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ORIGINAL ARTICLE

Genetic risk of TNFSF4 and FAM167A-BLK polymorphisms in children with asthma and allergic rhinitis in a Han Chinese population

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*Department of Otorhinolaryngology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, People's Republic of China***Abstract**

Background: Asthma and allergic rhinitis (AR) frequently occur as comorbid diseases of the upper airways. Single-nucleotide polymorphisms (SNPs) in the TNFSF4 and FAM167A-BLK genes have recently been shown to be associated with various immune-related disorders. **Objective:** Our aim was to determine whether TNFSF4 or FAM167A-BLK polymorphisms confer genetic susceptibility to asthma and AR in a Han Chinese population. **Methods:** We performed a case-control study of 290 asthmatic children and 252 healthy controls. Nine SNPs in the TNFSF4 region (rs1234313, rs1234314, rs1234315, rs12039904, rs844648 and rs10912580) and the FAM167A-BLK region (rs2254546, rs13277113 and rs1600249) were detected using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. **Results:** This study revealed that three SNPs in TNFSF4 (rs1234313, rs1234314 and rs1234315) and two SNPs in FAM167A-BLK (rs2254546 and rs1600249) were significantly correlated with asthma and AR, while SNP rs1600249 was associated with asthma without allergic rhinitis as a risk factor. Further, we demonstrated synergistic effects between the TNFSF4 and FAM167A-BLK SNPs. **Conclusion:** This study supports that the SNPs in TNFSF4 and FAM167A-BLK may be involved in asthma and AR gene risk in the Han Chinese cohort.

Keywords

Allergic rhinitis, asthma, FAM167A-BLK, polymorphisms, TNFSF4

History

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Introduction

Asthma and allergic rhinitis (AR) are common chronic, non-infectious airway disorders, which are induced by allergens and regulated by T lymphocytes and inflammatory mediators [1]. Both diseases frequently coexist in the same subject, and present a global health problem [2]. Several factors [3–6], including epidemiologic, pathologic and physiologic characteristics, are shared by both rhinitis and asthma and hence, support the concept of “one airway, one disease” [1]. Over the last two decades, the mechanisms of pathogenesis of asthma and AR have been studied widely and genetic factors are thought to be major players affecting the development and treatment of asthma and AR [7–9]. Various loci and candidate genes show associations with asthma and AR [10,11]. We have previously identified relationships between polymorphisms in the interleukin-23R [12], interleukin-12 receptor $\beta 1/\beta 2$ [13], interleukin-27 [14] and FCRL3 [15] genes and susceptibility to AR in a Chinese population. These findings suggested that an imbalance between Th1 and Th2 cells possibly participates in the pathogenic mechanisms of AR. However, the relation between etiology and pathogenesis remain largely unknown.

TNFSF4 (also known as OX40 ligand, OX40L) belongs to the tumor necrosis factor (TNF) superfamily. TNFSRF4 (OX40

or CD 134) and its ligands play an important role in costimulation of allergen-specific lymphocytes [16,17]. Recent studies have suggested that the OX40-OX40L interaction significantly contributes to the differentiation of some Th subsets, such as Th2 cells and Tregs [18–20]. Atopic diseases are also regulated by the innate and adaptive immune systems, especially the imbalance of Th1/Th2 cells. Two recent genome-wide association studies (GWAS) [21,22] involving Chinese populations reported TNFSF4 as a risk gene for systemic lupus erythematosus (SLE). Several studies have demonstrated that TNFSF4 gene polymorphisms are associated with susceptibility to immune-related diseases such as systemic sclerosis and primary Sjögren's syndrome (pSS) [23,24].

