

The association between sixteen genome-wide association studies-related allergic diseases loci and childhood allergic rhinitis in a Chinese Han population

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ABSTRACT

Background: Allergic rhinitis (AR) is one of the most common respiratory diseases in children. It is caused by a combination of genetic and environmental factors. Moderate-to-severe AR decreases the quality of life and social performance in children.

Objective: To investigate whether polymorphisms in previously published genome-wide association studies (GWAS) allergic disease loci are associated with childhood AR and the severity of AR symptoms in a Chinese Han population.

Methods: A case-control study was conducted in 503 pediatric patients with AR and 393 control Chinese school-aged subjects. AR severity was classified as mild or moderate-to-severe according to the AR and its Impact on Asthma (ARIA) guidelines. Overall, 16 tagged single-nucleotide polymorphisms (tSNPs) of published GWAS associations with allergic diseases were selected. All subjects were genotyped and analyzed for the 16 selected tSNPs using the improved multiplex ligation detection reaction (iMLDR) technique.

Results: Both rs7130588_AG and rs7927894_CT genotypes in *EMSY* region were associated with a significantly increased risk of AR (1.75-fold and 1.50-fold) compared to the AA and CC genotypes, respectively, specific to moderate-to-severe AR. The difference of rs7130588 genotypes in cases vs. controls was still statistically significant under the additive model after multiple test correction to adjust the false discovery rate (FDR) with an adjusted odds ratio of 1.818 and 95% confidence interval (CI) of 1.240–2.664 ($P_{\text{FDR}} = 0.0349$). The rs7130588_G allele was only associated with a high risk of moderate-to-severe AR (1.85-fold, $P_{\text{age and gender-adjustment}} = 0.003$). The rs2227284_TG genotype at the *IL4* locus was significantly associated with a 0.65-fold decreased risk of AR compared to the TT genotype. The protective effect of the rs2227284_G allele was also found in different severity of AR. Haplotype analysis showed a significantly increased AR risk associated with the haplotype G-T-T (rs7130588-rs2155219-rs7927894) and a protective effect with the haplotype C-G-C (rs2243250-rs2227284-s2243290).

Conclusions: The loci in *EMSY* and *IL4* can be considered as a hereditary marker for childhood AR. The rs7130588_G allele seems to predispose only to moderate-to-severe AR, though other mechanisms are also likely to be involved.

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1. Introduction

The prevalence of allergic diseases, including allergic rhinitis (AR), in children, appears to have increased since industrial revolution commenced. AR, a chronic inflammatory disease of the upper airway, is not only one of the most common chronic diseases in children, but also a global health problem [1]. The reported prevalence of AR varies widely, ranging from 7.83 to 48% in China, resulted from sampling strategy, the definition of AR, the ages of the children and the study results across cities with the different gross domestic product and health-system coverage [2–4]. In 2015, Li et al. [5] reported that the prevalence of AR was 12.9% of school-aged children in Shanghai, China. Besides presenting recognized common symptoms of AR such as sneezing, rhinorrhea, nasal obstruction, and compromised respiratory function, children suffering from AR also have additional impairments such as decreased school performance, sleep disturbance, and emotional and other psychosocial problems [6]. The severity of AR can therefore be classified as “mild” or “moderate-to-severe” according to the World Health Organization (WHO) guidelines entitled Allergic Rhinitis and its Impact on Asthma (ARIA) [1].

AR is typically a multifactorial disease caused by the interplay of genetic and environmental factors. Genetic variations are components of genetic factors. Genome-wide association studies (GWAS) have revealed allergic disease susceptibility loci in an unbiased and hypothesis-free manner. Many candidate genes in susceptibility loci suggest roles for innate immunity and immunoregulation including type 2 T helper (TH2) cell differentiation and effectors' functions, epithelial barrier functions, *IL1* family signaling, and regulatory T cells in the pathogenesis of allergic diseases. The first GWAS on allergic sensitization identified putative susceptibility loci including 11q13.5 near *EMSY* (previously known as *C11orf30*) and the HLA region at 6p21.32 in individuals of European origins with AR only sensitization to a single allergen (grass), [7] which was previously associated with atopic dermatitis (AD) [8] and asthma [9]. Polymorphisms in *IL-18* and *IL18RAP* in the *IL-1* family signaling were reported to be associated with AD in a Japanese population [10]. The association of *IL-13* SNP rs20541 with AR was found in an Asian population of ethnic Chinese in Singapore [11]. Polymorphisms in *IL-4* have been associated with increased total serum IgE levels [12], and asthma [13] in some populations but not associated with AR in the Asian populations [14]. Rs2897422 in *KIF3A*

encoding a component of the kinesin complex involved in the assembly of cilia, met genome-wide significance in AD cases and controls of European descent [15]. Independent replication of genotype-phenotype associations in distinct populations is generally thought to provide the most convincing evidence for the identification of a true disease susceptibility gene [16].

In view of the existing evidence, we conducted a study to test whether genetic loci associated with allergic diseases including (*EMSY*) and leucine rich repeat containing 32 (*LRRC32*) at 11q13.5, the HLA region at 6p21.32, *IL18R1* and *IL18RAP* at 2q12.1, and *IL13*, *IL4*, and *KIF3A* at 5q31.1 [11] are associated with AR risk and the severity of AR in a Chinese Han population. Confirming the association will strengthen the evidence for a causal relationship and elucidate the common genetic basis of AR and other allergic diseases.

2. Methods

2.1. Study subjects

We used a population-based case-control association study design to assess the risk of AR in Chinese children. Five hundred and three patients suffering from AR were recruited from the outpatient clinic of Otolaryngology Department at Shanghai Children's Medical Center from January 2015 to December 2015. Their ages ranged from 4 to 14 years. Age and gender matched controls were healthy children undergoing regular physical examination in our hospital. All subjects were ethnic Han Chinese, who had lived in the Shanghai region, China, since birth. The study approved by the ethics committee of Shanghai Children's Medical Center, and written informed consent was obtained from the parents or legal guardians. Of them, 156 patients with AR and 173 matched controls were enrolled in the preliminary study.

2.2. Clinical evaluation

We clinically diagnosed 503 patients with AR according to ARIA 2008 guidelines [1], with the recurrence of symptoms over a year; i.e., two or more persistent symptoms of water-like tears, nasal itching, congestion, or sneezing lasting for more than 1 h every day. One or more serum IgE species (SIgE) were found positive (≥ 0.35 kU/ml) by Western blotting using AllergyScreenTM human serum specific IgE allergen

Table 1
Clinical and demographic characteristics of the study subjects.

