



Association between polymorphisms in cytokine genes IL-17A and IL-17F and development of allergic rhinitis and comorbid asthma in Chinese subjects

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ABSTRACT

Background: Th17 cell lineage, a distinct pro-inflammatory lineage characterized by preferential synthesis of cytokines IL-17A and IL-17F, is thought to play an important role in the pathogenesis of allergic rhinitis (AR).

Objectives: Our aim was to investigate whether polymorphisms in and around IL-17A and IL-17F genes are associated with AR and comorbid asthma.

Methods: A case-control comparison was performed in a cohort of 279 AR patients, 197 allergic rhinitis with asthma (AR-A) patients and 281 control Chinese subjects, to investigate associations between 19 tagging single-nucleotide polymorphisms (SNPs) in IL-17A and IL-17F gene regions and manifestation of AR or AR-A. Genotyping was performed using the Sequenom MassARRAY platform.

Results: SNP rs3819024 in IL-17A gene, intergenic SNPs rs1892280 and rs10807439 were specifically associated with AR protective or risk effects, while rs3819024 in IL-17A gene, intergenic SNP rs13192563 in IL-17F gene were associated with AR-A protective or risk effects. Haplotype analysis showed significant AR risk in haplotype AA (rs1892280G–rs13192563A) and AR protective effect in haplotype GT (rs7758579A–rs11966760T); the haplotype AT(rs7758579–rs11966760) were considered AR-A risk.

Conclusions: Our findings preliminarily indicate IL17A and IL17F SNPs, and some intergenic variants have the potential association with AR and comorbid asthma in Chinese population.

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1. Introduction

Allergic rhinitis (AR) is one of the most common chronic disorders, which frequently co-exists with asthma [1]. As AR is similar to allergic asthma in many mechanistic and pathological aspects [2,3], one concept considers both AR and asthma as manifestations of “one airway” disease. However, not all AR patients have asthma and several studies have reported the existence of differences between AR and AR with asthma [4,5]; suggesting that AR with and without asthma may represent distinct phenotypes of AR.

Although AR does not exhibit a typical Mendelian hereditary pattern, studies in twins have demonstrated that the disease does nevertheless have a hereditary component [6]. To date, several studies have investigated single-nucleotide polymorphisms (SNPs) in genes encoding the molecules implicated in the pathogenesis of AR; including specific chemokines and their receptors [7,8],

interleukins (ILs) and their receptors [9,10], eosinophil peroxidase [11] and leukotriene C4 synthase [12].

Until recently it has been assumed that development and manifestation of allergic airway disease was primarily influenced by the activity of T helper (Th)1, Th2 cells and T-regulatory (Treg) cells in genetically susceptible individuals. Recent evidence suggests that Th17 cells are also likely to play an important role in the pathogenesis of allergic airways disease [13–15]. Th17 cell is a lineage distinct from Th1, Th2, and Treg cells; characterized by preferential synthesis of IL-17, IL-17F and IL-22 and involved in the induction and development of autoimmune inflammatory disease. Indeed, studies in patients with AR have demonstrated that IL-17 levels are significantly increased in nasal lavage [16], induced sputum [17], and serum [18]. One study demonstrated that serum IL-17 levels of patients with AR were significantly related to clinical symptoms, drug use and peripheral eosinophil counts [19]. Similarly, ex-vivo evaluation of peripheral blood mononuclear cells from persistent AR patients and healthy non-allergic subjects has demonstrated that the frequencies of IL-17 producing T cells were higher in the AR patients than in the healthy subjects [20].

Moreover, detailed studies of IL-17 have indicated that this is a family of six members designated IL-17A–F; of which IL-17A and

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IL-17F are expressed by adjacent genes localized on human chromosome 6 and appear to be most prominent in mediating the inflammatory activity of Th17 cells [21].

In view of these evidences, the aim of this study was therefore to examine whether polymorphisms in the IL-17A and IL-17F genes are associated with the development of AR with and without asthma, and what are the differences between these two phenotypes of AR.

2. Materials and methods

This was a case-control association analysis to assess the risk of AR conferred by SNPs in the IL-17A and IL-17F genes region in a Han Chinese cohort. The study was approved by the Ethics Committee of Beijing Tongren Hospital and written informed consent was obtained from all participants prior to entry into the study.

