction products by MALDI-TOF-MS: The samples were then manually desalted by using 6 mg of clean resin and a dimple plate and subsequently transferred to a 384-well Spectro-CHIP (Sequenom, Inc.) using a nano dispenser. Mass spectrum was acquired by Compact Mass Spectrometer and analyzed by MassARRAYTyper 4.0 Software (Sequenom, Inc.). The PCR assay

was arrayed with two no-template controls and four duplicated samples in each 384-well format as quality controls. All genotyping results were generated and checked by laboratory staff unaware of patient status.

Statistical analyses

Values are expressed as mean ± standard deviation or as numbers and percentages. Differences in age between case group and control group were evaluated using the t-test. Differences in gender and frequencies of the alleles and genotypes between case group and control group were evaluated using the χ^2 -test. Mann–Whitney test was used to compare serum total IgE level between case and control groups, and between different genetic models in dominant or recessive models. Kruskal–Wallis test was used to compare serum total IgE level among different genotypes in co-dominant model.

Hardy–Weinberg Equilibrium (HWE) was tested by the Chi square test for goodness of fit with a Web program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). It was tested in the control group by the Chi square test for goodness of fit, and a *P*-value of <0.05 was considered to be statistically significant. Akaike's information criteria were used to select the most parsimonious genetic model for each SNP [20]. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis with adjustment for age and gender. The significance level was set at *P*-value of 0.05 after corrections with 100,000 permutations. All *P* values are two tailed. These analyses were conducted with Stata statistical package (version 10.0; Stata Corp LP, College Station, TX, USA).

Results

Demographic characteristics of study population

The demographic characteristics of the study population are shown in Table 1. Both the AR and control groups were well matched with respect to age and gender. The mean ages of the AR and control groups were 36 and 37 years old, respectively and both groups consisted of similar sex ratio (AR group = 51.6%/48.4% males/females; control group = 49.7%/50.3% males/females). There were no significant difference were found in both age (*P* = 0.167) or the ratios for males/females (*P* = 0.372) between the control and AR groups. The median total serum IgE measurements were significantly increased in AR group than control group (125.0 and 29.2 IU/ml respectively; *P* = 0.0000). Furthermore, 273 (35.8%), 168 (22.0%), and 321 (42.2%) of AR subjects, respectively, were found to be allergic to HDMs alone, seasonal pollens alone, and mixed allergens.

Table 1 Demographic characteristics of study population

Demographic Index	Allergic rhinitis (n = 762)	Control (n = 760)
Age, Mean (Range) (years)	35.6 ± 12.7 (18–73)	37.4 ± 13.3 (18–78)
Sex, Male/Female, No. (%)	393 (51.6)/369 (48.4)	378 (49.7)/382 (50.3)
Duration(IQR) (months)	48.0(18.0–96.0)	–
Total IgE, Median (interquartile range) (kU/l)	125.0(61.95–277.0)	29.2(15.9–45.5)
Allergen category, no. (%)		
House dust mites	273 (35.8)	–
Seasonal pollens	168 (22.0)	–
Mixed allergens	321 (42.2)	–

Individual SNP association analysis

The 58 SNP IDs, locations, and allele frequencies are also given in Additional file 1: Table S1. A total of 3 variants, including rs6586513, rs9266772, rs6906021 did not pass Hardy–Weinberg Equilibrium (HWE) test ($P < 0.0001$). The minor allele frequencies (MAF) of four selected SNPs, such as rs6673480, rs17513503, rs7032572 and rs1250761 could not satisfy association study ($MAF < 0.01$). The four SNPs, rs3860069, rs11680788, rs9303280 and rs12973620 failed in assay design. Thus, we finally enrolled 47 SNPs to process the next analysis (Additional file 1: Table S2).

The genotype distributions of 47 selected SNPs in the case group and control group are summarized in Table 2 and Additional file 1: Table S3. Based on the analysis of unadjusted codominant model, the allele frequencies of rs6554809 in DNAH5, rs1438673 in WDR36-CAMK4 loci and rs7775228 in HLA region were significantly different between the case group and control group: rs6554809: C>T ($P = 0.006$), rs1438673: T>C ($P = 0.029$), rs7775228: T>C ($P = 0.001$), and they remain associated after 100,000 permutations ($P < 0.05$). The distribution of the genotypes of rs6554809, rs1438673 and rs7775228 showed significant difference between cases and controls.

Logistic regression analysis showed significantly related to AR risk compared with wild-type carriers in the codominant effect model assessed by AIC. The result showed that it was a risk factor with the SNP in rs9865818 in LPP ($P = 0.029$, OR = 1.469, 95% CI 1.041–2.074 for GG vs. AA); rs6554809 in DNAH5 ($P = 0.000$, OR = 1.597, 95% CI 1.246–2.048 for TC vs. CC); rs1438673 in WDR36-CAMK4 loci ($P = 0.037$, OR = 1.396, 95% CI 1.021–1.908 for CC vs. TT), rs7775228 in HLA region ($P = 0.000$, OR = 1.589, 95% CI 1.263–1.999 for TC vs. TT). However, it was a protective factor with the SNP in rs7203459 in CLEC16A ($P = 0.025$, OR = 0.731, 95% CI 0.555–0.962 for TC vs. TT). The detailed data was shown in Table 2. Moreover, 4 out of 5 polymorphisms' trend analysis showed positive results (rs9865818 in LPP, rs6554809 in DNAH5, rs1438673 in WDR36-CAMK4 and rs7775228 in HLA).

Table 2 Genotype frequencies of cases and controls and their associations with AR risk under co-dominant genetic model

Gene	SNP ID	Genotype	Case		Control		P (2 df) ^a	Logistic Regression		Armitage's trend test
			No.	Frequency (%)	No.	Frequency (%)		OR (95% CI)	P^b	
LPP	rs9865818	AA	325	42.71	361	47.50	0.052	1.000 (referent)		0.018
		GA	335	44.02	324	42.63		1.127 (0.903–1.407)	0.29	
		GG	101	13.27	75	9.87		1.469 (1.041–2.074)	0.029	
DNAH5	rs6554809	CC	541	71.18	593	78.03	0.006	1.000 (referent)		0.007
		TC	206	27.11	153	20.13		1.597 (1.246–2.048)	0.000	
		TT	13	1.71	14	1.84		1.235 (0.558–2.732)	0.603	
WDR36-CAMK4	rs1438673	TT	221	29.31	250	33.03	0.029	1.000 (referent)		0.014
		TC	382	50.66	393	51.92		1.045 (0.824–1.324)	0.718	
		CC	151	20.03	114	15.06		1.396 (1.021–1.908)	0.037	
HLA-DQB1-HLA-DQB2	rs7775228	TT	422	55.60	493	64.95	0.001	1.000 (referent)		0.001
		TC	275	36.23	216	28.46		1.589 (1.263–1.999)	0.000	
		CC	62	8.17	50	6.59		1.500 (0.996–2.259)	0.052	
CLEC16A	rs7203459	TT	633	83.07	604	79.58	0.177	1.000 (referent)		0.123
		TC	120	15.75	147	19.37		0.731 (0.555–0.962)	0.025	
		CC	9	1.18	8	1.05		0.962 (0.355–2.603)	0.939	

Italic values indicate significance of P value ($P < 0.05$)

^a Global P values [2 degrees of freedom (df)]: genotype frequencies in AR and control group were compared using a χ^2 test with 2 df

^b P values from unconditional logistic regression analyses, adjusted for age and gender

Under dominant model, the significant AR risks were observed in rs6554809 in DNAH5 ($P=0.000$, OR=1.567, 95% CI 1.231–1.995) and in rs7775228 ($P=0.000$, OR=1.571, 95% CI 1.267–1.948), while protective effect was found in rs7203459 in CLEC16A ($P=0.030$, OR=0.743, 95% CI 0.568–0.971). In addition, under recessive model, rs7617456 in TMEM18 was also revealed with protective effect ($P=0.040$, OR=0.628, 95% CI 0.403–0.980), and rs1438673 was evaluated with AR risks ($P=0.029$, OR=1.359, 95% CI 1.031–1.790). The detailed data was shown in Table 3 and Additional file 1: Table S4.

The functions and effects of these positive SNPs were retrieved from HaploReg v4.1 tool (<http://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) and reviewed in Table 4. The frequencies of SNPs were different among the common ethnic groups based on 1000 Genomes. Consistent with the result of this study, these SNPs were associated with IgE grass sensitization [10], self-reported allergy [12], hay fever [21] and asthma [22]. The loci mentioned above can affect the expression of genes, which are located in the histone modified regulatory region.