Demographic index	AR						Control n = 393
	Mild n = 254	p^a	Moderate-to-severe n = 249	p^b	Total n = 503	p^c	
<i>Gender, n (%)</i>							
Male	164(64.8)	0.100	147(59.0)	0.806	311(61.8)	0.271	228(58.0)
Female	90(35.4)		102(41.0)		192(38.1)		165(41.9)
<i>Age(years), SD</i>	6.1(2.6)	0.114	5.82(2.9)	0.569	5.9(2.7)	0.156	5.61(1.8)
<i>Weight(kg), SD</i>	25.1(9.2)	0.100	24.2(11.2)	0.447	24.7(9.6)	0.113	23.1(7.5)
<i>Height(cm), SD</i>	120.3(19.1)	0.224	118.9(18.6)	0.522	119.6(18.8)	0.205	117.1(12.6)
<i>Allergen category, n (%)</i>							
House dust mite	180(70.87)		179(71.89)		359(71.37)		n
Cat/dog hair	97(38.19)		80(31.50)		177(35.19)		n
Molds	61(24.02)		47(18.88)		108(21.47)		n
Seasonal grass/tree pollens	25(9.84)		22(8.84)		47(9.34)		n
Cockroaches	17(6.69)		19(7.63)		36(7.16)		n
ragweed	16(6.30)		17(6.82)		31(6.16)		n
Artemisia argyi	14(5.51)		13(5.22)		27(5.37)		n
<i>Penicillium notatum</i>	14(5.51)		16(6.42)		30(5.96)		n
<i>Aspergillus fumigatus</i>	12(4.72)		11(4.41)		23(4.57)		n

^a P value for the comparison between mild and controls calculated by χ^2 test for categorical variables or t -test for continuous variables.

^b P value for the comparison between moderate/severe AR and controls calculated by χ^2 test for categorical variables or t -test for continuous variables.

^c P value for the comparison between AR and controls calculated by χ^2 test for categorical variables or t -test for continuous variables.

detection kit for specific inhalant allergens including house dust mites, cat/dog hair, molds, seasonal grass/ tree and pollens, cockroaches; ragweed, *Artemisia argyi*, *Penicillium notatum*, *Aspergillus fumigatus*. Besides the positive diagnosis of AR as shown above, 249 moderate-to-severe and 254 mild AR subjects were classified according to the ARIA guidelines [1]. The moderate-to-severe disease was defined as confirming one or more of the followings: poor sleep, poor academic performance, impaired daily activities such as entertainments and sports, or other troublesome symptoms.

To identify relationships between genetic loci and AR, we set the following exclusion criteria: (1) patients with co-morbid asthma, eczema, or any other allergic disease; (2) tumor in the nasal cavity or any other inflammatory nasal disease or respiratory issue; or (3) autoimmune disease, hematological disease, congenital heart disease or other chronic diseases.

The 393 healthy controls who presented no clinical features related to nasal diseases when they did not have a cold or the flu were recruited. None of these subjects had a history of allergic disease nor an atopic family history. Moreover, all the controls were examined for the SIgE test combined with any other test, and were negative for antigen-specific IgE against any of the common allergens described above in the blood sample (Table 1).

2.3. Selection of sixteen common variants associated with allergic diseases identified by published GWAS

Based on the published literature such as recent GWAS [17] of allergic diseases and public SNP databases provided by the CHB(Han Chinese in Beijing, China) from the HapMap Project (<http://www.hapmap.org>, HapMap data rel 27 Phase II + III, Feb 2009), SNPs within or near *EMSY* (previously known as *C11orf30*) and *LRRC32* (11q13.5), HLA region (6p21.32), *IL18R1* and *IL18RAP* (2q12.1), and *IL13*, *IL4*, and *KIF3A* (5q31.1) were assessed in the preliminary study. After we genotyped the preliminary set of samples for 21 tSNPs, linkage disequilibrium (LD, D') patterns of these SNPs were assessed to observe the non-random association of alleles at different loci in the preliminary dataset compared to the 82 samples in the CHB population from the HapMap database (Fig. 1). According to the findings. Tag SNPs (tSNPs) exhibited strong LD in several groups of SNPs with correlation coefficients (r^2) greater than or equal to 0.8, indicating that most common SNPs can be represented by a subset of tSNPs using our preliminary dataset. Further pruning of the tSNPs was made using a pair-wise tagging algorithm to remove SNPs with the Hardy-Weinberg p value < 0.01, minor allele frequency (MAF) < 0.05, and r^2 thresholds > 0.8. A final selection of 16 tSNPs representing the entire loci were used to