2.1. Subjects

A total of 476 male and female AR patients (aged 4–82 years old) attending the Department of Otolaryngology Head and Neck Surgery outpatient clinic at Beijing Tongren Hospital, Beijing, China, were recruited from June to October 2010. Overall, 197 patients suffered from allergic rhinitis with comorbid asthma (AR-A). In contrast, patients with accompanying atopic dermatitis were excluded from the study. A total of 281 healthy volunteers were also recruited as the control group from the local population to determine the background population allele frequencies. All subjects were of Han Chinese ethnic origin and from the Beijing region.

A diagnosis of AR was based on medical history, the presence of positive skin prick test (SPT) responses to a panel of common allergens and typical symptoms as defined by the ARIA 2008 guidelines [22]; i.e. two or more AR symptoms (nasal congestion, rhinorrhoea, nasal itching, sneezing) persisting for 4 or more days a week during the past year. The allergens included seasonal grass pollens (Giant Ragweed, Mugwort, Lamb's quarters, Humulus, Chenopodium album, etc.), house dust mite (HDM) (Der f and Der p), animal fur (especially dog and cat), moulds (indoor and outdoor mustiness or floricultural environment) and cockroach; with a positive SPT result defined as a wheal greater than or equal to one half of the diameter of the histamine control and at least 3 mm larger than the diameter of the negative control. AR with asthma was classified based on a positive diagnosis of AR as shown above and positive doctor-diagnosed asthma with wheezing symptoms. A subset of the asthmatic subjects were also assessed for lung function and peak flow reversibility and completed the asthma control test (ACT). Similarly, the non-atopic status of healthy control individuals was confirmed on the basis of negative SPT to common allergens and no history of allergic conditions, diagnosis, or symptoms. SPTs were performed by specialist nurses, while the diagnoses for AR and asthma were made by rhinologists and physicians.

2.2. Selection of polymorphisms

IL-17A and IL-17F genes are located adjacently on human chromosome 6 at regions chr6: 52159144–52163395 and chr6: 52209443–52217257, respectively. Thus, region chr6: 52147144–52229257, encompassing 12 kb upstream of IL-17A and 12 kb downstream of IL-17F together with the intergenic region between the two genes, was investigated. In order to use a minimal set of markers to capture all alleles in the target region, tag SNPs were selected as target SNPs. Tag SNPs (tSNP) were selected using the Tagger software implemented in the Haploview software according to the Han-Chinese Beijing data set (URL: <http://www.hapmap.org/index.html.en>), with an r^2 threshold of 0.8 and a minor allele

frequency (MAF) of >5%. Twenty-two SNPs were selected as tSNP to represent the whole region. One tSNP (rs13192459) was excluded due to assay design criteria and another SNP (rs1892280) tagged by tSNP rs13192563 was added. After genotyping, three SNPs (rs721430, rs10948691 and rs7741835) were excluded for not being detected. Table 2 shows the position of the remaining 19 tSNPs.

2.3. DNA preparation and SNP genotyping

Genomic DNAs were extracted from peripheral blood samples collected from each study subject, using the DNA isolation kit for mammalian blood (Roche, Indianapolis, USA). The extracted genomic DNAs were analyzed by agarose gel electrophoresis, quantified by spectrophotometer, and stored at -80°C until use. Genotyping of allele-specific primer extension products, generated from amplified DNA sequences, was performed using the Sequenom MassARRAY iPLEX Gold platform (Sequenom, San Diego, California). Primers were designed using MassARRAY Assay Design 3.1 software (Sequenom, San Diego, California) and genotyping was performed by an investigator blinded to the sample status (i.e. from case or control subjects). Duplicate samples and positive and negative controls were included to confirm the accuracy of genotyping. Furthermore, direct sequencing of randomly selected samples was also carried out as quality control.

2.4. Statistical analysis

Quality of the genotype data was evaluated by the HWE proportion tests. The statistical power of sample size was calculated with G*Power 3.1. Pairwise linkage disequilibrium (LD) among the SNPs was examined using Lewontin's standardized coefficient D' and LD coefficient r^2 [23], and haplotype blocks were defined by the method of Gabriel et al. [24] in Haploview 4.2 with default settings (the CI for a strong LD was minimal for upper 0.98 and low 0.7 and maximal for a strong recombination of 0.9, and a fraction of strong LD in informative comparisons was at least 0.95). In addition, PHASE 2.1 Bayesian algorithm [25] was used to estimate the haplotype frequencies.