The FAM167A-B lymphoid tyrosine kinase (BLK) gene region is associated with SLE [21,22,25], rheumatoid arthritis [26] and systemic sclerosis [27]. Zhou et al. [28] reported that there were possible interactions of BLK, TNFSF4, TRAF1, TNFAIP3 and REL in SLE, which may represent a synergic effect of T cells and B cells through the nuclear factor (NF)- κ B pathway in determining immunologic aberration. NF- κ B plays an important role as a regulator of genes of several pro-inflammatory mediators including cytokines, chemokines, adhesion molecules, iNOS and costimulatory molecules [29,30]. Since various genetic susceptibility factors and signaling pathways are similar between inflammatory and autoimmune diseases, asthma and AR may share a common pathogenesis mechanism. The role of SNPs in TNFSF4 and FAM167A-BLK in the development of asthma and AR has not been investigated until date.

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We hypothesized that the TNFSF4 and FAM167A-BLK genes may be involved in the pathogenic process of asthma and AR. To test this hypothesis, we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to identify SNPs in these genes that correlate with both comorbid diseases in a Han Chinese population.

Materials and methods

Ethics statement

The local ethics authorities (the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China) provided permission and helped to obtain informed consent from all participants. Written informed consent was obtained from the next of kin, caretakers or guardians on behalf of all minors participating in this study.

Subjects

We recruited 290 male and female asthmatic children between 1 and 12 years of age from September 2013 to September 2014. All diagnoses were made and treatments were continued at Children's Hospital of Chongqing Medical University, Chongqing, China. One hundred forty-one children had asthma without allergic rhinitis and 149 had asthma with comorbid AR. Two hundred fifty-two healthy volunteers of the same ethnicity as the patients were recruited as the control group. The diagnosis of asthma was based on the patient's signs and symptoms according to the Global Initiative for Asthma (GINA) guidelines. Positive responses to a skin prick test (SPT; Allergopharma, Hamburg, Germany) and to a panel of common allergens were used to diagnose asthma. A positive SPT result was defined as formation of a wheal with a diameter equal to or larger than one-half that of the histamine control wheal, and at least 3 mm larger than that of the negative control. Eighteen inhaled allergens were tested, including house dust, grass, tree, mold, food and cat and dog dander. Asthma with AR was determined based on positive diagnosis of asthma as described above and factors such as medical history, nasal/ endoscopic inspection and positive SPT as defined by the ARIA 2008 guidelines [31]. SPTs were performed by specialists, technicians and nurses, while diagnoses of asthma and asthma with AR were made by clinical physicians and rhinologists. Children with accompanying systemic disease were excluded from the study. Blood samples were collected from all patients and control subjects in ethylene-diaminetetraacetate tubes and stored at -70°C until use.

DNA preparation

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes using the Wizard[®] Genomic DNA Purification Kit (Promega, Beijing, China) following the manufacturer's instructions. Briefly, 300 μL of blood was mixed with cell lysis solution. Leukocytes were spun down and lysed with nuclei lysis solution. Proteins were separated using protein precipitation solution and removed by centrifugation. DNA was recovered by precipitation with methyl alcohol and reconstituted in 100 μL DNA rehydration solution.

SNP selection and genotyping

Six SNPs (rs1234313, rs1234314, rs1234315 and rs12039904, rs844648 and rs10912580) for TNFSF4 and three SNPs (rs2254546, rs13277113 and rs1600249) for FAM167A-BLK, which have been previously shown to be associated with certain immune-related diseases, were genotyped. PCR-RFLP was adopted to analyze these SNPs. The primer sequences and reaction conditions used in this study are shown in Supplemental Table S1. The PCR products were incubated with restriction enzymes for 1 or 16 h. The digestion products were visualized on a 4% agarose gel and stained with Gold View (SBS Genentech, Beijing, China). RFLP genotyping results were validated by direct sequencing of the PCR-amplified DNA samples (20% of the samples); the results were 100% concordant.