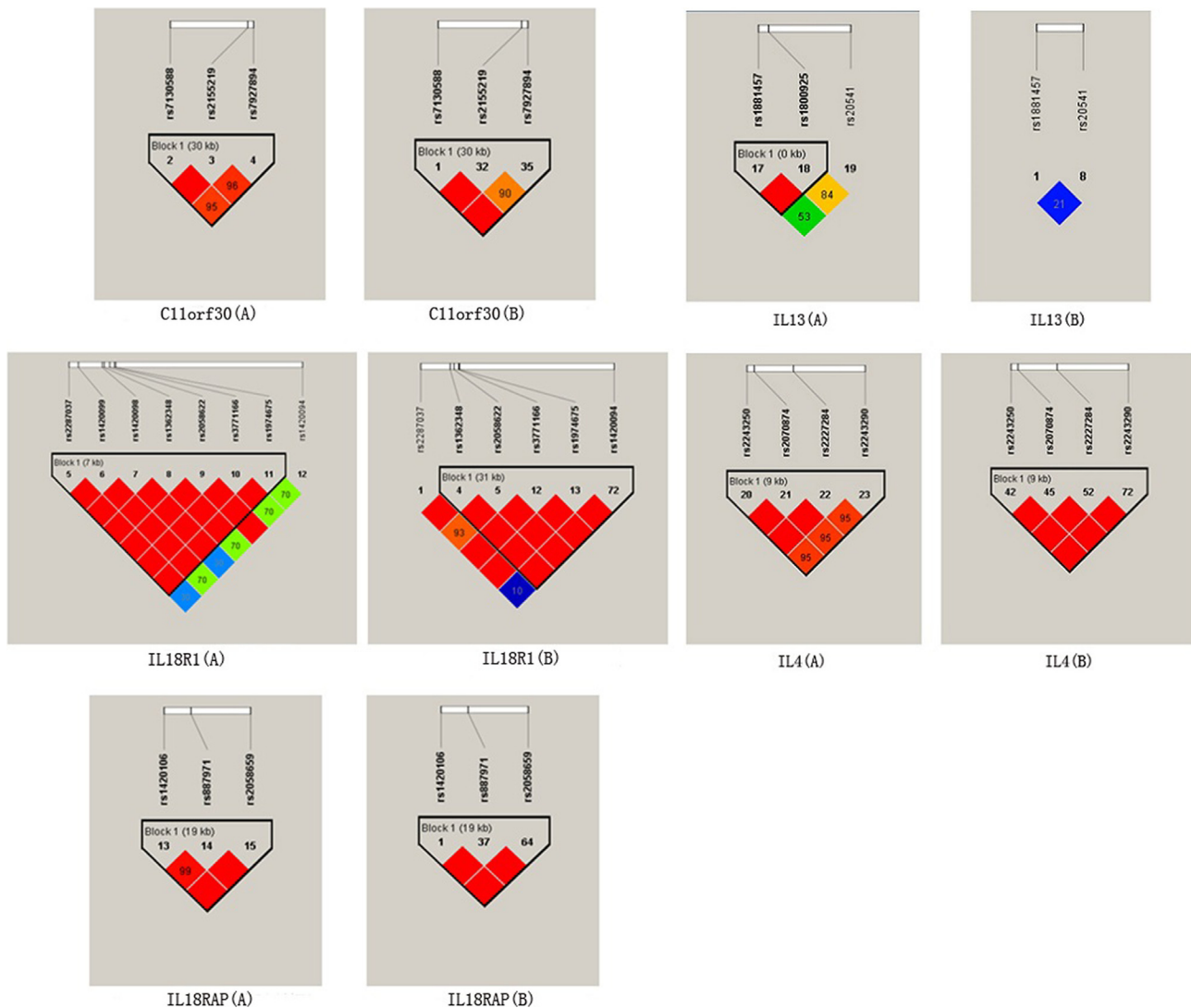


Fig. 1. Linkage disequilibrium (LD, D') map across the *C11orf30* (now known as *EMSY* region), *IL18R1*, *IL18RAP*, *IL13*, *KIF3A*, *IL4* gene region. Test data in the preliminary study (A) vs HapMap-CHB data (82 normal population) (B). The measure of LD (D') among all possible pairs of SNPs is shown graphically according to the depth of red color. The numbers in squares are D' values ($D' \times 100$). Haplotype block structure was estimated with the Haploview program. (D').

Table 2
Characteristics of 16 GWAS-related tSNPs for association with allergic diseases.

Gene symbol (chromosome locus)	SNP_ID (risk allele)	Chromosome coordinate (hg19)	Region	Minor allele frequencies		P^a	P^b	P value for HWE test
				AR	Control			
<i>EMSY-LRRC32</i> (11q13.5)	rs7130588(G)	76270683	intergenic	0.108	0.070	0.002	0.022	0.158
	rs2155219(G)	76299194	intergenic	0.435	0.449	0.554	0.992	0.634
	rs7927894(T)	76301316	intergenic	0.182	0.146	0.039	0.429	0.229
<i>HLA region</i> (6p21.32)	rs7775228(C)	32658079	intergenic	0.209	0.220	0.327	0.938	0.922
	rs2287037(T)	102979028	intronic	0.350	0.386	0.117	0.762	0.517
<i>IL18R1</i> (2q12)	rs1974675(A)	102986375	intronic	0.137	0.143	0.690	0.992	0.781
	rs1420094(T)	103015687	<i>IL18R1</i> downstream	0.136	0.143	0.645	0.992	0.329
<i>IL18RAP</i> (2q12)	rs887971(C)	103041167	intronic	0.346	0.380	0.115	0.762	0.247
	rs2058659(A)	103054556	intronic	0.135	0.138	0.831	0.992	0.255
	rs1881457(C)	131992409	<i>IL13</i> upstream	0.243	0.277	0.104	0.762	0.794
	rs1800925(T)	131992809	<i>IL13</i> upstream	0.172	0.181	0.582	0.992	0.908
<i>IL13</i> (5q31)	rs20541(A)	131995964	exonic	0.310	0.318	0.719	0.992	0.244
<i>IL4</i> (5q31)	rs2243250(C)	132009154	<i>IL4</i> upstream	0.181	0.211	0.120	0.762	0.749
	rs2227284(G)	132012725	intronic	0.113	0.156	0.007	0.106	0.242
	rs2243290(C)	132018169	intronic	0.175	0.203	0.137	0.762	0.381
<i>KIF3A</i> (5q31)	rs2897442(T)	132049027	intronic	0.105	0.129	0.109	0.762	0.621

HWE: Hardy-Weinberg equilibrium.

Bold text indicates a statistically significant difference with a p value less than 0.05.

Genomic coordinates are based on GRCh37/hg19 Human Reference Genome.

^a P value for the difference in frequencies in AR group vs. in controls.

^b P value for the difference in frequencies in AR group vs. in controls after FDR_BH adjustment.

genotype all samples (Table 2).

2.4. Specimen collection and DNA extraction

DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and stored at 4 °C prior to the further investigation within two days. DNA concentrations were measured with the NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.5. SNP genotyping and quality control

SNP genotyping work was performed using an improved multiplex ligation detection reaction (iMLDR) technique developed by Genesky Biotechnologies Inc. (Shanghai, China). A multiplex PCR-ligase detection reaction method was used in the iMLDR. For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were further distinguished by different extended lengths at the 3' end. Two negative controls were set: one with double-distilled water as the template and the other with the DNA template but without primers, while all other conditions are kept the same in one plate. A randomly selected set of 45 (5%) samples was tested in duplicate by different persons, and the results were 100% concordant.

3. Statistical analyses

Continuous data passing the Kolmogorov-Smirnov test were expressed as mean (standard deviation, SD). The differences in basic characteristics between groups were tested with chi-square (χ^2) test for categorical variables or t -test for continuous variables. The frequency genotypes were expressed as the number or percentage of the total number. Deviation from the Hardy-Weinberg equilibrium (HWE) for each SNP was tested using the Pearson's χ^2 test with 1 degree of freedom in control subjects. Pair-wise LD measures were performed using Haploview version 4.0 with default settings. The r^2 for each pair of SNPs was calculated, and haplotype blocks were defined by the

method of Gabriel et al. [18] Haplotype blocks were defined based on the pairwise D' values > 0.9 .

The comparison between genotype and estimated haplotype frequencies of AR and controls was tested by χ^2 . Odds ratios (ORs) and 95% confidence intervals (CIs) of SNP associations with the binomial disease phenotype were calculated by using an unconditional logistic regression model with adjustment for age and gender in full genotype and common genetic models including co-dominant, dominant, recessive, and additive models, and haplotype analysis as described below [19]:

$$\text{Logit}(Y_i) = \mu + \beta\alpha + \gamma\text{COV} + e_i$$

where: Y_i = binomial response phenotype of the i th individual; μ = overall mean; β = regression coefficient for the additive/co-dominant/dominant/recessive effect of the SNP; α = the coded coefficients for different genotypes; COV = covariates; γ = regression coefficient for the covariates; e_i = random error.

In order to reduce the impact of confounding factors, we matched cases and controls by age, BMI, reported ethnicity, gestational age, method of delivery, parental educational level, family income, living space per capita, and family sizes although the matching between cases and controls was not unique. The results were then stratified by the severity of AR symptoms. All statistical analyses were conducted using SPSS 17.0 (Statistical Package for the Social Sciences Inc, Chicago, IL, USA) and PLINK 1.07 software. In data statistics, $p < 0.05$ is considered of suggestive significance. All statistical tests were two-tailed. Moreover, the Benjamini-Hochberg (BH) [20] method was applied for multiple comparisons to control the false discovery rate (FDR), which was much more powerful than comparable procedures to control the traditional family-wise error rate [21]. FDR at 5% level was controlled and FDR adjusted P value of 0.05 implies that 5% of significant tests will result in false positives, which were calculated at this level to assess the statistical significance of each tSNP after correction for multiple comparisons.

The statistical power for the study was calculated using G*Power 2 software (<http://www.psych.uni-duesseldorf.de/aap/projects/gpower/>). The statistical power for comparison of AR vs. control was 94.6% with the current sample size; the α was 0.05 and the β was 0.2.