Genotype and haplotype frequencies of case patients and control subjects were compared using χ^2 tests. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis with adjustment for age and gender.

All single-marker and haplotype association analyses were carried out using the STATA software (version 11.0; StataCorp LP, College Station, TX, USA) and $P < 0.05$ was considered significant. Moreover, significant P values were corrected with the Bonferroni correction by multiplying with N (the number of SNPs in one gene). All statistical tests were two sided.

3. Results

3.1. Characteristics of study subject

Clinical and demographic characteristics of cases and controls are summarized in Table 1. All groups were comparable with respect to mean age and gender ratio. The mean ages for control, AR and AR-A groups were 37.08 ± 16.17 , 26.09 ± 15.10 , and 24.60 ± 17.57 years, respectively, and not significantly different for AR and AR-A groups compared to control group ($P = 0.166$ and 0.056 , respectively; measured by t -test). The Pearson Chi-Square test of the ratios for male/female between control and two case groups were tested ($P = 0.273$ and 0.011 , respectively).

The statistic power of AR vs. control and AR-A vs. control was 92.6% and 87.0%, respectively.

Table 1

Clinical and demographic characteristics of the study subjects.

Demographic Index	AR ^a	AR-A ^b	Control
Gender [Male/Female]	140/139	113/84	128/153
Age [Mean (range)]	26.09 (4–82)	24.60 (4–66)	37.08 (3–75)
Perennial/Seasonal [n(%)]	220(78.85%)/59(21.15%)	174(88.32%)/23(11.68%)	NA ^c
Allergen category [n(%)]			
House dust mite	135(48.39%)	106(53.81%)	NA ^c
Pollens	59(21.15%)	23(11.67%)	NA ^c
Mixed allergens	85(30.46%)	68(34.52%)	NA ^c

^a Allergic rhinitis.^b Allergic rhinitis with asthma.^c No data available.

3.2. Single marker association analysis

Allele frequencies of each tSNPs selected for investigation are as shown in Table 2. Allele of SNP rs3819024 located at 5' of IL-17A gene and rs1892280 located between IL-17A and IL-17F gene was marginally but significantly distributed between AR and control ($P = 0.048$ and $P = 0.047$, respectively). In contrast, allele of SNP rs7758579 located between IL-17A and IL-17F gene, but not allele of SNP rs3819024 and rs1892280, was significantly distributed between AR-A and control ($P = 0.0106$). However, associations with all these SNPs were lost after 100,000 permutations ($P > 0.05$).

At the genotype level, the rs1974226 was excluded because of deviation from HWE. As shown in Table 3, the IL-17A gene SNP rs3819024 was associated with both AR ($P = 0.012$, OR = 0.572, 95% CI [0.369–0.886], for GA vs. AA comparison; $P = 0.010$, OR = 0.497, 95% CI [0.292–0.848], for GG vs. AA comparison) and AR-A ($P = 0.031$, OR = 0.583, 95% CI [0.357–0.952], for GA vs. AA comparison; $P = 0.046$, OR = 0.549, 95% CI [0.305–0.990], for GG vs. AA comparison). Intergenic SNPs rs1892280 ($P = 0.042$,

OR = 1.814, 95% CI [1.021–3.223], AA vs. GG comparison) and rs10807439 ($P = 0.038$, OR = 0.631, 95% CI [0.408–0.975], for AA vs. GG comparison) were specifically associated with AR protective or risk effects, while intergenic SNPs rs13192563 ($P = 0.009$, OR = 1.775, 95% CI [1.158–2.723], for AG vs. AA comparison) and rs669141 ($P = 0.042$, OR = 1.842, 95% CI [1.022–3.319], for TT vs. CC comparison) in IL-17F gene were specifically associated with AR-A protective or risk effects. The rs3819024 (GG genotype) and rs13192563 (AG genotype) were significant even after Bonferroni correction ($P = 0.050$ and 0.045 , respectively). Data for other SNPs without statistical significance are not shown in Table 3.

3.3. LD and haplotype analysis

To better understand the relationship between the 18 tSNPs in and around IL-17A and IL-17F genes, LD analysis was performed and blocks were defined by confidence intervals. Few pair-wise alleles showed linkage disequilibrium except rs1892280 and rs13192563, with $r^2 = 0.84$ and 0.85 , in AR vs. control and AR-A

Table 2

Information about 19 genotyped SNPs of IL17A and IL17F genes.