Statistical analysis

Quality of the genotype data was evaluated from the Hardy-Weinberg equilibrium (HWE), which was estimated using the chi-square test (χ^2 test). Statistical power was estimated using Power and Sample Size Estimation Software (<http://bios-tat.mc.vanderbilt.edu/PowerSampleSize>). Pairwise linkage disequilibrium (LD) among the SNPs was examined using Lewontin's standardized coefficient D' and LD coefficient r^2 , and haplotype blocks were defined by the solid spine of LD method in Haploview 4.2 (<http://www.broadinstitute.org/haploview>). This method defines a haplotype block if the first and last SNP in a region are in strong LD ($D' \geq 0.8$) with all intermediate SNPs, while the intermediate SNPs not necessarily are in LD with each other. Genotype and allele frequencies of patients and controls were compared using χ^2 tests. Mean clinical parameter values were compared using the independent samples t -test, count data were compared using the χ^2 test. The online software platform SHEsis (<http://analysis2.bio-x.cn/myanalysis.php>) was used to analyze the haplotype data and probabilities. The odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression with adjustment for age and gender. All statistical analyses were performed using the SPSS software, (version 20.0 (SPSS Inc., Chicago, IL) and $p < 0.05$ was considered significant.

We employed a multistage strategy for analyzing gene-gene interactions by using the multifactor dimensionality reduction (MDR) method [32]. This method includes a combined cross-validation/permutation-testing procedure that minimizes false-positive results by multiple examinations of the data. Each best model was tested for its accuracy, cross-validation consistency (CVC) and significance level determined with permutation testing. The MDR analysis was performed by using version 3.0.2 of the open-source MDR software package available online (<http://www.multifactor dimensionality reduction.org/>).

Results

Comparison of clinical features

Socio-demographic characteristics of the subjects enrolled in this study are summarized in Table 1. There were no significant differences between the two patient groups and controls in terms of mean age and gender distribution. Seventy-three

Table 1. Selected clinical features and demographic characteristics of study population and controls.

Characteristics	Asthma	Asthma-AR	Control	<i>p</i> ^a	<i>p</i> ^b
Gender [male/female]	80/61	85/64	140/112	0.821	0.873
Age [mean(range)] years	9.72 (1–12)	9.62 (1–12)	9.99 (2–12)	0.285	0.153
Allergen category [%]					
House dust mite	73 (51.8)	83 (55.7)	—	—	—
Pollen	56 (39.7)	51 (34.2)	—	—	—
Multiple allergens	12 (8.5)	15 (10.1)	—	—	—

AR, allergic rhinitis.
^a*p* Value for simple asthma patients.
^b*p* Value for asthma comorbid allergic rhinitis.

(51.8%) asthmatic children and 83 (55.7%) asthmatic children with comorbid AR were sensitive to house dust mite, 56 (39.7%) and 51 (34.2%), respectively, were sensitive to tree pollen, and sensitivity to multiple allergens was noted for 12 (8.5%) and 15 (10.1%), respectively.

Representation of selected SNPs

Primary information for the SNPs in the TNFSF4 and FAM167A-BLK genes is shown in Supplemental Table S2. The genotype distribution of the nine SNPs was in Hardy–Weinberg equilibrium in controls (*p* > 0.05). In addition, the call rates were >98% for each of the nine SNPs, with minor allele frequencies >5%.

To determine the statistical power of the study, we assumed an alpha value of 0.05. The power was thus >0.991 for rs1234313, rs1234314 and rs1234315; 0.449 for rs12039904; 0.988 for rs10912580; 0.859 for rs2254546; 0.465 for rs13277113; 0.707 for rs1600249; 0.065 for rs844648.

Frequencies of alleles and genotypes between patients and controls

The genotype and allele frequencies in the controls and children with asthma without allergic rhinitis are shown in Table 2. There were obvious differences between patients and controls in the frequencies of rs1234313 and rs1234315. The frequencies of the rs1234313 A A genotype and the A allele were obviously higher in the patients with asthma without allergic rhinitis than in controls (*p* = 1.0 × 10^{−4}, OR 6.16, 95% CI 2.47–15.4 and *p* = 1.3 × 10^{−5}, OR 2.47, 95% CI = 1.65–3.71, respectively). Significantly increased prevalence of the rs 1234315 CT genotype was found in patients with asthma without allergic rhinitis compared to the controls (*p* = 6.6 × 10^{−4}, OR 4.99, 95% CI 1.98–12.62). The frequencies of the rs 1234314 CG genotype and the C allele were lower in the patients with asthma without allergic rhinitis than in controls. The frequency of the rs2254546 A allele was higher in patients with asthma without allergic rhinitis than in controls, although the significance was marginal.