4. Results

4.1. Characteristics of study subjects

Basic characteristics of our current subjects are shown in Table 1. All groups were comparable with respect to gender, age, weight, and height, of which were not significantly different for case group vs. the control group or for mild AR vs. moderate-to-severe AR ($P > 0.05$). Similar proportions of certain allergens of AR in sub-phenotypes for severity of the AR symptoms were distributed in the AR group. House dust mite and animal fur were the two most common allergens to detect in AR subjects.

4.2. Hardy-Weinberg equilibrium

All the SNPs analyzed were in Hardy-Weinberg equilibrium in AR patients and controls, which indicated that the sampled subjects were representative of the population without any deviation of genotype frequencies ($P > 0.05$) (Table 2).

4.3. Individual SNP association analysis

The results of genotypic and allelic frequency analysis for each tSNPs selected are as shown in Table 2. The G allele of rs7130588 and the T allele of rs7927894 located between *EMSY* and *LRRC32* on chr11q13.5 were more prevalent in the patients with AR than in controls ($p = 0.005$, $p = 0.039$, respectively); however, the G allele of rs2227284 in *IL4* was associated with a lower risk for AR ($p = 0.007$).

In the codominant model, the AG genotype of rs7130588 suggested a significant increase of AR risk (age and gender adjusted $p = 0.011$, OR = 1.752, 95% CI [1.143–2.685], $p_{FDR} = 0.487$) compared with the AA homozygote, including confounding factors if any. The CT genotype

of rs7927894 was observed to suggest an increased risk of AR (age and gender adjusted $p = 0.027$, OR = 1.502, 95% CI [1.048–2.153], $p_{FDR} = 0.994$). Meanwhile, a significant decrease of AR risk was revealed in TG vs TT of rs2227284 (age and gender adjusted $p = 0.023$, OR = 0.645, 95% CI [0.441–0.943], $p_{FDR} = 0.851$).

Furthermore, comparison of common models in AR (Table 3) showed that rs7130588 G allele was significantly associated with AR risk in either a dominant (age and gender adjusted $p = 0.012$, OR = 1.851, 95% CI [1.220–2.804]), or an additive model (age and gender adjusted $p = 0.002$, OR = 1.818, 95% CI [1.240–2.664]), but not a recessive model. Similarly, rs7927894:T was significantly associated with AR in a dominant model (age and gender adjusted $p = 0.037$, OR = 1.447, 95% CI [1.023–2.047]). Interestingly, rs2227284:G was associated with a significant protective effect on AR in dominant ($p = 0.022$, OR = 0.648, 95% CI [0.447–0.940]) and additive ($p = 0.029$, OR = 0.680, 95% CI [0.481–0.961]) models. However, associations of these SNPs with AR were not significant after FDR adjustment except rs7130588 in the additive model ($p_{FDR} = 0.0349$). Data for other SNPs without statistical significance are not shown in Table 3.

4.4. LD analysis and haplotype analysis

To understand better the relationship between the 16 tSNPs within and near relevant genes, we performed LD analysis and identified blocks by confidence intervals. Five haplotype blocks were found in AR subjects. Table 4 summarizes associations between frequencies of haplotypes in blocks and risk of AR. The haplotypes G-T-T (rs7130588-rs2155219-rs7927894) investigated was found to be associated with an increased risk of AR ($p = 0.003$, adjusted OR = 1.854, 95% CI [1.243–2.765]), while haplotype C-G-C (rs2243250-rs22272874-rs22432990) were associated with a decreased risk of AR ($p = 0.020$,

Table 3

Relationships between 3 SNPs (rs7130588, rs7927894, and rs2227284) and AR in different genetic models.

SNP (type) Gene (locus)	Model	Genotype	p^a	Logistic regression	p^b	FDR _{BH} adjusted ^c
				OR (95%CI)		
rs7130588 (intergenic) <i>EMSY-LRRC32</i> (11q13.5)	Codominant	AA	0.021	1 (reference)	–	–
		AG		1.752 (1.143–2.685)	0.011	0.487
		GG		4.365 (0.838–22.73)	0.081	0.487
	Dominant	AA	0.012	1 (reference)	–	–
		AG + GG		1.851 (1.220–2.804)	0.004	0.059
	Recessive	AA + AG	0.084	1 (reference)	–	–
rs7927894 (intergenic) <i>EMSY-LRRC32</i> (11q13.5)	Codominant	GG	–	3.971 (0.764–20.65)	0.101	0.434
		–		1.818 (1.240–2.664)	0.002	0.035
		–		–	–	–
	Dominant	CC	0.092	1 (reference)	–	–
		CT		1.502 (1.048–2.153)	0.027	0.994
		TT		1.029 (0.397–2.670)	0.953	0.994
rs2227284 (intronic) <i>IL4</i> (5q31)	Codominant	CC	0.041	1 (reference)	–	–
		CT + TT		1.447 (1.023–2.047)	0.037	0.197
		–		–	–	–
	Recessive	CC + CT	0.86	1 (reference)	–	–
		TT		0.9188 (0.356–2.370)	0.861	0.936
	Additive ^d	–	–	1.305 (0.968–1.759)	0.081	0.433
rs2227284 (intronic) <i>IL4</i> (5q31)	Codominant	TT	0.078	1 (reference)	–	–
		TG		0.645 (0.441–0.943)	0.023	0.851
		GG		0.710 (0.175–2.876)	0.631	0.851
	Dominant	TT	0.024	1 (reference)	–	–
		TG + GG		0.648 (0.447–0.940)	0.022	0.177
	Recessive	TT + TG	0.735	1 (reference)	–	–
	Additive ^d	GG	–	0.791 (0.196–3.193)	0.741	0.850
		–		0.680 (0.481–0.961)	0.029	0.230

Bold text indicates a statistically significant difference with a p value less than 0.05.

^a Global P value across all genotype frequencies in different genetic models between patients and control subjects using χ^2 test.

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

^c An FDR adjusted p -value by Benjamini-Hochberg (BH) method was applied.

^d Additive model: Encodes bi-allelic genotypes based on the number of copies of the minor allele.

Table 4
Associations between common haplotypes and AR risk.