The above results suggested that allele “C” was regard as a risk allele in both rs7775228 and rs1438673 for AR subjects. Similarly with the result of AR risk, Under dominant model, the levels of serum total IgE significantly decreased in individuals with rs7775228_TT genotype compared to TC and CC carriers (median 49.8 KU/L vs. 66.7 KU/L, $P<0.0001$). No significant association with serum total IgE was observed under recessive model for rs7775228. The co-dominant model analysis for rs7775228 found a significant decrease of serum total IgE among TT carries (median 49.8 KU/L) compared to CC (median 67.9 KU/L, $P=0.025$) and TC carries (median 65.4 KU/L, $P=0.0004$), respectively (Fig. 1a). Under recessive model analysis for rs1438673, the levels of serum total IgE significantly increased among CC carries (median 60.5 KU/L) compared to TC and TT carries (median 53.8 KU/L) ($P=0.0097$), as well as under co-dominant model, significant elevation of serum total IgE was detected among CC carries (median 60.50 KU/L) comparing with TC carries (median 54.6 KU/L) ($P=0.0413$) (Fig. 1b). No significant associations between another AR-associated SNPs and serum

Table 3 Association analysis of SNPs under dominant and recessive genetic model

Gene	SNP ID	Dominant model			Recessive model		
		Genetic model	OR (95% CI)	P^a	Genetic model	OR (95% CI)	P^a
TMEM108	rs7617456	(GA + AA) vs. GG	0.980 (0.794–1.209)	0.849	AA vs. (GG + GA)	0.628 (0.403–0.980)	0.040
DNAH5	rs6554809	(TC + TT) vs. CC	1.567 (1.231–1.995)	0.000	TT vs. (CC + TC)	1.098 (0.498–2.420)	0.817
WDR36-CAMK4	rs1438673	(TC + CC) vs. TT	1.124 (0.897–1.409)	0.308	CC vs. (TT + TC)	1.359 (1.031–1.790)	0.029
HLA-DQB1-HLA-DQB2	rs7775228	(TC + CC) vs. TT	1.571 (1.267–1.948)	0.000	CC vs. (TT + TC)	1.276 (0.855–1.906)	0.233
CLEC16A	rs7203459	(TC + CC) vs. TT	0.743 (0.568–0.971)	0.030	CC vs. (TT + TC)	1.017 (0.377–2.748)	0.973

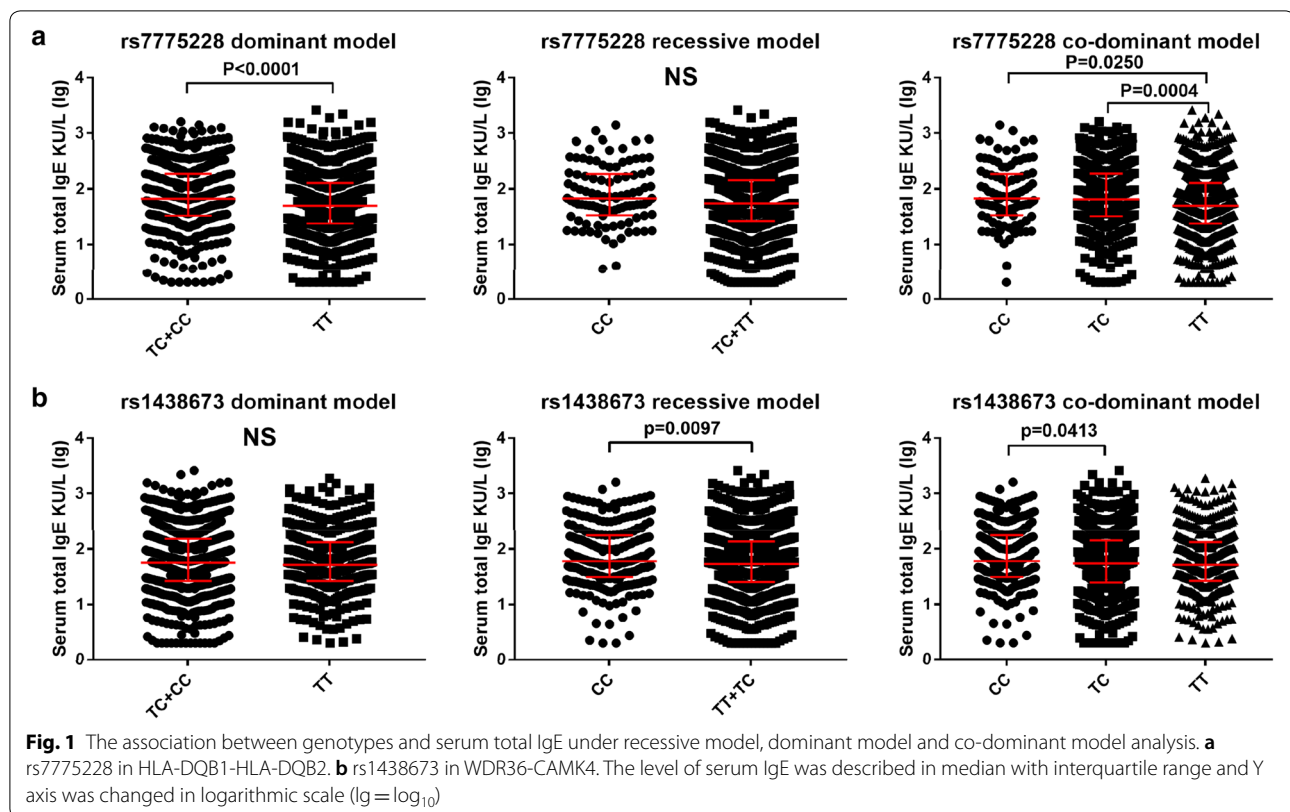
Italic values indicate significance of P value ($P < 0.05$)

^a P values from unconditional logistic regression analyses, adjusted for age and gender

Table 4 Functional annotation of positive SNPs in HaploReg v4.1 tool

SNP	Frequency in 1000 Genomes				Association phenotype with GWAS	eQTL	Histone markers
	AFR	AMR	ASN	EUR			
rs9865818	0.11	0.37	0.33	0.42	Allergic sensitization [11]	NA	H3K4me1_Enh H3K27ac_Enh
rs7617456	0.21	0.47	0.24	0.40	IgE grass sensitization [10]	NA	H3K4me3_Pro
rs6554809	0.49	0.25	0.12	0.16	IgE grass sensitization [10]	NA	H3K4me1_Enh
rs1438673	0.84	0.6	0.54	0.52	Asthma and hay fever [21] Self-reported allergy [12]	TSLP	H3K4me1_Enh
rs7775228	0.17	0.2	0.28	0.15	Asthma [22] IgE grass sensitization [10]	HLA-DPA1/HLA-DPA2/HLA-DQB1/HLA-DQB2	H3K4me1_Enh
rs7203459	0.18	0.21	0.09	0.26	Self-reported allergy [12]	LOC642755, C16orf75/SOCS1	H3K4me1_Enh H3K27ac_Enh

AFR African, AMR Amerindian, ASN Asian, EUR European, eQTL Expression quantitative trait loci, NA not available because of the lack of data



total IgE were noted under recessive, dominant and co-dominant genetic model (Additional file 1: Figure S3).

Discussion

In present study we carried out a replication study of susceptibility variants associated with AR and allergy in AR as well as healthy population [11–13, 15]. We demonstrated that variants in or near LPP (rs9865818), DNAH5 (rs6554809), WDR36-CAMK4 (rs1438673), HLA (rs7775228) and CLEC16A (rs7203459) were significantly associated AR in Han Chinese.

Actually, it is almost 4 decades since an association between genetic variants in the HLA region and specific allergen sensitization was reported [23], and various loci in the HLA region have been implicated in candidate gene studies of asthma or allergic phenotypes [24]. A recent study investigated common genetic variant associations with prevalent AR and grass sensitization using existing GWAS data in 4 large European adult cohorts for AR (3933 self-reported cases vs. 8965 control subjects) and grass sensitization (2315 cases vs. 10,032 control subjects). Three loci reached genome-wide significance for either phenotype. The HLA variant rs7775228-C was strongly associated with grass sensitization and weakly with AR ($P_{\text{grass}} = 1.6 \times 10^{-9}$; $P_{\text{AR}} = 8.0 \times 10^{-3}$) and it cis-regulates HLA-DRB4¹⁰, similar with our present findings.

DNAH5 (rs6554809, $P = 3.3 \times 10^{-6}$) was firstly detected as a candidate gene for AR and grass sensitization through a genome-wide meta-analysis by Ramasamy et al. [10] in 2011, the same locus presented positive in present study. DNAH5 mutations are a common cause of primary ciliary dyskinesia with outer dynein arm defects [25] and DNAH5 has been regarded as a force generating of respiratory cilia. Sugier et al. combined GWAS and epistasis analysis and exhibited that DNAH5 and adhesion G protein-coupled receptor V1 (ADGRV1) interactions might represent a novel mechanism underlying for ciliary function in atopy [26].

Hinds et al. [12] conducted a meta-analysis of genome-wide associations with self-reported cat, dust-mite and pollen allergies in 53,862 individuals and identified 16 shared susceptibility loci with association $P < 5 \times 10^{-8}$, including rs9860547 in LPP ($P = 1.2 \times 10^{-9}$). Bønnelykke et al. [11] performed the first large-scale GWAS of allergic sensitization in 5789 affected individuals and 10,056 controls and followed up the top SNP at each of 26 loci in 6114 affected individuals and 9920 controls. They identified 10 susceptibility loci with significant association with allergic sensitization including rs9865818 in LPP ($P = 3.4 \times 10^{-6}$). Here we also replicated rs9865818 of LPP using diagnosed AR population. Several new allergy-associated loci are in or near genes involved in T-helper cell

differentiation. The association between LPP and allergy may be mediated by an effect on the expression of BCL6 (B cell lymphoma 6), a transcription factor that represses the STAT6-mediated response to IL-4 and IL-13 and IgE class switching [27] and inhibits Th2 cell differentiation in a mouse model [28]. Concerning TSLP, a recent research suggested a role of TSLP in directly promoting T helper 2(Th2) cell effector function and support the notion of TSLP as a key driver of Th2 inflammation [29], while the crucial effects of Th2 lineage in the pathogenesis of allergy have been always highlighted with the associations identified in or near key Th2 pathways genes. Interestingly, here we couldn't successfully repeat the allergy susceptible loci in TSLP while we identified a signal (rs1438673) in 5q22.1 in WDR36-CAMK4 intergenic region, which are about 50,000 bp far away from TSLP gene. The Genotype-Tissue Expression (GTEx) data exhibit a strong association between rs1438673 genotype and TSLP expression. Likewise, another our previous study also demonstrated that the SNPs in TSLP locus ensured complete genetic coverage were not associated with AR susceptibility in Chinese subjects [30].