Gene: locus and OMIM No. ^a	SNP_ID	Chromosome position ^b	Genic location	Allele (1/2)	AR ^c number (11/12/22)	AR-A ^d number (11/12/22)	Control number (11/12/22)	P^e	P^f	P value for HWE ^g test
Intergenic	rs6926641	52147355	–	G/C	183/65/5	125/53/3	184/72/5	0.6928	0.8139	0.501
	rs10085177	52150294	–	C/T	142/100/17	99/76/10	141/113/12	0.9655	0.9478	0.071
	rs10948682	52152128	–	A/C	152/75/8	112/59/5	147/89/7	0.4814	0.5735	0.133
	rs16882166	52154106	–	C/G	216/49/2	158/31/1	231/38/2	0.2084	0.6092	0.752
IL17A: 6p12; OMIM: 603149	rs4711998	52158312	5' near gene	A/G	141/107/11	89/76/19	139/108/17	0.4626	0.1831	0.512
	rs8193036	52158452	5' near gene	C/T	134/112/19	81/83/21	131/108/24	0.6272	0.19	0.798
	rs3819024	52158745	5' near gene	A/G	84/128/51	55/90/38	62/140/61	0.048	0.1902	0.294
	rs3819025	52159233	Intron 1	G/A	182/74/6	128/52/5	176/77/9	0.462	0.5951	0.872
	rs3804513	52161156	Intron 2	A/T	179/70/5	146/34/0	201/55/5	0.1284	0.1643	0.588
	rs1974226	52163294	3' UTR	A/G	23/246/0	23/166/0	23/242/0	0.9831	0.6023	1.36E–42
Intergenic	rs1892280	52164941	–	G/A	106/121/41	69/108/15	126/116/29	0.047	0.2239	0.766
	rs13192563	52173195	–	A/G	104/127/17	67/101/5	127/113/17	0.1828	0.2746	0.219
	rs7758579	52194240	–	G/A	158/89/24	99/72/22	170/87/19	0.3078	0.0106	0.096
	rs11966760	52194993	–	T/G	85/130/44	47/99/34	71/128/61	0.0524	0.622	0.822
	rs10807439	52206071	–	C/T	81/126/60	50/92/46	61/147/58	0.2709	0.882	0.086
IL17F: 6p12; OMIM: 606496	rs13209590	52207610	3' near gene	A/G	176/76/5	144/40/0	193/63/7	0.3533	0.1001	0.501
	rs7771466	52212068	Intron 1	C/A	191/43/3	135/38/2	195/50/2	0.7646	0.6299	0.534
	rs1266828	52216021	Intron 1	T/C	179/57/11	122/48/7	162/78/7	0.2743	0.6794	0.511
	rs669161	52228974	5' near gene	C/T	80/125/57	41/98/46	75/139/51	0.9639	0.0823	0.347

^a OMIM, Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/Omim>).^b SNP position in chromosome 6 in the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>).^c Allergic rhinitis.^d Allergic rhinitis with asthma.^e P value for difference in allele distributions between AR and controls.^f P value for difference in allele distributions between AR-A and controls.^g HWE, Hardy–Weinberg equilibrium.

Table 3
Associations of genotype with allergic rhinitis or allergic rhinitis plus asthma risk.

Groups	SNP ID (gene)	Genotype	<i>P</i> ^c	Logistic Regression	
				OR (95% CI)	<i>p</i> ^d
AR ^a vs. control	rs3819024 (IL17A)	AA	0.093	1.000 (referent)	
		GA		0.572 (0.369–0.886)	0.012
		GG		0.497 (0.292–0.848)	0.010
	rs1892280 (intergenic)	GG	0.144	1.000 (referent)	
		AG		1.285 (0.873–1.894)	0.204
		AA		1.814 (1.021–3.223)	0.042
	rs10807439 (intergenic)	CC	0.107	1.000 (referent)	
		TC		0.631 (0.408–0.975)	0.038
		TT		0.615 (0.364–1.037)	0.068
AR-A ^b vs. control	rs3819024 (IL17A)	AA	0.308	1.000 (referent)	
		GA		0.583 (0.357–0.952)	0.031
		GG		0.549 (0.305–0.990)	0.046
	rs13192563 (intergenic)	AA	0.008	1.000 (referent)	
		AG		1.775 (1.158–2.723)	0.009
		GG		0.679 (0.228–2.021)	0.487
	rs669141 (IL17F)	CC	0.202	1.000 (referent)	
		TC		1.506 (0.921–2.464)	0.103
		TT		1.842 (1.022–3.319)	0.042

^a Allergic rhinitis.
^b Allergic rhinitis with asthma.
^c Global *P* values across all genotype frequencies.
^d *P* values from unconditional logistic regression analyses, adjusted for age and gender.