The frequencies of rs 1234315 and rs2254546 were significantly different between the asthmatic children with comorbid AR and controls (Table 3). Significantly increased prevalence of the rs 1234315 CT genotype was found in the patients compared to the controls (*p* = 5.8 × 10^{−4}, OR 6.82, 95% CI 2.29–20.36). The frequency of the rs2254546 AG genotype in the patients’ group was significantly lesser than that in the controls (*p* = 7.4 × 10^{−5}, OR 0.30, 95% CI 0.17–0.55). In addition, we found that the frequencies of rs1234313, rs1234314

and rs1600249 were different between the asthmatic children with comorbid AR and the control individuals (*p* = 0.008, OR 3.76, 95% CI 1.42–9.95; *p* = 0.005, OR 0.30, 95% CI 0.13–0.69; and *p* = 0.001, OR 0.25, 95% CI 0.11–0.57, respectively).

Haplotype frequencies in patients and controls

Using the solid spine of the LD method for haplotype block partitioning, three blocks were identified. As shown in Supplemental Figure S1, rs1234313, rs1234314 and rs1234315 (*D'* > 0.82) belonged to haplotype block 1 in TNFSF4, and they generated eight haplotypes. The haplotype GGC had higher frequency in the patients with asthma without allergic rhinitis (4.0%, Table 4) than in the controls (0.6%) and the distribution rates of the GCC and GCT were lower in cases than in controls (3.2% in cases and 11.7% in controls, 0.7% in cases and 3.8% in controls, respectively). The SNPs rs12039904, rs844648 and rs10912580 (*D'* > 0.88) belonged to haplotype block 2 in TNFSF4, and they generated eight haplotypes. The frequencies of CAG, TGA and TGG haplotypes were higher in the patients with asthma without allergic rhinitis than in the controls (5.0% in cases and 1.4% in controls, 3.8% in cases and 1.4% in controls, 3.8% in cases and 1.4% in controls, respectively). The SNPs rs2254546, rs13277113 and rs1600249 (*D'* > 0.93) belonged to block 1’ in FAM167A-BLK, and haplotypes A AC and GGC had higher frequencies (3.7% and 12.4%) in the children with asthma without allergic rhinitis than in the controls (0.6% and 4.4%). Haplotype GAA had a lower frequency (42.9%) in the patients than in controls (53.1%).

The haplotype frequencies in the asthma patients with comorbid AR and controls are summarized in Table 5. In block 1, the distribution rates of the AGT and GGC haplotypes were higher (23.4% in cases and 12.6% in controls, 3.3% in cases and 0.6% in controls, respectively) and those of GCT and GGT were lower (0.8% and 19.5%) in the patients than in the controls (3.8% and 26.2%). In block 2, the frequencies of TAA, TGA and TGG haplotypes were higher (6.5%, 5.4%, and 4.7%, respectively) in the patients and the frequency of the TAG haplotype was lower (16.2%) in the patients than in the controls (24.2%). In block 1’, the GGC haplotype frequency was obviously higher in the patients than in the controls (17.5% in cases and 4.4% in controls), and the distribution rates of AGC and GAA were lower (11.2% and 43.0%) in the patients than in the controls (19.0% and 53.1%).

Locus-locus and gene-gene interactions

After cross-validation and permutation tests of the gene-gene interactions in relation to the asthma without allergic

Table 2. Genotype frequencies of nine SNPs in controls and patients with asthma without allergic rhinitis.