In this study, we found that the SNPs in rs1438673 and in rs7775228 had strongly association with serum total IgE in AR group. The variant near the HLA-DQB1 was regarded as a predictor of total serum IgE levels in multiple race-ethnic groups, which was identified by an independently meta-analysis [31]. The serum total IgE level was used to diagnosis of AR in the in vitro, and high total IgE level suggests that in vitro testing would confirm specific sensitizations in AR patients [32]. The serum total IgE might play a role as a mediator in the development of allergic diseases. The high serum total IgE concentration was associated with the risk for allergic sensitization [33]. In addition, high serum total IgE increased macrophage expression of TLR4 in induced sputum in asthmatic subjects [34]. This outcome may result from a link between innate immunity and IgE-mediated adaptive immune responses in asthma. The variation of serum total IgE also showed have an association with asthma control [35], which might also increase the risk of AR. Besides, our findings also reinforced the notion that there was a shared genetic etiology of allergic and autoimmune disease, with discovered susceptibility loci for allergy, many of which were previously associated with autoimmune disease [12]. In present study, we exhibited rs7203459 in CLEC16A which had been proven to be associated with type 1 diabetes mellitus [36–38], multiple sclerosis [39] and other autoimmune disease [40] was associated with AR (OR=0.731), indicating such risk allele for autoimmune disease seemed to be protective against allergy in Han Chinese. Comparably, previous study reported that the data in same locus in CLEC16A revealed that autoimmune

disease and allergy were associated with the same risk alleles (OR=1.07) [12]. Such inconsistent results to some degree suggested that the complex context of autoimmune diseases as well as allergy as many autoimmune diseases were associated with increased activation of Th1 responses, whereas allergy had been associated with Th2 activity.

It is striking that in the present study, variants in only 5 of 58 previously identified candidate genes demonstrated significant association with Han Chinese AR. Of these variants, rs6554809-T allele in DNAH5 was the risk factor for AR in our study, which was opposite to Ramasamy et al. [10]. These observations suggest that the genetic determination of AR as well as allergic diseases might perhaps be more complicated than what we suspected. Although AR and other allergic diseases commonly coexist and share some susceptibility genes, there appeared to be more genetic heterogeneity among patients with atopic disorders [41]. Most GWASs enrolled admixture of subjects with diverse phenotypes and it is difficult to determine disease-specific or overlapping genes with confidence. In the present study, under the premise of a limited study population, we specifically excluded subjects with comorbid asthma, eczema and other allergic disease to reduce the effect of comorbidity as potential confounding factors and mainly focused on these selected genes involved in susceptibility to pure AR. It's worthy to mention that all the AR individuals here were diagnosed AR in terms of skin test response and serum allergen specific IgE, guaranteeing the clear diagnosis of our study population. However, it is to be noted that our study showed a poor reproducibility of reported associations from 6 previous genome-wide studies in AR and allergy, and especially lack of association for 5 SNPs previously associated with asthma. In addition to the “allergic disease genes”, there are “tissue-specific genes” that contribute to the expression to a certain atopic disease [41], such as GSDMB locus, where none of association was detected in the present study, exerting greatest effect in bronchi tissues. Possibly different combinations of susceptibility genes, such as gene–gene interactions, are involved in different allergic phenotypes [1]. In addition, the nature of variant susceptibility in different populations exhibits heterogeneity. On the one hand, the levels of linkage disequilibrium exist different among distinct populations, such as the risk allele of rs6554809 in DNAH5 in Chinese population was lower than European population (0.09 vs. 0.16). On the other hand, investigation from recent association of rare and low-frequency variants with asthma suggested some loci are ethnicity specific, including associated variants in GRASP and GSDMB in patients with Latino ancestry and variants in MTHFR in patients with African ancestry [42]. Convincingly

replication in different populations would be necessary to identify some loci or genes that are more frequently involved in the pathogenesis of AR and allergy and would provide some potential targeting of preventative treatments and are therefore of great interest in the clinical perspective. For example, we reported the rs777528 (CC + CT) genotypes in HLA-DQB1-HLA-DQB2 loci presented risk role for AR and were associated with an increased total serum IgE levels, suggesting central role in allergic and immune response.

However, the findings of our study are slightly limited, particularly in view of associations in only 6 genome-wide studies due to a limited number of SNPs in two genotyping assays. Furthermore, this study is somewhat limited in the sample size and the results did not exclude the potential false positive and negative associations, although we enrolled well-diagnosed AR to avoid population stratification as well as adjustment for multiple testing involved in 47 SNPs. Further studies would also be performed in larger cohorts and assess associations in a larger number of loci for identification of susceptibility to AR.

Intriguingly, whereas, it's indubitable that the analysis driven by statistical and knowledge-based evidence represents a promising approach for identifying new genes or targets involved in complex traits and an important next step will be to more carefully characterize the extent to which individual associations lead to a global predisposition to allergy compared to effects on specific target tissues, such as skin, lung or mucosa.

Conclusions

In summary, here we replicated shared and AR-specific susceptibility loci in LPP, DNAH5, HLA, CLEC16A and WDR36-CAMK4 which were also plausible candidates in terms of their biological function in the development of AR. Further understanding the molecular mechanisms underlying these associations may provide new insights into the etiology of allergic disease in Han Chinese.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13223-020-04111-9>.

Additional file 1. Additional tables and figures.

Abbreviations

AR: Allergic rhinitis; ARIA: Allergic Rhinitis and its Impact on Asthma; CIs: Confidence intervals; Der f: Dermatophagoides farinae; Der p: Dermatophagoides pteronyssinus; GINA: Global Initiative for Asthma; GWASs: Genome-wide association studies; HDM: House dust mite; HWE: Hardy-Weinberg Equilibrium; IgE: Immunoglobulin E; ORs: Odds ratios; slgE: Specific IgE; SNPs: Single nucleotide polymorphisms.

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Authors' contributions

GY and LJ collected the data and performed statistical analyses. LJ, ZY and ZL conceived and designed the experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Beijing TongRen Hospital and all subjects provided written informed consent prior to entry in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table S1. SNPs associated with AR and allergy phenotypes in 6 genome-wide studies.

Reference	Gene	SNP_ID	Chr.Position ^a (GRCh38.p12)	Genic location	Minor/major allele	Population	Associated phenotype	Reported <i>P</i> value	Reported OR (95% CI)
Ramasamy, <i>et al.</i> (2011)	TSLP	rs1898671	chr5:111072304	Intron	T/C	European-the British	AR	5.2×10 ⁻⁶	1.15 (1.08-1.22)
	PPM1A-DHRS7	rs216518	chr14:60216712	Intergenic region	A/C	1958 Birth		1.9×10 ⁻⁶	1.21 (1.12-1.31)
	GLI3	rs4724100	chr7:42225080	Intron	C/T	Cohort; the		2.2×10 ⁻⁶	1.14 (1.08-1.21)
	ENTPD6	rs1044573	chr20:25226018	3' UTR	G/A	follow-up		9.7×10 ⁻⁷	1.15 (1.09-1.21)
	SEMA6A-TNFAIP8	rs6898653	chr5:116639960	Intergenic region	G/A	of the		1.0×10 ⁻⁶	1.23 (1.13-1.34)
	MST1L-CROCC	rs6586513	chr1:16889836	Intergenic region	A/C	European		3.9×10 ⁻⁶	1.24 (1.13-1.36)
	CLEC16A	rs887864	chr16:11065028	Intron	G/A	Community		1.1×10 ⁻⁶	1.16 (1.09-1.23)
	TMEM108	rs7617456	chr3:133009059	Intergenic region	A/G	Respiratory Health	Grass sensitization	3.3×10 ⁻⁶	1.18 (1.10-1.26)
	NOD1	rs7789045	chr7:30454406	5' near gene	A/T	Survey; the		6.2×10 ⁻⁵	1.15 (1.08-1.24)
	IL2	rs2069772	chr4:122451978	Intron	C/T	Northern		1.1×10 ⁻⁶	1.19 (1.11-1.28)
	HLA-DQB1-HLA-DQB2	rs7775228	chr6:32690302	Intergenic region	C/T	Finland Birth		1.6×10 ⁻⁹	1.33 (1.21-1.45)
	C16orf72-GRIN2A	rs631208	chr16:9305867	Intergenic region	G/A	Cohort of		2.0×10 ⁻⁶	1.18 (1.10-1.26)
	TLR1-TLR6	rs3860069	chr4:38860500	Intergenic region	C/A	1966; and		4.4×10 ⁻⁵	1.21 (1.11-1.33)
	EPS15	rs6673480	chr1:51393570	Intron	T/C	the Swiss		2.2×10 ⁻⁶	1.35 (1.19-1.52)
	DNAH5	rs6554809	chr5:13740867	Intron	T/C	Study on		3.3×10 ⁻⁶	1.29 (1.16-1.43)
	ABL2	rs1325195	chr1:179102621	3' UTR	G/A	Air		4.6×10 ⁻⁶	1.17 (1.10-1.26)
	C11orf30-LRRC32	rs2155219	chr11:76588150	Intergenic region	T/G	Pollution and Lung Disease in Adults	AR and Grass sensitization	3.8×10 ⁻⁸ for AR and 9.4×10 ⁻⁹ for Grass sensitization	1.17 (1.11-1.24) for AR and 1.22 (1.14-1.31) for grass sensitization