Haplo-type ^a	Cases		Controls		Crude OR	<i>P</i> ^b	Adjusted OR	<i>P</i> ^c
	No.	Frequency (%)	No.	Frequency (%)				
Block1: rs7130588AG-rs2155219GT-rs7927894CT at 11q13.15								
A-G-C	430	42.70	346	44.00	1 (reference)	–	1 (reference)	–
A-T-C	386	38.40	320	40.80	0.903 (0.705–1.158)	0.52	0.840 (0.667–1.059)	0.142
A-T-T	77	7.700	63	8.00	0.827 (0.526–1.303)	0.41	0.827 (0.533–1.282)	0.396
G-T-T	103	10.20	50	6.40	1.769 (1.165–2.685)	0.01	1.854 (1.243–2.765)	0.003
Block2: rs2287037-rs1974675 at 2q12								
C-G	515	51.20	369	46.90	1 (reference)	–	1 (reference)	–
C-A	138	13.70	113	14.40	0.806 (0.570–1.141)	0.23	0.853 (0.613–1.189)	0.35
T-G	353	35.10	304	38.70	0.856 (0.671–1.093)	0.21	0.899 (0.711–1.135)	0.372
Block3: rs887971-rs2058659 at 2q12								
T-G	521	51.80	376	47.80	1 (reference)	–	1 (reference)	–
T-A	136	13.50	109	13.90	0.892 (0.633–1.257)	0.515	0.961 (0.692–1.334)	0.814
C-G	349	34.70	301	38.30	0.829 (0.649–1.060)	0.135	0.851 (0.673–1.077)	0.181
Block4: rs1881457-rs1800925 at 5q31								
A-C	761	75.60	568	72.30	1 (reference)	–	1 (reference)	–
C-T	173	17.20	143	18.20	0.971 (0.724–1.303)	0.848	0.999 (0.746–1.336)	0.995
C-C	72	7.20	75	9.50	0.712 (0.467–1.086)	0.115	0.706 (0.464–1.073)	0.103
Block5:rs2243250-rs2227284-rs2243290 at 5q31								
T-T-A	818	81.30	617	78.50	1 (reference)	–	1 (reference)	–
C-G-C	113	11.20	119	15.14	0.720 (0.512–0.997)	0.019	0.708 (0.504–0.995)	0.020
C-T-C	59	5.90	38	4.83	1.168 (0.709–1.925)	0.462	1.215 (0.738–1.999)	0.443

Bold text indicates a statistically significant difference with a *p* value less than 0.05.

^a Not available for the rarity of haplotype less than 0.05 for all the subjects.

^b *P* value from unconditional logistic regression analyses.

^c *P* value from unconditional logistic regression analyses, adjusted for age and gender.

adjusted OR = 0.708, 95%CI [0.504–0.995]). The LD plot indicated the two identical blocks with high LD which were significantly associated with the risk of AR (Fig. 2). However, no other haplotype was found to be associated with either risk or protection against AR (see Table 5).

4.5. Genotypic groups based on severity of symptoms

To address the question of whether risk genotypes we studied were correlated with specific subtypes of AR or not, the relationships were analyzed with stratification by severity of symptoms. Regarding these three SNPs and two blocks with significant relationship of the risk of AR, individuals with the G-allele of rs7130588 genotype had an almost

1.85-fold increased risk of AR only with moderate-to-severe after adjusting for age and gender (*p* = 0.001, adjusted OR = 1.855, 95% CI [1.265–2.727]). However, there was an approximate 0.7-fold decreased risk of AR in individuals with a G-allele of rs2227284, regardless of the severity of symptoms. (age and gender adjusted mild: *p* = 0.025, adjusted OR = 0.717, 95% CI [0.535–0.962]; moderate-to-severe: *p* = 0.035, adjusted OR = 0.696, 95% CI [0.497–0.795]). Also, we found the rs7130588_AG (age and gender adjusted 1.76-fold, *p* = 0.010, adjusted OR = 1.762, 95% CI [1.150–2.698]) and rs7927894_CT (1.49-fold, *p* = 0.033, adjusted OR = 1.487, 95% CI [1.039–2.129]) were significantly associated with a high risk of moderate-to-severe AR compared to the reference genotype. The protective

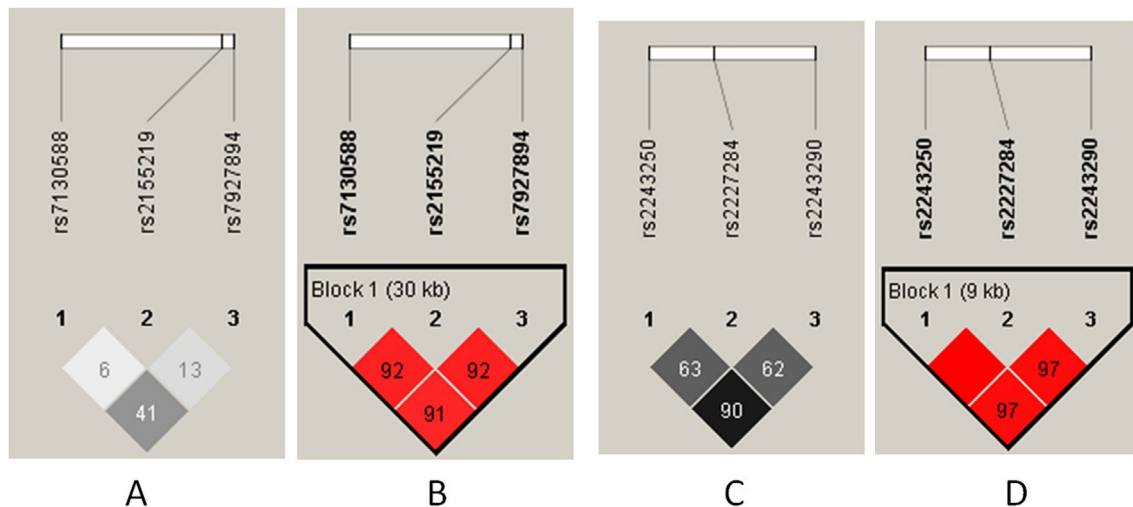


Fig. 2. Pairwise LD between the *EMSY-LRRC32* region (A, B) polymorphisms and *IL-4* (C, D) in AR. Values of r^2 ($r^2 \times 100$) are shown (A, C), and those squares in shades of color, where white represents very low r^2 and scarlet represents very high r^2 . Haplotype block structure was estimated with the Haploview program. The measure of LD (D') among all possible pairs of SNPs is shown graphically according to the depth of red color (B, D). The numbers in squares are D' values ($D' \times 100$).

Table 5
ORs for significant alleles and haplotypes stratified by severity of symptoms.

Genotypes(s)	Sub-phenotypes (%)		Controls N (%)	Mild vs. controls		Moderate-to-severe vs. controls		Mild vs. moderate-to-severe	
	Mild	Moderate-to-Severe		OR (95% CI)	<i>P</i> ^a	OR (95%CI)	<i>P</i> ^b	OR (95%CI)	<i>P</i> ^c
rs7130588									
G allele	48 (9.45)	61 (12.2)	55 (7.00)	1.350 (0.932–1.956)	0.112	1.855 (1.265–2.722)	0.001	1.388 (0.897–1.996)	0.153
rs7927894									
T allele	93 (18.3)	91 (18.3)	115 (14.6)	1.251 (0.975–1.606)	0.079	1.305 (0.965–1.763)	0.083	0.998 (0.725–1.374)	0.989
rs2227284									
G allele	57 (11.2)	57 (11.5)	123 (15.7)	0.717 (0.535–0.962)	0.025	0.696 (0.497–0.975)	0.035	0.980 (0.694–1.385)	0.910
Block1:rs7130588-rs2155219-rs7927894									
G-T-T	47 (9.40)	56 (11.2)	50 (6.40)	1.443 (1.001–2.080)	0.049	1.854 (1.243–2.765)	0.003	0.895 (0.631–1.268)	0.531
Block5:rs2243250-rs2227284-rs2243290									
C-G-C	57 (11.4)	56 (11.2)	119 (15.1)	0.747 (0.557–1.000)	0.049	0.708 (0.504–0.995)	0.047	0.990 (0.701–1.339)	0.990

^a *P* value from unconditional logistic regression analyses for the comparison of risk (or protective) and reference alleles/haplotypes between mild and controls, adjusted for age and gender.