SNP ID	Genotypes Allele	Asthma (%) (N = 141)	Control (%) (N = 252)	χ^2	p^*	Adjusted** OR (95% CI)	Unadjusted OR (95% CI)
rs1234313	GG	11 (7.8)	43 (17.1)			Reference	Reference
	AA	61 (43.3)	82 (32.5)	4.49	1.0×10^{-4}	6.16 (2.47–15.4)	3.01 (1.43–6.34)
	AG	69 (48.9)	127 (50.4)	0.08	0.02	2.80 (1.22–6.40)	2.16 (1.04–4.46)
	G	91 (32.3)	213 (42.3)			Reference	
rs1234314	A	191 (67.7)	291 (57.7)	7.61	1.3×10^{-5}	2.47 (1.65–3.71)	
	GG	33 (23.4)	47 (18.7)			Reference	Reference
	CC	39 (27.7)	78 (31.0)	0.47	0.11	0.41 (0.14–1.21)	0.71 (0.40–1.29)
	CG	69 (48.9)	127 (50.4)	0.08	0.004	0.28 (0.12–0.66)	0.77 (0.46–1.33)
rs1234315	G	135 (47.9)	221 (43.8)			Reference	
	C	147 (52.1)	283 (56.2)	1.18	0.023	0.57 (0.35–0.92)	
	TT	18 (12.8)	59 (23.4)			Reference	Reference
	CC	37 (26.2)	83 (32.9)	1.91	0.14	2.35 (0.75–7.37)	1.47 (0.76–2.82)
rs12039904	CT	86 (61.0)	110 (43.7)	10.87	6.6×10^{-4}	4.99 (1.98–12.62)	2.57 (1.41–4.67)
	T	122 (43.3)	228 (45.2)			Reference	
	C	160 (56.7)	276 (54.8)	0.29	0.040	1.71 (1.02–2.84)	
	TT	11 (7.8)	21 (8.3)			Reference	Reference
rs844648	CC	55 (39.0)	125 (49.6)	4.09	0.33	0.55 (0.16–1.84)	0.84 (0.38–1.86)
	CT	75 (53.2)	106 (42.1)	4.51	0.43	0.65 (0.22–1.91)	1.36 (0.62–2.98)
	T	97 (34.4)	148 (29.4)			Reference	
	C	185 (65.6)	356 (70.6)	2.13	0.33	0.77 (0.45–1.31)	
rs10912580	GG	39 (27.7)	72 (28.6)			Reference	Reference
	AA	28 (19.9)	56 (22.2)	0.30	0.52	0.73 (0.27–1.94)	0.92 (0.51–1.68)
	AG	74 (52.5)	124 (49.2)	0.39	0.29	0.70 (0.37–1.36)	1.11 (0.68–1.80)
	G	152 (53.9)	268 (53.2)			Reference	
rs13277113	A	130 (46.1)	236 (46.8)	0.04	0.70	0.94 (0.698–1.27)	
	GG	14 (9.9)	26 (10.3)			Reference	Reference
	AA	50 (35.5)	127 (50.4)	8.15	0.13	0.46 (0.17–1.27)	0.73 (0.35–1.51)
	AG	77 (54.6)	99 (39.3)	8.59	0.80	1.13 (0.45–2.82)	1.44 (0.71–2.95)
rs2254546	G	105 (37.2)	151 (30.0)			Reference	
	A	177 (62.8)	353 (70.0)	4.36	0.16	0.54 (0.33–0.89)	
	GG	61 (43.3)	153 (60.7)			Reference	Reference
	AA	5 (3.5)	19 (7.5)	2.52	0.08	0.29 (0.08–1.19)	0.66 (0.23–1.84)
rs1600249	AG	75 (53.2)	80 (31.7)	17.41	0.36	1.29 (0.75–2.22)	2.38 (1.54–3.67)
	G	197 (69.9)	386 (76.6)			Reference	
	A	85 (30.1)	118 (23.4)	4.27	0.012	1.53 (1.10–2.14)	
	GG	14 (9.9)	26 (10.3)			Reference	Reference
rs13277113	AA	38 (27.0)	120 (47.6)	16.07	0.18	0.44 (0.14–1.45)	0.60 (0.28–1.26)
	AG	89 (63.1)	106 (42.1)	16.04	0.74	0.83 (0.27–2.54)	1.58 (0.78–3.22)
	G	117 (41.5)	158 (31.3)			Reference	
	A	165 (58.5)	346 (68.7)	8.17	0.168	0.77 (0.52–1.12)	
rs1600249	CC	17 (12.1)	32 (12.7)			Reference	Reference
	AA	19 (13.6)	89 (35.3)	21.64	0.08	0.40 (0.14–1.11)	0.40 (0.19–0.87)
	AC	105 (74.5)	131 (52.0)	19.05	0.70	1.20 (0.48–2.96)	1.51 (0.79–2.87)
	C	139 (49.3)	195 (38.7)			Reference	
	A	143 (50.7)	309 (61.3)	8.32	0.085	0.72 (0.49–1.05)	