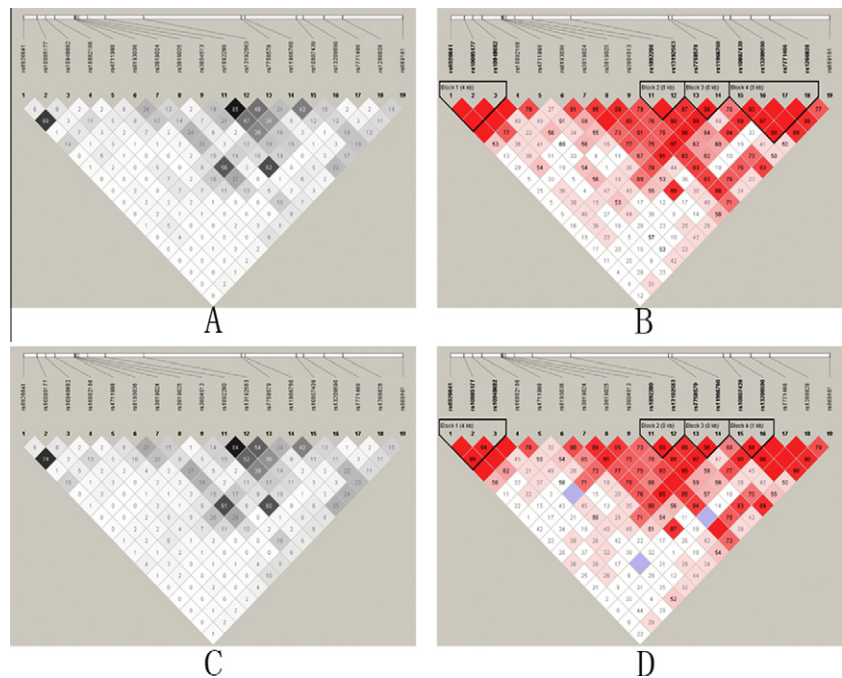


Fig. 1. Graphical representation of the SNP locations and LD structure of each gene. The SNP distribution and haplotype block structure across IL17A and IL17F genes are shown. The measure of LD (r^2) among all possible pairs of SNPs is shown graphically according to the shade of color (A/C), where white represents very low r^2 and scarlet represents very high r^2 . The numbers in squares are r^2 values ($r^2 \times 100$). The measure of LD (D') among all possible pairs of SNPs is shown graphically according to the shade of color (B/D), where white represents very low D' and dark represents very high D' . The numbers in squares are D' values ($D' \times 100$). The above two graphics were drawn in AR vs. control group, and the bottom two graphics were drawn in AR-A vs. control group.

vs. control, respectively (Fig. 1A/C). Four haplotype blocks were found in the AR group (Fig. 1B) and in the AR-A group (Fig. 1D). Significant risk for AR was associated with haplotype AA (rs1892280G/–rs13192563A; $P = 0.049$, OR = 1.310, 95% CI [1.001–1.714] for AA vs. GA haplotype comparison), where as protective effect against AR was associated with haplotype GT (rs7758579A/–rs11966760T; $P = 0.024$, OR = 0.716, 95% CI [0.536–0.947], for GT vs. GG haplotype comparison) (Table 4.1). In contrast, for AR-A haplotype AT (rs7758579–rs11966760) was

considered a risk factor ($P = 0.016$, OR = 1.517, 95% CI [1.080–2.132], for AT vs. GG haplotype comparison); with other haplotypes not being associated with either risk or protection against AR-A (Table 4.2).

4. Discussion

Although the Th17 cytokines, IL-17A and IL-17F [26,27], and SNPs of IL-17A or IL-17F gene [28–31] have previously been shown

Table 4.1

Associations between common haplotypes and allergic rhinitis risk.