^b *P* value from unconditional logistic regression analyses for the comparison of risk (or protective) and reference alleles/haplotypes between moderate/severe AR and controls, adjusted for age and gender.

^c *P* value from unconditional logistic regression analyses for the comparison of risk (or protective) and reference alleles/haplotypes between mild AR and moderate/severe AR, adjusted for age and gender.

effect of the rs2227284_TG was significantly associated with a nearly 0.70-fold decreased risk of AR in both mild (age and gender adjusted $p = 0.033$, OR = 0.662, 95% CI [0.455–0.963]) and moderate-to-severe (age and gender adjusted 1.76-fold, $p = 0.031$, OR = 0.650, 95% CI [0.445–0.950]) subgroups compared to the TT reference genotype (Table S1).

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.cyto.2018.08.022>.

5. Discussion

AR and other allergic diseases are complex immunological diseases caused by a combination of environmental and genetic factors. Recent GWAS have implicated that genetic components of allergic diseases include genes involved in underlying immunological pathways [17,22]. The candidate gene approach is hypothesis-driven and has been performed in various ethnic populations to obtain supporting evidence through linkage analysis [16]. We selected genetic loci identified by recent GWAS [7,17] in regions such as *EMSY-LRRC32*(11q13.15), HLA region (6p21), *IL18R1* and *IL18RAP*(2q12), *IL4* and *IL13*, and *KIF3A* (5q31) as positional candidate regions and further investigated the association of variants in these loci with AR to identify underlying causal allergic disease-associated genes.

LRRC32 located on chromosome 11q13.5, 38 kb downstream of *EMSY*, is essential for the expression of latent TGF- β on the cell surface. *LRRC32* mRNA is highly expressed in activated FOXP3⁺ regulatory T cells [8]. The T regulatory cells response in allergy that is characterized by a down-regulation of allergen-specific T cell proliferation and inhibition of both Th1 and Th2 cytokines production [23]. In the pathogenesis pathway of AR, Th2 differentiation, an important characteristic of chronic allergic inflammation, is a critical step that results in T helper cell polarization to Th2 and increased serum IgE levels [24]. The mucosa inflammation is characterized by mast cell and eosinophil activation. Genes encoding classic and non-classic HLA class I molecules are located on chromosome 6p21. They play a central role in suppression of immune responses, long-term immune escape, and tolerance and modulation of inflammation [25]. *IL18R1* and *IL18RAP* are family members of *IL1RL1*, which may also be important in the

development of asthma and atopy. *IL18R* is a key regulator of Th1 cells. Binding of *IL18* to *IL18R* stimulates Th1 cytokine release, but also Th2 cytokines, depending on its cytokine milieu.[26] *IL4*, *IL13*, and *KIF3A* are located at 5q31, that encompasses a cluster of cytokine and immune-related genes and has been associated with several autoimmune and inflammatory diseases including asthma [27]. *IL-4* and *IL-13* promote IgE synthesis, upregulate adhesion molecules selective for eosinophil recruitment, and cause increased mucus production and airway hyper-reactivity [28]. These pathophysiologic events promote the production of allergen-specific IgE.

SNPs in coding sequences of genes, non-coding regions of genes, or intergenic regions may be involved in the timing, amount, or function of proteins produced from encoding genes and contribute to disease risk. We report in this study that increased copies of the rs7130588:G allele is significantly associated with AR in the additive model after FDR_{BH} adjustment. Additive risk between genes on the logarithmic scale of the logistic regression implies that ORs across genes will be multiplicative on the penetrance scale [29] which indicates that the risk of AR is increased 1.8-fold for genotype rs7130588_GA and by 3.2-fold for genotype rs7130588_GG in our results. A common dominant model indicates that either one or two copies of the rs7927894:T allele confer a 1.4-fold increase in disease risk, whereas, one or two copies of the rs2227284:G allele lower the risk to 0.6 fold. However, associations of these alleles with AR were not significant after multiple testing adjustment. A GWAS in an Australian population has suggested an association of rs7130588 on chromosome 11q13.5 near the *EMSY-LRRC32* region with asthma with genome-wide significance [30]. Interestingly, rs7927894 was reported to be associated with two immune-related diseases, Crohn's disease [31] and AD [8], which is in complete LD ($r^2 = 1$) with rs7130588 in the Australian population but not in our study ($r^2 = 0.41$). However, the two predisposing alleles (rs7130588:G and rs7927894:T) occur on the same haplotype in the Chinese population. The rs2155219 locus showed no association with AR risk in our study, although it was found to be associated with AR and grass sensitization in a large-scale genome-wide meta-analysis [7] and a recent GWAS identified this locus to be associated with AR in an Asian population of ethnic Chinese in Singapore [32]. In addition, two SNPs within 11q13.5(rs7936562 and rs7124842) were reported not to be

associated with asthma but with AD in the Chinese Han Population [33]. With regard to rs2227284, there was some discrepancy concerning the previous reports in different populations. One study indicated that the GG genotype was significantly inversely associated with the risk of rhinoconjunctivitis (adjusted OR: 0.60; 95% CI:0.37–0.98) in young Japanese adult women [34], but another study reported opposite findings that the GG genotype was the most frequent genotype in Pakistani patients with asthma and AR [35].

We performed haplotype analysis to further clarify the role of significant SNPs in this study. We found the haplotype G-T-T (rs7130588-rs2155219-rs7927894) significantly enriched in cases over controls after adjusting for age and gender, especially in individuals with moderate-to-severe AR, suggesting a risk role of the haplotype for moderate-to-severe AR. The risk effect of rs7927894 in a haplotype was reported in Caucasians and Asian population with AD or with persistent AR [36]. Moreover, haplotype C-G-C (rs2243250-rs22272874-rs22432990) had a significant protective effect against AR; however, we observed only marginal patterns of association after stratifying subjects by the severity of symptoms. Therefore, specific haplotypes in the *EMSY-LRRC32* and *IL4* regions associated with childhood AR may be of considerable clinical interest.

In addition, such difference in statistics and the distribution of corresponding genotypes and alleles drove us to analyze in more depth, since there is distinctive phenotypes classification by severity symptoms in childhood AR. We found that approximately 12% of Chinese school-aged individuals with AR are carriers of the rs7130588:G allele and their risk of developing moderate-to-severe AR is 1.9 times that of non-carriers. Also, the AG genotype at this locus has significantly increased the risk of moderate-to-severe AR. The findings imply the important role of the *EMSY-LRRC32* region in moderate-to-severe AR. The protective effect of rs22272874:G allele and TG genotype confers a nearly 0.7 times decreased risk of AR relative to that of non-carriers in the mild or moderate-to-severe group regardless of the severity classification. Our results indicate that further investigation is warranted in patients of other ethnicities with moderate-to-severe AR who developed severe asthma symptoms.