								grass sensitization	
	SLC25A46-TSLP	rs17513503	chr5:110810746	Intergenic region	G/C			7.4×10 ⁻⁷ for AR and 1.2×10 ⁻⁸ for grass sensitization	1.28 (1.16-1.41) for AR and 1.39 (1.24-1.56) for grass sensitization
Bønnelykke, <i>et al.</i> (2013)	C11orf30-LRRC32	rs2155219	chr11:76588150	Intergenic region	T/G	European	Allergen sensitization	1.4×10 ⁻¹⁸	1.18(1.13-1.22)
	STAT6	rs1059513	chr12:57095926	3' UTR	C/T			1.0×10 ⁻¹⁴	1.30(1.21-1.39)
	SLC25A46-TSLP	rs10056340	chr5:110854353	Intergenic region	G/T			5.2×10 ⁻¹⁴	0.83(0.78-0.87)
	HLA-DQB1-AS1	rs6906021	chr6:32658534	2KB Upstream	C/T			2.2×10 ⁻¹²	0.87(0.83-0.90)
	IL1RL1	rs3771175	chr2:102343750	3' UTR	A/G			4.9×10 ⁻¹¹	0.83(0.78-0.88)
	TLR1- TLR6	rs17616434	chr4:38811255	Intergenic region	C/T			5.2×10 ⁻¹¹	1.23(1.18-1.29)
	LPP	rs9865818	chr3:188354725	Intron	G/A			2.7×10 ⁻¹⁰	0.89(0.86-0.92)
	PVT1	rs4410871	chr8:127802783	Intron	T/C			5.4×10 ⁻¹⁰	1.14(1.09-1.19)
	ADAD1-IL2	rs17454584	chr4:122432277	Intergenic region	G/A			5.5×10 ⁻¹⁰	0.87(0.83-0.91)
	HLA-B-MICA	rs6932730	chr6:31386405	Intergenic region	C/T			4.2×10 ⁻⁸	1.14(1.09-1.20)
Hinds, <i>et al.</i> (2013)	TLR1- TLR6	rs2101521	chr4:38809930	Intergenic region	G/A	Individuals having >97 % European	Self-reported allergic sensitization- for pollen	5.3×10 ⁻²¹	1.15(1.11-1.18)
	WDR36-CAMK4	rs1438673	chr5:111131801	Intergenic region	C/T			2.3×10 ⁻²⁰	1.12(1.09-1.14)
	C11orf30-LRRC32	rs2155219	chr11:76588150	Intergenic region	G/T			1.6×10 ⁻¹⁹	1.11(1.09-1.14)

	IL1RL1-IL1RL2	rs10189629	chr2:102263004	Intergenic region	A/C	ancestry	allergy, dust	1.8×10^{-16}	1.16(1.12-1.20)
	HLA-DQB1-AS1	rs6906021	chr6:32658534	2KB Upstream	C/T	and	mite	7.1×10^{-15}	1.10(1.07-1.13)
	HLA-B-MICA	rs9266772	chr6:31384336	Intergenic region	C/T	pregnant	Allergy and	3.2×10^{-12}	1.11(1.08-1.14)
	PTGER4-DAB2	rs7720838	chr5:40486794	Intergenic region	G/T	women	cat allergy	8.2×10^{-11}	1.08(1.06-1.11)
	PLCL1	rs10497813	chr2:198049348	Intron	G/T	resident in		6.1×10^{-10}	1.08(1.05-1.10)
	LPP	rs9860547	chr3:188411191	Intron	A/G	Avon, UK		1.2×10^{-9}	1.08(1.05-1.10)
	GSDMB	rs9303280	chr17:39917778	Intron	T/C			8.9×10^{-9}	1.07(1.05-1.10)
	RANBP6-IL33	rs7032572	chr9:6172380	Intergenic region	G/A			1.7×10^{-9}	1.12(1.08-1.16)
	NFATC2	rs6021270	chr20:51524725	Intron	C/T			6.9×10^{-9}	1.16(1.11-1.23)
	SMAD3	rs17228058	chr15:67157967	Intron	G/A			1.2×10^{-8}	1.08(1.05-1.11)
	GATA3-CELF2	rs962993	chr10:9011169	Intergenic region	T/C			1.5×10^{-8}	1.07(1.05-1.10)
	ADAD1	rs17388568	chr4:122408207	Intron	A/G			3.9×10^{-8}	1.07(1.05-1.10)
	TTC6	rs1998359	chr14:37607943	Intron	G/C			4.8×10^{-8}	1.08(1.05-1.12)
	TPD52-ZBTB10	rs6473223	chr8:80355920	Intergenic region	T/C			7.7×10^{-8}	1.07(1.04-1.10)
	RNF144A-ID2	rs10174949	chr2:8302118	Intergenic region	A/G			1.0×10^{-7}	1.07(1.05-1.10)
	CLEC16A	rs7203459	chr16:11136846	Intron	C/T			2.0×10^{-7}	1.07(1.04-1.10)
	IL21R-IL4R	rs2107357	chr16:27399508	Intergenic region	A/G			3.3×10^{-7}	1.09(1.06-1.13)
	PEX14	rs2056417	chr1:10521601	Intron	A/G			3.7×10^{-7}	1.07(1.04-1.10)
	KIRREL3-ETS1	rs10893845	chr11:128316987	Intergenic region	G/T			6.4×10^{-7}	1.09(1.04-1.09)
Bunyavanich, <i>et al.</i> (2014)	TWIST1-TMEM19 6	rs7780001	chr7:19438358	Intergenic region	A/T	European	AR	2.0×10^{-8}	0.73(0.62-0.84)
	LINC00486	rs11680788	chr2:32834029	Intron	T/C	American,		3.8×10^{-8}	0.47(0.19-0.82)
	DLG1-BDH1	rs6583203	chr3:197352715	Intergenic region	T/C	Latino and		1.4×10^{-8}	1.65(1.48-1.83)
	EIF5AL1-ZCCHC2 4	rs1250761	chr10:79500204	Intergenic region	A/G	African		4.5×10^{-9}	1.80 (1.61-2.00)
	ZNF776	rs12973620	chr19:57738275	Intergenic region	C/G			5.0×10^{-8}	1.56(1.40-1.72)

Bønnelykke, <i>et al.</i> (2014)	GSDMB	rs2305480	chr17:39905943	Missense Variant	A/G	European	Early childhood		6.4×10^{-23}	1.32(1.23-1.39)
	IL33	rs928413	chr9:6213387	2KB Upstream	A/G		asthma with		8.8×10^{-13}	1.24(1.17-1.32)
	RAD50	rs6871536	chr5:132634182	Intron	C/T		severe		7.6×10^{-7}	1.17(1.10-1.25)
	IL1R1	rs1558641	chr2:102149405	Intron	A/G		exacerbations		6.9×10^{-9}	1.11(1.04-1.19)
	CDHR3	rs6967330	chr7:106018005	Missense Variant	A/G				2.7×10^{-14}	1.26(1.18-1.33)
Dizier, <i>et al.</i> (2014)	C1orf87-NFIA	rs12122228	chr1:60890848	Intergenic region	T/G	European	Asthma with	AR	2.0×10^{-6}	0.61(0.47-0.80)

a. SNP position in the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>).

Table S2. Allele frequencies and HWE testing for replicated SNPs.