Block	AR		Control		P^a	P^b	Logistic regression	
	No.	Frequency (%)	No.	Frequency (%)			OR (95% CI)	P^c
<i>Block1: rs6926641–rs10085177–rs10948682</i>								
GCA	284	53.80	284	53.40	0.899	0.698	1.000 (referent)	
GTA	136	25.80	137	25.80	0.984		1.057 (0.566–1.974)	0.863
CCC	79	15.00	84	15.80	0.722		0.924 (0.684–1.249)	0.607
GCC	28	5.40	27	5.00	0.800		0.949 (0.656–1.374)	0.783
<i>Block2: rs1892280–rs13192563</i>								
GA	335	62.20	368	67.60	0.063	0.101	1.000 (referent)	
AG	181	33.60	160	29.30	0.131		1.351 (0.659–2.770)	0.412
AA	22	4.20	16	3.00	0.316		1.310 (1.001–1.714)	0.049
<i>Block3: rs7758579–rs11966760</i>								
GG	221	40.50	256	46.20	0.055	0.085	1.000 (referent)	
GT	188	34.50	173	31.20	0.259		0.716 (0.536–0.957)	0.024
AT	137	25.00	124	22.30	0.290		0.909 (0.648–1.275)	0.582
AG	0	0.00	1	0.30	NA ^c		NA ^d	NA ^d
<i>Block4: rs10807439–rs13209590–rs7771466–rs1266828</i>								
TACT	185	35.40	204	38.60	0.285	0.168	1.000 (referent)	
CACT	111	21.20	93	17.50	0.134		1.381 (0.984–1.939)	0.062
CACC	84	16.10	97	18.40	0.311		1.095 (0.754–1.590)	0.633
CGCT	85	16.30	74	14.00	0.291		1.408 (0.960–2.067)	0.808
TAAT	54	10.40	55	10.50	0.964		1.103 (0.695–1.750)	0.679
Others	4	0.60	2	1.00	0.410		0.615 (0.102–3.705)	0.596

^a P value for difference in individual haplotype frequencies.^b Global P values across all genotype frequencies.^c P values from unconditional logistic regression analyses, adjusted for age and gender.^d NA, Not available because of the rarity of genotype.**Table 4.2**

Associations between common haplotypes and allergic rhinitis plus asthma risk.

Block	AR-A		Control		P^a	P^b	Logistic Regression	
	No.	Frequency (%)	No.	Frequency (%)			OR (95% CI)	P^c
<i>Block1: rs6926641–rs10085177–rs10948682</i>								
GCA	204	54.80	286	53.80	0.738	0.798	1.000 (referent)	
GTA	95	25.60	136	25.60	0.994		0.923 (0.662–1.286)	0.635
CCC	60	16.10	84	15.80	0.905		0.975 (0.654–1.454)	0.903
GCC	11	2.90	25	4.80	0.168		0.691 (0.310–1.542)	0.367
Others	2	0.60	0	0.00	NA ^c		NA ^c	NA ^c
<i>Block2: rs1892280–rs13192563</i>								
GA	246	64.00	368	67.60	0.212	0.418	1.000 (referent)	
AG	124	32.00	160	29.30	0.386		1.187 (0.881–1.600)	0.259
AA	16	4.00	16	3.00	0.402		1.371 (0.620–3.028)	0.436
GG	0	0.00	1	0.01	NA ^c		NA ^d	NA ^d
<i>Block3: rs7758579–rs11966760</i>								
GG	170	43.50	256	46.30	0.402	0.084	1.000 (referent)	
GT	103	26.50	173	31.20	0.121		1.008 (0.724–1.404)	0.960
AT	115	29.50	123	22.20	0.011		1.517 (1.080–2.132)	0.016
AG	1	0.50	1	0.30	0.802		1.208 (0.063–23.082)	0.900
<i>Block4: rs10807439–rs13209590</i>								
TA	184	48.80	260	49.40	0.979	0.221	1.000 (referent)	
CA	151	40.10	194	36.40	0.251		1.151 (0.857–1.545)	0.350
CG	41	10.90	75	14.20	0.148		0.790 (0.501–1.244)	0.309
TG	2	0.20	0	0.00	NA ^c		NA ^c	NA ^c

^a P value for difference in individual haplotype frequencies.^b Global P values across all genotype frequencies.^c P values from unconditional logistic regression analyses, adjusted for age and gender.^d NA, Not available because of the rarity of genotype.

to be associated with asthma, to our knowledge this is the first study to report a genetic association between IL-17A and IL-17F genes and allergic rhinitis. While, one study reported a correlation

of IL-17A SNP rs8193036, but not rs2275913 or rs3819024, with pediatric asthma in Chinese Han population in Taiwan [28], another study reported that IL-17A SNP rs2275913 conferred genetic

susceptibility to childhood asthma in a Chinese Han cohort in Chongqing [29].