A limitation of this study was that we were not able to determine random individual peculiarities that may bias the results. In genome-wide association studies (GWAS), principal components analyses (PCA) are used to demonstrate population-specific variations in allele distributions. PCA were not performed in this study, because our cases and controls were of a homogeneous ethnic background and all subjects were self-reported ethnic Han Chinese, who had lived in the Shanghai region, China, since birth and were recruited from the same local hospital. Moreover, it has been previously reported that Chinese population is genetically more homogeneous compared to Europeans [37]. In addition, this study was not a GWAS, thus genome-wide genotype data unavailable for the PCA. There are potentially other random individual factors that we are not aware of. Further studies are warranted.

In conclusion, results of this study suggest that intergenic variants in *EMSY-LRRC32* region and non-coding variants near *IL4* are potentially associated with manifestation and severity of AR in a Chinese Han school-aged population. However, these findings need to be validated in larger patient cohorts in multicenter studies, since all our subjects are limited to Han people of Shanghai in Eastern China. Further studies are required to explicate causative roles of genes implicated by this study. Functional characterization of the impact of disease-associated variants on genes can be assessed with genetic manipulations in animal models, and with chemical or pharmacological inhibitors *in vivo* or *in vitro* studies. The interactions between genes and environmental exposures should be explored.

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Table S1. ORs for 16 t-SNPs stratified by severity of symptoms

Genotypes(s)		subphenotypes(%)		Controls(%)	Mild vs Controls		Moderate-to-Severe vs Controls		Mild vs Moderate-to-Severe	
		Mild	Moderate/severe		OR(95%CI)	P ^a	OR(95%CI)	P ^b	OR(95%CI)	P ^c
rs7130588	AA	210 (82.68)	193 (77.51)	340 (86.51)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	AG	40 (15.75)	51 (20.48)	51 (12.98)	1.270 (0.811-1.988)	0.299	1.762 (1.150-2.698)	0.010	0.721 (0.456-1.139)	0.166
	GG	4 (1.6)	5 (2.01)	2 (0.51)	3.238 (0.588-17.833)	0.211	4.404 (0.846-22.916)	0.106	0.735 (0.195-2.778)	0.743
	A	460 (90.56)	437 (87.75)	731 (93.00)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	G	48 (9.45)	61 (12.25)	55 (7.00)	1.350 (0.932-1.956)	0.112	1.855 (1.265-2.722)	0.001	1.388 (0.897-1.996)	0.153
rs7927894	CC	172 (67.72)	165 (66.27)	290 (73.79)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	CT	71 (27.95)	77 (30.92)	91 (23.16)	1.315 (0.915-1.892)	0.160	1.487 (1.039-2.129)	0.033	0.867 (0.590-1.272)	0.494
	TT	11 (4.33)	7 (2.81)	12 (3.05)	1.437 (0.624-3.309)	0.394	0.918 (0.357-2.365)	1.000	1.565 (0.597-4.104)	0.473
	C	415 (81.69)	407 (81.73)	671 (85.37)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	T	93 (18.31)	91 (18.27)	115 (14.63)	1.251 (0.975-1.606)	0.079	1.305 (0.9652-1.763)	0.083	0.998 (0.725-1.374)	0.989
rs2227284	TT	199 (78.35)	195 (78.31)	276 (70.23)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	TG	53 (20.87)	51 (20.48)	111 (28.24)	0.662 (0.455-0.963)	0.033	0.650 (0.445-0.950)	0.031	1.018 (0.661-1.569)	1.000
	GG	2 (0.78)	3 (1.21)	6 (1.53)	0.462 (0.092-3.314)	0.478	0.708 (0.175-2.864)	0.742	0.653 (0.108-3.952)	0.684
	T	451 (88.78)	441 (88.55)	663 (84.35)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	G	57 (11.2)	57 (11.5)	123 (15.7)	0.717 (0.535-0.962)	0.025	0.696 (0.497-0.975)	0.035	0.980 (0.694-1.385)	0.91
rs2155219	TT	84 (33.07)	73 (29.32)	118 (30.03)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	TG	126 (49.61)	126 (50.60)	197 (50.13)	0.898 (0.628-1.285)	0.583	1.034 (0.716-1.493)	0.925	0.869 (0.583-1.295)	0.542
	GG	44 (17.32)	48 (19.28)	78 (19.85)	0.792 (0.498-1.260)	0.350	0.912 (0.569-1.460)	0.721	0.917 (0.544-1.545)	0.791
	T	294 (57.87)	272 (54.62)	433 (55.09)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	G	214 (42.13)	222 (44.58)	353 (44.91)	0.893 (0.713-1.119)	0.33	1.001 (0.798-1.255)	1.000	0.892 (0.695-1.145)	0.373

rs7775228	TT	160 (62.99)	155 (62.25)	233 (59.29)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	CC	86 (33.86)	79 (31.73)	140 (35.62)	0.895 (0.640-1.251)	0.551	0.848 (0.602-1.195)	0.385	1.055 (0.723-1.535)	0.848
	CC	8 (3.15)	15 (6.02)	20 (5.09)	0.583 (0.250-1.355)	0.235	1.127 (0.560-2.269)	0.723	0.583 (0.250-1.355)	0.235
	T	406 (79.92)	389 (78.11)	606 (77.10)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	C	102 (20.08)	109 (21.89)	180 (22.90)	0.846 (0.643-1.112)	0.242	0.943 (0.720-1.236)	0.681	0.897 (0.662-1.215)	0.487
rs2287037	CC	109 (42.91)	104 (41.77)	151 (38.42)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	CT	117 (46.06)	110 (44.18)	180 (45.80)	0.900 (0.642-1.264)	0.546	0.887 (0.629-1.252)	0.539	1.015 (0.698-1.475)	1.000
	TT	28 (11.02)	35 (14.06)	62 (15.78)	0.626 (0.376-1.042)	0.080	0.820 (0.505-1.330)	0.465	0.763 (0.434-1.343)	0.391
	C	335 (65.94)	318 (63.86)	482 (61.32)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	T	173 (34.06)	180 (36.14)	304 (38.68)	0.819 (0.649-1.034)	0.099	0.897 (0.711-1.132)	0.376	0.912 (0.704-1.182)	0.509
rs1420094	CC	189 (74.41)	185 (74.30)	293 (74.55)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	CT	60 (23.62)	61 (24.50)	87 (22.14)	1.069 (0.734-1.558)	0.773	1.110 (0.763-1.617)	0.63	0.963 (0.639-1.451)	0.917
	TT	5 (1.97)	3 (1.20)	13 (3.31)	0.596 (0.209-1.700)	0.461	0.820 (0.505-1.330)	0.465	1.631 (0.384-6.925)	0.724
	C	438 (86.22)	431 (86.55)	673 (85.62)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	T	70 (13.78)	67 (13.55)	113 (14.38)	0.819 (0.649-1.034)	0.099	0.365 (0.103-1.300)	0.123	0.912 (0.704-1.182)	0.509
rs1974675	GG	183 (72.05)	190 (76.31)	293 (73.28)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	GA	67 (26.38)	55 (22.09)	87 (24.68)	1.233 (0.853-1.782)	0.297	0.975 (0.664-1.431)	0.922	1.265 (0.839-1.906)	0.297
	AA	4 (1.57)	4 (1.61)	13 (2.04)	0.493 (0.158-1.534)	0.309	0.474 (0.152-1.477)	0.216	1.038 (0.256-4.213)	1.000
	G	433 (85.24)	435 (87.35)	673 (85.62)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	A	75 (14.76)	63 (12.65)	113 (14.38)	1.032 (0.752-1.415)	0.872	0.863 (0.620-1.201)	0.406	1.196 (0.834-1.715)	0.360
rs2058659	GG	190 (74.80)	185 (74.30)	297 (75.57)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	GA	59 (23.23)	61 (24.50)	83 (21.12)	1.111 (0.760-1.625)	0.626	1.180 (0.808-1.722)	0.437	0.942 (0.624-1.421)	0.834
	AA	5 (1.97)	3 (1.20)	13 (3.31)	0.601 (0.211-1.714)	0.461	0.370 (0.104-1.318)	0.125	1.632 (0.382-6.888)	0.724
	G	439 (86.42)	431 (86.55)	677 (86.13)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-