Gene	SNP_ID	Minor/ major Allele	MAF ^a			<i>P</i> ^c	<i>P</i> for HWE ^d	Genotyping Rate (%) ^e	Analysis
			NCBI ^b	AR	Control				
PEX14	rs2056417	A/G	0.140	0.188	0.189	0.898	0.112	100.00	47 SNPs were analyzed in this study
C1orf87-NFIA	rs12122228	T/G	0.342	0.237	0.232	0.763	0.685	100.00	
ABL2	rs1325195	G/A	0.442	0.501	0.493	0.690	0.888	100.00	
RNF144A-ID2	rs10174949	A/G	0.275	0.237	0.263	0.094	0.413	100.00	
IL1R1	rs1558641	T/C	0.275	0.233	0.223	0.519	0.834	98.36	
IL1RL1-IL1RL2	rs10189629	A/C	0.092	0.082	0.086	0.727	0.469	100.00	
IL1RL1	rs3771175	A/T	0.117	0.081	0.091	0.360	0.319	99.93	
PLCL1	rs10497813	T/G	0.292	0.230	0.242	0.438	0.835	98.69	
TMEM108	rs7617456	A/G	0.225	0.236	0.257	0.171	0.133	99.80	
LPP	rs9865818	G/A	0.367	0.353	0.312	0.016	0.853	99.93	
	rs9860547	A/G	0.450	0.406	0.373	0.062	0.969	99.93	
DLG1-BDH1	rs6583203	T/C	0.033	0.033	0.036	0.610	0.300	100.00	
TLR1- TLR6	rs2101521	G/A	0.333	0.377	0.361	0.377	0.544	100.00	
	rs17616434	T/C	0.317	0.369	0.352	0.335	0.650	100.00	
ADAD1	rs17388568	A/G	0.100	0.142	0.130	0.322	0.472	99.93	
ADAD1-IL2	rs17454584	G/A	0.075	0.142	0.130	0.330	0.320	100.00	
IL2	rs2069772	C/T	0.100	0.144	0.129	0.236	0.445	100.00	
DNAH5	rs6554809	T/C	0.092	0.153	0.119	0.007	0.265	99.87	
PTGER4-DAB2	rs7720838	T/G	0.158	0.247	0.270	0.159	0.543	100.00	
SLC25A46-TSLP	rs10056340	G/T	0.142	0.152	0.138	0.248	0.181	100.00	
TSLP	rs1898671	T/C	0.050	0.046	0.045	0.943	0.716	100.00	
WDR36-CAMK4	rs1438673	C/T	0.458	0.454	0.410	0.016	0.044	99.28	

SEMA6A-TNFAIP8	rs6898653	G/A	0.267	0.143	0.155	0.352	0.851	99.93		
RAD50	rs6871536	C/T	0.208	0.186	0.168	0.190	0.739	99.87		
HLA-B-MICA	rs6932730	C/T	0.150	0.144	0.144	0.982	0.879	98.95		
TWIST1-TMEM196	rs7780001	T/A	0.292	0.238	0.220	0.250	0.153	99.93		
NOD1	rs7789045	T/A	0.333	0.380	0.378	0.874	0.183	99.93		
GLI3	rs4724100	T/C	0.325	0.243	0.238	0.765	0.327	100.00		
CDHR3	rs6967330	A/G	0.075	0.074	0.062	0.178	0.953	100.00		
TPD52-ZBTB10	rs6473223	C/T	0.433	0.393	0.391	0.911	0.046	99.87		
PVT1	rs4410871	T/C	0.358	0.353	0.351	0.900	0.610	99.93		
IL33	rs928413	G/A	0.050	0.065	0.080	0.119	0.557	100.00		
GATA3-CELF2	rs962993	T/C	0.175	0.091	0.078	0.178	0.768	100.00		
C11orf30-LRRC32	rs2155219	G/T	0.483	0.431	0.444	0.468	0.247	99.93		
KIRREL3-ETS1	rs10893845	G/T	0.300	0.242	0.251	0.583	0.788	98.16		
STAT6	rs1059513	C/T	0.092	0.068	0.075	0.462	0.909	99.67		
TTC6	rs1998359	G/C	0.092	0.108	0.109	0.933	0.443	100.00		
SMAD3	rs17228058	G/A	0.217	0.019	0.022	0.518	0.527	99.87		
C16orf72-GRIN2A	rs631208	G/A	0.208	0.265	0.272	0.651	0.507	100.00		
CLEC16A	rs887864	G/A	0.175	0.196	0.213	0.265	0.561	100.00		
	rs7203459	C/T	0.117	0.091	0.107	0.120	0.776	99.93		
IL21R-IL4R	rs2107357	A/G	0.225	0.285	0.274	0.503	0.103	99.80		
GSDMB	rs2305480	A/G	0.350	0.283	0.302	0.248	0.737	98.95		
ENTPD6	rs1044573	A/G	0.075	0.061	0.072	0.236	0.254	100.00		
NFATC2	rs6021270	C/T	0.050	0.060	0.052	0.315	0.968	100.00		
HLA-DQB1-HLA-DQB2	rs7775228	C/T	0.308	0.263	0.208	0.000	0.0002	99.74		
PPM1A-DHRS7	rs216518	A/C	0.067	0.037	0.041	0.563	0.0005	100.00		
HLA-B-MICA	rs9266772	C/T	0.150	0.109	0.120	0.321	< 0.0001	100.00	HWE	test

									$P < 0.0001$
HLA-DQB1-AS1	rs6906021	C/T	0.442	0.443	0.405	0.035	< 0.0001	96.91	
MST1L-CROCC	rs6586513	A/C	0.178	0.160	0.145	0.261	< 0.001	99.47	
EIF5AL1-ZCCHC24	rs1250761	A/G	0.000	0.000	0.000	-	-	-	MAF<0.01
RANBP6-IL33	rs7032572	G/A	0.183	0.004	0.004	0.996	0.913	100.00	
SLC25A46-TSLP	rs17513503	G/C	0.022	0.000	0.001	0.856	0.985	99.61	
EPS15	rs6673480	T/C	0.000	0.001	0.003	0.455	0.942	100.00	
TLR1-TLR6	rs3860069	NA	NA	NA	NA	NA	NA	NA	the genotyping assays failed for these SNPs
LINC00486	rs11680788	NA	NA	NA	NA	NA	NA	NA	
GSDMB	rs9303280	NA	NA	NA	NA	NA	NA	NA	
ZNF776	rs12973620	NA	NA	NA	NA	NA	NA	NA	

a. MAF, minor allele frequency.

b. MAF for Chinese in the NCBI dbSNPs database.

c. P value for difference in allele frequencies between AR and control group.

d. HWE, Hardy–Weinberg equilibrium in control group.

e. Genotyping Rate (%) is the percentage of successfully genotyped individuals in the current study.

f. NA, not available because of the failed to replicate in this study.

Table S3. Genotype frequencies of 47 among cases and controls and their associations with AR risk under co-dominant genetic model.

Gene	SNP ID	Genotype	Case		Control		<i>P</i> (2 df) ^a	logistic Regression		Armitage's trend test
			No.	Frequency	No.	Frequency		OR (95%CI)	<i>P</i> ^b	
PEX14	rs2056417	GG	506	66.40%	506	66.58%	0.849	1.000 (referent)	0.973	0.901
		AG	226	29.66%	220	28.95%		0.996 (0.791-1.254)		
		AA	30	3.94%	34	4.47%		0.881 (0.522-1.487)		
C1orf87-NFIA	rs12122228	GG	442	58.01%	446	58.68%	0.954	1.000 (referent)	0.811	0.761
		GT	279	36.61%	275	36.18%		1.027 (0.825-1.279)		
		TT	41	5.38%	39	5.13%		1.049 (0.652-1.686)		
ABL2	rs1325195	GG	198	25.98%	196	25.79%	0.714	1.000 (referent)	0.715	0.693
		GA	365	47.90%	378	49.74%		0.954 (0.741-1.228)		
		AA	199	26.12%	186	24.47%		1.094 (0.818-1.462)		
RNF144A-ID2	rs10174949	GG	450	59.06%	417	54.87%	0.244	1.000 (referent)	0.208	0.100
		GA	263	34.51%	286	37.63%		0.867 (0.695-1.082)		
		AA	49	6.43%	57	7.50%		0.817 (0.538-1.241)		
IL1R1	rs1558641	CC	431	57.31%	449	60.27%	0.229	1.000 (referent)	0.134	0.508
		CT	292	38.83%	260	34.90%		1.184 (0.949-1.476)		
		TT	29	3.86%	36	4.83%		0.784 (0.465-1.322)		
IL1RL1-IL1RL2	rs10189629	CC	645	84.65%	634	83.42%	0.34	1.000 (referent)	0.236	0.729
		CA	109	14.30%	122	16.05%		0.839 (0.628-1.121)		
		AA	8	1.05%	4	0.53%		1.446 (0.423-4.936)		
IL1RL1	rs3771175	TT	643	84.49%	626	82.37%	0.374	1.000 (referent)	0.164	0.357
		AT	112	14.72%	130	17.11%		0.817 (0.615-1.086)		
		AA	6	0.79%	4	0.53%		1.077 (0.296-3.921)		
PLCL1	rs10497813	GG	454	60.53%	431	57.31%	0.245	1.000 (referent)	0.092	0.445
		GT	247	32.93%	278	36.97%		0.825 (0.659-1.032)		