In accordance with the findings from studies in asthmatic patients, our study has demonstrated that several SNPs, in particular IL-17A SNP rs3819024 was associated with AR and AR-A in a cohort of Chinese Han subjects in Beijing. In contrast, no association was demonstrated between IL-17A SNP rs8193036 with AR or AR-A. In HapMap HCB population, rs3819024 shows marginal LD with rs2275913, with the r^2 of 0.72. Furthermore, in our study rs2275913 was represented by rs11966760 (with r^2 set at 0.8), which was also not found to be associated with AR or AR-A. Similarly, despite the reported association of IL-17F (H161R) variant with asthma [31], our study failed to confirm the association between AR and s763780, which was represented by rs7771466 (with r^2 of 1) in our study. The discrepancy concerning the association of IL-17A/IL-17F polymorphisms with AR and asthma may be due to variations in inheritance, although differences in ethnic groups studied cannot be ruled out.

AR and AR-A may present two phenotypes of AR. One previous study compared the biomarkers between AR and AR with concomitant asthma and found that AR had larger wheal response and higher BslgE-level to grass pollen, but lower HDM-specific BslgE-levels and overall less allergies ($P=0.017$), compared with the AR-A. These results suggesting that AR and AR-A may be distinct phenotypes of the allergic airways disease [4]. Indeed, another study has reported significant evidence for heterogeneity of linkage to 1p31 between AR and AR-A, permitting to characterize phenotypic entities resulting from specific genetic factors [5]. Our study has demonstrated that although one SNP (rs3819024/IL-17A) was associated with both AR and AR-A, other SNPs were specifically associated with either AR or AR-A. In particular intergenic SNPs rs1892280, rs10807439 and haplotypes GA (rs1892280G/rs13192563A) were uniquely associated with AR, whereas IL-17F SNP rs669141 and intergenic SNP rs13192563 were uniquely associated with AR-A. Moreover, haplotype AT (rs7758579/rs11966760T) had AR protective but AR-A risk effect. The existence of unique protective or risk variants further support the evidence for AR with and without asthma as two separate phenotypes of one disease.

Types of SNPs include coding sequences of genes, non-coding regions of genes, or intergenic regions between genes. SNPs that are not in protein-coding regions may play a regulatory role by affecting gene splicing, transcription factor binding, or the sequence of non-coding RNA. Indeed, studies have demonstrated that intergenic variants may play a role in pathophysiological processes of related diseases [32,33]. However, to date no study has examined the association of intergenic SNPs between the IL-17A and IL-17F genes with development of disease. Results of our study support the indication that the intergenic region between IL-17A and IL-17F is a candidate region that may confer genetic susceptibility for AR and AR-A in Chinese population, and merits further investigation in individuals of other ethnicity. Presently, the intrinsic function of intergenic region is still largely unknown. Intergenic region between IL-17A and IL-17F gene in this study may play an important regulatory role in the generation and development of AR inflammation by altering mRNA splicing and posttranscriptional processes of IL-17A or IL-17F gene.

In conclusion, the results of this preliminary study suggest that some IL-17A and IL-17F SNPs and intergenic variants are potentially associated with the manifestation of AR or AR-A in Chinese population, and AR or AR-A may represent two phenotypes of AR. These findings, however, need to be confirmed in larger patient cohorts in multicentre studies. Moreover, as SNPs with a minor allele frequency (MAF) of <5% were excluded from the present study, and in view of evidence from recent studies which suggests that low-frequency SNPs may contribute strongly to genetic susceptibility

to common diseases [34–36], future studies should include those low-frequency SNPs and further investigate the regulatory effect of SNPs on IL-17A or IL-17F expression highlighted by the present study.

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Disclosure of potential conflict of interest

The authors declare that they have no conflict of interest.

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