rs887971	A	69 (13.58)	67 (13.45)	109 (13.87)	0.976 (0.706-1.351)	0.934	0.966 (0.696-1.339)	0.868	1.011 (0.704-1.451)	1.000
	TT	111 (43.70)	103 (41.37)	158 (40.20)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	TC	109 (42.91)	120 (48.19)	169 (43.00)	0.918 (0.652-1.292)	0.663	1.089 (0.774-1.532)	0.664	0.843 (0.580-1.224)	0.393
	CC	34 (13.39)	26 (10.44)	66 (16.79)	0.733 (0.454-1.185)	0.231	0.604 (0.360-1.014)	0.060	1.213 (0.382-2.160)	0.560
	T	331 (65.16)	326 (65.46)	485 (61.70)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
rs20541	C	177 (34.84)	172 (34.54)	301 (38.30)	0.862 (0.683-1.087)	0.216	0.850 (0.673-1.074)	0.192	1.014 (0.782-1.314)	0.974
	GG	125 (49.21)	110 (44.18)	179 (45.55)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	GA	113 (44.49)	111 (44.58)	178 (45.29)	0.909 (0.655-1.262)	0.616	1.015 (0.726-1.419)	1.000	0.896 (0.621-1.292)	0.576
	AA	16 (6.30)	28 (11.24)	36 (9.16)	0.636 (0.338-1.197)	0.171	1.266 (0.732-2.189)	0.400	0.503 (0.258-1.000)	0.050
	G	363 (71.46)	331 (66.47)	536 (68.19)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
rs1881457	A	145 (28.54)	167 (33.53)	250 (31.81)	0.856 (0.671-1.093)	0.217	1.082 (0.852-1.374)	0.541	0.792 (0.606-1.035)	0.089
	GG	150 (59.06)	137 (55.02)	204 (51.91)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	GA	89 (35.04)	98 (39.36)	160 (40.71)	0.757 (0.542-1.057)	0.109	0.912 (0.655-1.271)	0.613	0.829 (0.574-1.199)	0.348
	AA	15 (5.91)	14 (5.62)	29 (7.38)	0.703 (0.364-1.358)	0.333	0.719 (0.367-1.410)	0.408	0.979 (0.456-2.101)	1.000
	G	389 (76.57)	372 (74.70)	568 (72.26)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
rs1800925	A	119 (23.43)	126 (25.30)	218 (27.74)	0.797 (0.616-1.032)	0.092	0.883 (0.684-1.139)	0.365	0.903 (0.677-1.205)	0.509
	CC	177 (69.69)	166 (66.67)	264 (67.18)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	CT	70 (27.56)	77 (30.92)	115 (29.26)	0.908 (0.638-1.292)	0.654	1.065 (0.752-1.508)	0.723	0.853 (0.579-1.255)	0.432
	TT	6 (2.36)	7 (2.81)	14 (3.56)	0.639 (0.241-1.695)	0.485	0.795 (0.314-2.011)	0.819	0.804 (0.265-2.441)	0.782
	C	426 (83.86)	407 (81.73)	643 (81.81)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
rs2243250	T	82 (16.14)	91 (18.27)	143 (18.19)	0.866 (0.643-1.166)	0.368	1.005 (0.752-1.344)	1.000	0.861 (0.620-1.195)	0.404
	TT	172 (67.72)	165 (66.27)	242 (61.58)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	TC	74 (29.13)	75 (30.12)	136 (34.61)	0.766 (0.543-1.080)	0.141	0.809 (0.573-1.141)	0.258	0.947 (0.644-1.392)	0.844
	CC	8 (3.15)	9 (3.61)	15 (3.82)	0.750 (0.311-1.809)	0.664	0.880 (0.376-2.058)	0.833	0.853 (0.321-2.263)	0.807

rs2243290	T	418 (82.28)	405 (81.33)	620 (78.88)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	C	90 (17.72)	93 (18.67)	166 (21.12)	0.804 (0.605-1.069)	0.153	0.858 (0.646-1.138)	0.318	0.938 (0.681-1.292)	0.744
	AA	173 (68.11)	168 (67.47)	245 (62.34)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	AC	72 (28.35)	75 (30.12)	136 (34.61)	0.750 (0.531-1.059)	0.118	0.804 (0.570-1.134)	0.225	0.932 (0.633-1.373)	0.767
	CC	9 (3.54)	6 (2.41)	12 (3.05)	1.062 (0.438-2.576)	1.000	0.792 (0.268-1.981)	0.629	1.457 (0.507-4.182)	0.601
rs2897422	A	418 (82.28)	411 (82.53)	626 (79.64)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	C	90 (17.72)	87 (17.47)	160 (20.36)	0.842 (0.633-1.122)	0.249	0.828 (0.620-1.106)	0.217	1.017 (0.735-1.407)	0.934
	CC	204 (80.32)	199 (79.92)	295 (75.06)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	CT	48 (18.90)	46 (18.47)	94 (23.92)	0.738 (0.500-1.092)	0.144	0.725 (0.488-1.078)	0.117	1.018 (0.650-1.595)	1.000
	TT	2 (0.79)	4 (1.61)	6 (1.02)	0.482 (0.096-2.412)	0.482	0.988 (0.275-3.547)	1.000	0.488 (0.088-2.693)	0.477
	C	456 (87.76)	444 (89.16)	684 (87.02)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
	T	52 (10.24)	54 (10.84)	102 (12.98)	0.765 (0.537-1.090)	0.159	0.816 (0.574-1.158)	0.293	0.938 (0.627-1.402)	0.759

^a: *P* value from unconditional logistic regression analyses for the comparison of risk (or protective) and reference genotypes/alleles/haplotypes between mild and controls, adjusted for age and gender.

^b: *P* value from unconditional logistic regression analyses for the comparison of risk (or protective) and reference genotypes/alleles/haplotypes between moderate/severe AR and controls, adjusted for age and gender.

^c: *P* value from unconditional logistic regression analyses for the comparison of risk (or protective) and reference genotypes/alleles/haplotypes between mild AR and moderate/severe AR, adjusted for age and gender.