TMEM108	rs7617456	TT	49	6.53%	43	5.72%		1.057 (0.678-1.648)	0.806	
		GG	438	57.63%	427	56.26%		1.000 (referent)		
		GA	286	37.63%	274	36.10%	0.063	1.051 (0.843-1.310)	0.658	0.173
LPP	rs9865818	AA	36	4.74%	58	7.64%		0.640 (0.407-1.007)	0.053	
		AA	325	42.71%	361	47.50%		1.000 (referent)		
		GA	335	44.02%	324	42.63%	0.052	1.127 (0.903-1.407)	0.29	0.018
LPP	rs9860547	GG	101	13.27%	75	9.87%		1.469 (1.041-2.074)	0.029	
		GG	274	36.01%	299	39.34%		1.000 (referent)		
		GA	356	46.78%	355	46.71%	0.155	1.080 (0.860-1.357)	0.507	0.064
DLG1-BDH1	rs6583203	AA	131	17.21%	106	13.95%		1.330 (0.971-1.823)	0.076	
		TT	712	93.44%	705	92.76%		1.000 (referent)		
		CT	50	6.56%	55	7.24%	0.603	0.845 (0.560-1.276)	0.424	0.603
TLR1-TLR6	rs2101521	CC	0	0.00%	0	0.00%		NA ^c	NA ^c	
		AA	299	39.24%	314	41.32%		1.000 (referent)		
		AG	352	46.19%	343	45.13%	0.677	1.079 (0.862-1.351)	0.507	0.381
TLR1-TLR6	rs17616434	GG	111	14.57%	103	13.55%		1.165 (0.844-1.608)	0.354	
		CC	306	40.16%	322	42.37%		1.000 (referent)		
		TC	350	45.93%	341	44.87%	0.631	1.077 (0.861-1.347)	0.517	0.338
ADAD1	rs17388568	TT	106	13.91%	97	12.76%		1.174 (0.846-1.630)	0.336	
		GG	561	73.72%	578	76.05%		1.000 (referent)		
		GA	184	24.18%	167	21.97%	0.574	1.138 (0.888-1.457)	0.307	0.326
ADAD1-IL2	rs17454584	AA	16	2.10%	15	1.97%		1.079 (0.511-2.279)	0.841	
		AA	562	73.75%	578	76.05%		1.000 (referent)		
		GA	183	24.02%	166	21.84%	0.583	1.135 (0.886-1.455)	0.316	0.336
IL2	rs2069772	GG	17	2.23%	16	2.11%		1.092 (0.529-2.254)	0.811	
		TT	560	73.49%	579	76.18%	0.48	1.000 (referent)		0.240

DNAH5	rs6554809	CT	185	24.28%	166	21.84%		1.161 (0.906-1.487)	0.238	
		CC	17	2.23%	15	1.97%		1.186 (0.567-2.480)	0.65	
		CC	541	71.18%	593	78.03%		1.000 (referent)		
		TC	206	27.11%	153	20.13%	0.006	1.597 (1.246-2.048)	0.000	0.007
		TT	13	1.71%	14	1.84%		1.235 (0.558-2.732)	0.603	
PTGER4-DAB2	rs7720838	GG	428	56.17%	402	52.89%		1.000 (referent)		
		GT	291	38.19%	306	40.26%	0.36	0.957 (0.770-1.191)	0.695	0.154
		TT	43	5.64%	52	6.84%		0.837 (0.538-1.303)	0.431	
SLC25A46-TSLP	rs10056340	TT	545	71.52%	561	73.82%		1.000 (referent)		
		GT	202	26.51%	189	24.87%	0.436	1.093 (0.861-1.387)	0.466	0.239
		GG	15	1.97%	10	1.32%		1.293 (0.560-2.985)	0.547	
TSLP	rs1898671	CC	695	91.21%	693	91.18%		1.000 (referent)		
		TC	64	8.40%	65	8.55%	0.901	0.975 (0.671-1.417)	0.896	0.944
		TT	3	0.39%	2	0.26%		1.312 (0.216-7.953)	0.768	
WDR36-CAMK4	rs1438673	TT	221	29.31%	250	33.03%		1.000 (referent)		
		TC	382	50.66%	393	51.92%	0.029	1.045 (0.824-1.324)	0.718	0.014
		CC	151	20.03%	114	15.06%		1.396 (1.021-1.908)	0.037	
SEMA6A-TNFAIP8	rs6898653	AA	565	74.24%	543	71.45%		1.000 (referent)		
		GA	174	22.86%	198	26.05%	0.332	0.840 (0.658-1.071)	0.16	0.361
		GG	22	2.89%	19	2.50%		1.098 (0.578-2.084)	0.776	
RAD50	rs6871536	TT	504	66.14%	524	69.13%		1.000 (referent)		
		CT	233	30.58%	214	28.23%	0.419	1.312 (0.706-2.438)	0.39	0.187
		CC	25	3.28%	20	2.64%		1.132 (0.899-1.425)	0.291	
PPM1A-DHRS7	rs216518	CC	706	92.65%	703	92.50%		1.000 (referent)		
		CA	56	7.35%	52	6.84%	0.076	1.063 (0.706-1.600)	0.769	0.572
		AA	0	0	5	0.66%		NA ^c	NA ^c	

HLA-B-MICA	rs6932730	TT	552	73.21%	551	73.27%		1.000 (referent)		
		TC	187	24.80%	186	24.73%	1	1.020 (0.799-1.302)	0.872	0.982
		CC	15	1.99%	15	1.99%		0.833 (0.396-1.752)	0.63	
HLA-DQB1-HLA-DQB2	rs7775228	TT	422	55.60%	493	64.95%		1.000 (referent)		
		TC	275	36.23%	216	28.46%	0.001	1.589 (1.263-1.999)	0.000	0.001
		CC	62	8.17%	50	6.59%		1.500 (0.996-2.259)	0.052	
TWIST1-TMEM196	rs7780001	AA	443	58.14%	455	59.95%		1.000 (referent)		
		TA	276	36.22%	274	36.10%	0.29	0.997 (0.800-1.242)	0.978	0.245
		TT	43	5.64%	30	3.95%		1.575 (0.952-2.606)	0.077	
NOD1	rs7789045	AA	294	38.63%	303	39.87%		1.000 (referent)		
		TA	355	46.65%	340	44.74%	0.753	1.063 (0.847-1.333)	0.598	0.876
		TT	112	14.72%	117	15.39%		1.049 (0.765-1.438)	0.768	
GLI3	rs4724100	CC	438	57.48%	446	58.68%		1.000 (referent)		
		CT	278	36.48%	266	35.00%	0.828	1.060 (0.850-1.323)	0.603	0.768
		TT	46	6.04%	48	6.32%		0.952 (0.613-1.478)	0.827	
CDHR3	rs6967330	GG	653	85.70%	669	88.03%		1.000 (referent)		
		AG	105	13.78%	88	11.58%	0.4	1.210 (0.884-1.657)	0.235	0.177
		AA	4	0.52%	3	0.39%		1.525 (0.310-7.510)	0.604	
TPD52-ZBTB10	rs6473223	TT	285	37.50%	269	35.39%		1.000 (referent)		
		CT	353	46.45%	388	51.05%	0.156	0.861 (0.685-1.081)	0.198	0.910
		CC	122	16.05%	103	13.55%		1.077 (0.781-1.484)	0.653	
PVT1	rs4410871	CC	310	40.74%	323	42.50%		1.000 (referent)		
		TC	364	47.83%	340	44.74%	0.443	1.142 (0.914-1.426)	0.243	0.900
		TT	87	11.43%	97	12.76%		0.927 (0.659-1.304)	0.665	
IL33	rs928413	AA	665	87.27%	645	84.87%		1.000 (referent)		
		GA	95	12.47%	109	14.34%	0.196	0.875 (0.644-1.187)	0.39	0.119

		GG	2	0.26%	6	0.79%		0.421 (0.082-2.163)	0.3	
GATA3-CELF2	rs962993	CC	629	82.55%	646	85.00%		1.000 (referent)		
		CT	127	16.67%	110	14.47%	0.398	1.160 (0.870-1.546)	0.313	0.176
		TT	6	0.79%	4	0.53%		2.027 (0.523-7.861)	0.307	
C11orf30-LRRC32	rs2155219	TT	234	30.75%	227	29.87%		1.000 (referent)		
		GT	398	52.30%	391	51.45%	0.673	0.977 (0.770-1.240)	0.849	0.455
		GG	129	16.95%	142	18.68%		0.878 (0.644-1.197)	0.411	
KIRREL3-ETS1	rs10893845	TT	439	58.38%	418	56.33%		1.000 (referent)		
		GT	262	34.84%	276	37.20%	0.637	0.910 (0.728-1.138)	0.408	0.589
		GG	51	6.78%	48	6.47%		1.052 (0.682-1.623)	0.819	
STAT6	rs1059513	TT	660	86.84%	648	85.60%		1.000 (referent)		
		CT	97	12.76%	105	13.87%	0.754	0.902 (0.663-1.227)	0.511	0.460
		CC	3	0.39%	4	0.53%		0.491 (0.106-2.267)	0.362	
TTC6	rs1998359	CC	612	80.31%	606	79.74%		1.000 (referent)		
		CG	136	17.85%	143	18.82%	0.755	0.953 (0.728-1.248)	0.727	0.935
		GG	14	1.84%	11	1.45%		1.228 (0.538-2.801)	0.626	
SMAD3	rs17228058	AA	732	96.19%	725	95.52%		1.000 (referent)		
		GA	29	3.81%	34	4.48%	0.513	0.776 (0.461-1.307)	0.34	0.513
		GG	0	0.00%	0	0.00%		NA ^c	NA ^c	
C16orf72-GRIN2A	rs631208	AA	411	53.94%	406	53.42%		1.000 (referent)		
		GA	298	39.11%	294	38.68%	0.783	1.011 (0.812-1.258)	0.925	0.652
		GG	53	6.96%	60	7.89%		0.870 (0.578-1.308)	0.502	
CLEC16A	rs887864	AA	498	65.35%	474	62.37%		1.000 (referent)		
		GA	229	30.05%	249	32.76%	0.477	0.874 (0.697-1.096)	0.245	0.273
		GG	35	4.59%	37	4.87%		0.775 (0.471-1.276)	0.317	
CLEC16A	rs7203459	TT	633	83.07%	604	79.58%	0.177	1.000 (referent)		0.123

IL21R -IL4R	rs2107357	TC	120	15.75%	147	19.37%	0.26	0.731 (0.555-0.962)	0.025	0.499
		CC	9	1.18%	8	1.05%		0.962 (0.355-2.603)	0.939	
		GG	391	51.52%	392	51.58%		1.000 (referent)		
		AG	304	40.05%	320	42.11%		0.920 (0.740-1.144)	0.452	
		AA	64	8.43%	48	6.32%		1.191 (0.787-1.804)	0.408	
GSDMB	rs2305480	GG	388	51.60%	365	48.41%	0.464	1.000 (referent)		0.248
		GA	302	40.16%	322	42.71%		0.918 (0.737-1.143)	0.444	
		AA	62	8.24%	67	8.89%		0.884 (0.600-1.302)	0.531	
ENTPD6	rs1044573	GG	674	88.45%	657	86.45%	0.498	1.000 (referent)		0.247
		GA	83	10.89%	97	12.76%		0.795 (0.576-1.096)	0.161	
		AA	5	0.66%	6	0.79%		0.768 (0.226-2.613)	0.673	
NFATC2	rs6021270	TT	674	88.45%	683	89.87%	0.54	1.000 (referent)		0.318
		CT	84	11.02%	75	9.87%		1.129 (0.803-1.586)	0.485	
		CC	4	0.52%	2	0.26%		2.160 (0.374-12.472)	0.389	

a. Global *P* values [2 degrees of freedom (df)]: genotype frequencies in AR and control group were compared using a χ^2 test with 2 df.

b. *P* values from unconditional logistic regression analyses, adjusted for age and gender.

c. NA, not available because of the rarity of genotype.

Table S4. Association analysis of 47 SNPs under dominant and recessive genetic model.

Gene	SNP ID	Genetic model	Case	Control	logistic Regression	
					OR (95%CI)	P ^a
PEX14	rs2056417	(AG+AA) vs.GG	256/506	254/506	0.981 (0.787-1.222)	0.861
		AAvs.(GG+AG)	30/732	34/726	0.882 (0.526-1.478)	0.633
C1orf87-NFIA	rs12122228	(GT+TT) vs.GG	320/442	314/446	1.030 (0.834-1.271)	0.784
		TTvs.(GG+GT)	41/721	39/721	1.037 (0.650-1.655)	0.878
ABL2	rs1325195	(GA+AA) vs.GG	564/198	564/196	0.999 (0.788-1.267)	0.996
		AAvs.(GG+GA)	199/563	186/574	1.127 (0.887-1.432)	0.326
RNF144A-ID2	rs10174949	(GA+AA) vs.GG	312/450	343/417	0.860 (0.697-1.061)	0.158
		AAvs.(GG+GA)	49/713	57/703	0.865 (0.575-1.300)	0.485
IL1R1	rs1558641	(CT+TT) vs.CC	321/431	296/449	1.133 (0.916-1.403)	0.250
		TTvs.(CC+CT)	29/723	36/709	0.735 (0.439-1.230)	0.240
IL1RL1-IL1RL2	rs10189629	(CA+AA) vs.CC	117/645	126/634	0.861 (0.649-1.144)	0.302
		AAvs.(CC+CA)	8/754	4/756	1.485 (0.435-5.065)	0.528
IL1RL1	rs3771175	(AT+AA) vs.TT	118/643	134/626	0.826 (0.624-1.092)	0.180
		AAvs.(TT+AT)	6/755	4/756	1.111 (0.306-4.031)	0.873
PLCL1	rs10497813	(GT+TT) vs.GG	296/454	321/431	0.858 (0.693-1.061)	0.158
		TTvs.(GG+GT)	49/701	43/709	1.129 (0.731-1.744)	0.586
TMEM108	rs7617456	(GA+AA) vs.GG	322/438	332/427	0.980 (0.794-1.209)	0.849
		AAvs.(GG+GA)	36/724	58/701	0.628 (0.403-0.980)	0.040
LPP	rs9865818	(GA+GG) vs.AA	436/325	399/361	1.192 (0.967-1.469)	0.100
		GGvs.(AA+GA)	101/660	75/685	1.385 (0.999-1.922)	0.051
LPP	rs9860547	(GA+AA) vs.GG	487/274	461/299	1.138 (0.918-1.410)	0.239
		AAvs.(GG+GA)	131/630	106/654	1.275 (0.956-1.701)	0.099
DLG1-BDH1	rs6583203	(CT+CC) vs.TT	50/712	55/705	0.845 (0.560-1.277)	0.425

		CCvs.(TT+CT)	0/762	0/760	NA ^b	NA ^b
TLR1-TLR6	rs2101521	(AG+GG) vs.AA	463/299	446/314	1.099 (0.889-1.358)	0.384
		GGvs.(AA+AG)	111/651	103/657	1.118 (0.829-1.509)	0.464
TLR1-TLR6	rs17616434	(TC+TT) vs.CC	456/306	438/322	1.098 (0.889-1.357)	0.384
		TTvs.(CC+TC)	106/656	97/663	1.130 (0.832-1.534)	0.435
ADAD1	rs17388568	(GA+AA) vs.GG	200/561	182/578	1.133 (0.891-1.440)	0.307
		AAvs.(GG+GA)	16/745	15/745	1.048 (0.497-2.207)	0.902
ADAD1-IL2	rs17454584	(GA+GG) vs.AA	200/562	182/578	1.132 (0.890-1.439)	0.312
		GGvs.(AA+GA)	17/745	16/744	1.061 (0.516-2.184)	0.872
IL2	rs2069772	(CT+CC) vs.TT	202/560	181/579	1.163 (0.915-1.478)	0.217
		CCvs.(TT+CT)	17/745	15/745	1.146 (0.550-2.390)	0.716
DNAH5	rs6554809	(TC+TT) vs.CC	219/541	167/593	1.567 (1.231-1.995)	0.000
		TTvs.(CC+TC)	13/747	14/746	1.098 (0.498-2.420)	0.817
PTGER4-DAB2	rs7720838	(GT+TT) vs.GG	334/428	358/402	0.940 (0.763-1.159)	0.563
		TTvs.(GG+GT)	43/719	52/708	0.853 (0.553-1.314)	0.470
TMEM232-TSLP	rs10056340	(GT+GG) vs.TT	217/545	199/561	1.103 (0.874-1.393)	0.410
		GGvs.(TT+GT)	15/747	10/750	1.265 (0.550-2.912)	0.580
TSLP	rs1898671	(TC+TT) vs.CC	67/695	67/693	0.987 (0.684-1.424)	0.943
		TTvs.(CC+TC)	3/759	2/758	1.310 (0.216-7.931)	0.769
WDR36-CAMK4	rs1438673	(TC+CC) vs.TT	533/221	507/250	1.124 (0.897-1.409)	0.308
		CCvs.(TT+TC)	151/603	114/643	1.359 (1.031-1.790)	0.029
SEMA6A-TNFAIP8	rs6898653	(GA+GG) vs.AA	196/565	217/543	0.863 (0.683-1.091)	0.217
		GGvs.(AA+GA)	22/739	19/741	1.147 (0.606-2.170)	0.674
RAD50	rs6871536	(CT+CC) vs.TT	258/504	234/524	1.147 (0.919-1.434)	0.226
		CCvs.(TT+CT)	25/737	20/738	1.263 (0.683-2.338)	0.457
PPM1A-DHRS7	rs216518	(CA+AA) vs.CC	56/706	57/703	0.987 (0.661-1.474)	0.950

		AAvs.(CC+CA)	0/762	5/755	NA ^b	NA ^b
HLA-B-MICA	rs6932730	(TC+CC) vs.TT	202/552	201/551	1.005 (0.793-1.273)	0.970
		CCvs.(TT+TC)	15/739	15/737	0.829 (0.395-1.738)	0.619
HLA-DQB1-HLA-DQB2	rs7775228	(TC+CC) vs.TT	337/422	266/493	1.571 (1.267-1.948)	0.000
		CCvs.(TT+TC)	62/697	50/709	1.276 (0.855-1.906)	0.233
TWIST1-TMEM196	rs7780001	(TA+TT) vs.AA	319/443	304/455	1.050 (0.850-1.298)	0.648
		TTvs.(AA+TA)	43/719	30/729	1.577 (0.960-2.590)	0.072
NOD1	rs7789045	(TA+TT) vs.AA	467/294	457/303	1.059 (0.856-1.311)	0.598
		TTvs.(AA+TA)	112/649	117/643	1.015 (0.758-1.358)	0.922
GLI3	rs4724100	(CT+TT) vs.CC	324/438	314/446	1.043 (0.845-1.288)	0.695
		TTvs.(CC+CT)	46/716	48/712	0.930 (0.604-1.432)	0.743
CDHR3	rs6967330	(AG+AA) vs.GG	109/653	91/669	1.220 (0.895-1.662)	0.209
		AAvs.(GG+AG)	4/758	3/757	1.487 (0.302-7.322)	0.626
TPD52-ZBTB10	rs6473223	(CT+CC) vs.TT	475/285	491/269	0.907 (0.730-1.126)	0.375
		CCvs.(TT+CT)	122/638	103/657	1.173 (0.875-1.572)	0.287
PVT1	rs4410871	(TC+TT) vs.CC	451/310	437/323	1.094 (0.886-1.351)	0.405
		TTvs.(CC+TC)	87/674	97/663	0.866 (0.629-1.193)	0.380
IL33	rs928413	(GA+GG) vs.AA	97/665	115/645	0.854 (0.632-1.153)	0.302
		GGvs.(AA+GA)	2/760	6/754	0.428 (0.084-2.195)	0.309
GATA3-CELF2	rs962993	(CT+TT) vs.CC	133/629	114/646	1.185 (0.893-1.572)	0.239
		TTvs.(CC+CT)	6/756	4/756	1.970 (0.510-7.607)	0.325
C11orf30-LRRC32	rs2155219	(GT+GG) vs.TT	527/234	533/227	0.951 (0.758-1.192)	0.660
		GGvs.(TT+GT)	129/632	142/618	0.891 (0.679-1.169)	0.403
KIRREL3-ETS1	rs10893845	(GT+GG) vs.TT	313/439	324/418	0.931 (0.753-1.151)	0.510
		GGvs.(TT+GT)	51/701	48/694	1.089 (0.712-1.666)	0.694
STAT6	rs1059513	(CT+CC) vs.TT	100/660	109/648	0.883 (0.652-1.196)	0.422

		CCvs.(TT+CT)	3/757	4/753	0.498 (0.108-2.298)	0.371
TTC6	rs1998359	(CG+GG) vs.CC	150/612	154/606	0.973 (0.751-1.262)	0.839
		GGvs.(CC+CG)	14/748	11/749	1.240 (0.545-2.823)	0.608
SMAD3	rs17228058	(GA+GG) vs.AA	29/732	34/725	0.776 (0.461-1.307)	0.340
		GGvs.(AA+GA)	0/761	0/759	NA ^b	NA ^b
C16orf72-GRIN2A	rs631208	(GA+GG) vs.AA	351/411	354/406	0.987 (0.801-1.215)	0.899
		GGvs.(AA+GA)	53/709	60/700	0.866 (0.582-1.288)	0.478
CLEC16A	rs887864	(GA+GG) vs.AA	264/498	286/474	0.861 (0.693-1.069)	0.174
		GGvs.(AA+GA)	35/727	37/723	0.811 (0.495-1.326)	0.403
CLEC16A	rs7203459	(TC+CC) vs.TT	129/633	155/604	0.743 (0.568-0.971)	0.030
		CCvs.(TT+TC)	9/753	8/751	1.017 (0.377-2.748)	0.973
IL21R -IL4R	rs2107357	(AG+AA) vs.GG	368/391	368/392	0.956 (0.776-1.178)	0.674
		AAvs.(GG+AG)	64/695	48/712	1.236 (0.826-1.850)	0.302
GSDMB	rs2305480	(GA+AA) vs.GG	364/388	389/365	0.912 (0.740-1.124)	0.387
		AAvs.(GG+GA)	62/690	67/687	0.919 (0.632-1.336)	0.657
ENTPD6	rs1044573	(GA+AA) vs.GG	88/674	103/657	0.793 (0.580-1.085)	0.147
		AAvs.(GG+GA)	5/757	6/754	0.791 (0.233-2.685)	0.706
NFATC2	rs6021270	(CT+CC) vs.TT	88/674	77/683	1.155 (0.827-1.614)	0.398
		CCvs.(TT+CT)	4/758	2/758	2.134 (0.370-12.320)	0.397

a. *P* values from unconditional logistic regression analyses, adjusted for age and gender.

b. NA, not available because of the rarity of genotype.

Figure S1: Meta-analysis results based on multiple gene lists by Metascape. The results of enrichment network visualized from the three gene lists (Bønnelykke, *et al.* (2013), Hinds, *et al.* (2013) and Ramasamy *et al.* (2011)). The associations within each study are represented as pie charts in the nodes and cluster labels were added manually. The enrichment network shows that cytokine production, regulation of cytokine secretion and positive regulation of interleukin-6 production are generally shared among all three lists. Th17 cell differentiation is shared between the Bønnelykke and Hinds lists.

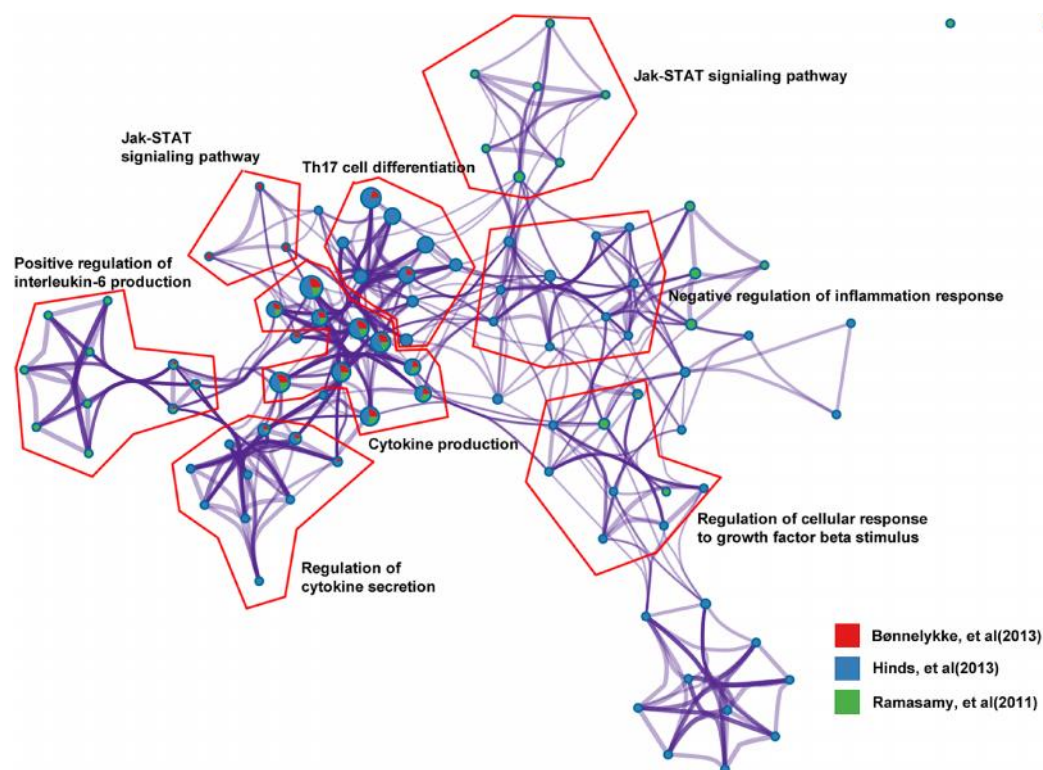


Figure S2.The flow diagram of recruitment subjects in AR group and control group

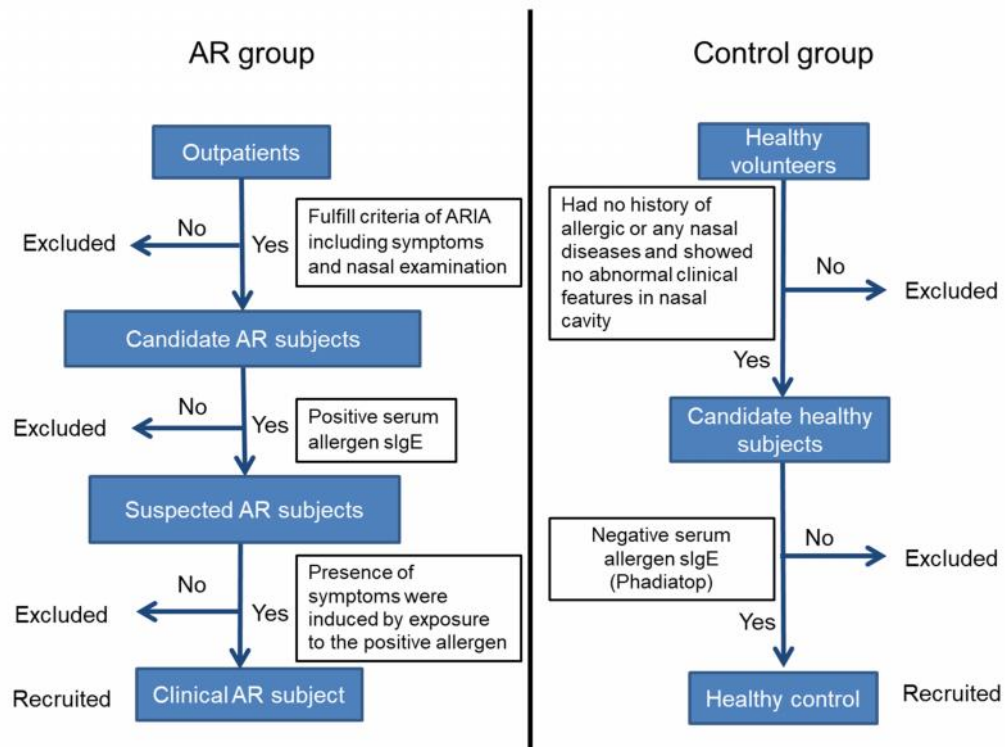


Figure S3. The association between genotypes and serum total IgE under recessive model, dominant model and codominant model analysis. (A) rs7617456 in TMEM108 gene. (B) rs6554809 in DNAH5 gene. (C) rs9865818 in LPP gene. (D) rs7203459 in CLEC16A gene. The level of serum IgE was described in median with interquartile range and Y axis was changed in logarithmic scale ($\lg = \lg_{10}$).