* p Value for Asthma without allergic rhinitis patients.

**OR values from logistic regression analyses, adjusted for age and gender.
OR, odds ratio.

rhinitis group, the best models included a 2-marker model (rs2254546 and rs13277113) with 63.78% balanced accuracy and a CVC of 9/10, a 3-marker model (rs2254546, rs13277113 and rs1600249) with 64.95% balanced accuracy and a 8/10 CVC, and a 4-marker model (rs1234315, rs2254546, rs13277113 and rs1600249) with 64.14% balanced accuracy and a 9/10 CVC (Table 6).

Regarding the role of these interactions in predicting asthma with comorbid AR, we identified a 2-marker model (rs2254546 and rs13277113) that had maximum CVC and a maximum balanced accuracy of 65.02%, a 3-marker model (rs2254546, rs13277113 and rs1600249) with 62.43% balanced accuracy and a 7/10 CVC, and a 4-marker model (rs12039904, rs844648, rs2254546 and rs13277113) with 62.59% balanced accuracy and a 5/10 CVC (Table 6).

Discussion

Single-nucleotide polymorphisms (SNPs) in the TNFSF4 gene have previously been shown to be associated with AR [33]. In the current study, we investigated the association of SNPs in TNFSF4 and FAM167A-BLK with asthma, whether or not with comorbid AR, in children in a Han Chinese population.

We found a significant difference between TNFSF4 and FAM167A-BLK polymorphisms in adults with AR in our previous study (600 AR patients and 700 controls, unpublished). The TNFSF4 gene is highly expressed by antigen-presenting cells, particularly dendritic cells [34]. In addition, B cells, macrophages and Langerhans cells can express TNFSF4 in inflammatory diseases [34]. The homologous receptor TNFRSF4, which binds TNFSF4, regulates cytokine

Table 3. Genotype frequencies of nine SNPs in controls and patients with asthma comorbid AR.

SNP ID	Genotypes Allele	Asthma-AR (%) (N = 149)	Control (%) (N = 252)	χ^2	p^*	Adjusted** OR (95% CI)	Unadjusted OR (95% CI)
rs1234313	GG	8 (5.4)	43 (17.1)			Reference	Reference
	AA	59 (39.6)	82 (32.5)	2.05	0.008	3.76 (1.42–9.95)	3.70 (1.62–8.49)
	AG	82 (55.0)	127 (50.4)	0.81	0.014	3.12 (1.26–7.77)	3.46 (1.55–7.75)
	G	98 (32.9)	213 (42.3)			Reference	
rs1234314	A	200 (67.1)	291 (57.7)	6.93	0.002	1.82 (1.23–2.65)	
	GG	35 (23.5)	47 (18.7)			Reference	Reference
	CC	33 (22.1)	78 (31.0)	3.63	0.01	0.24 (0.08–0.73)	0.59 (0.32–1.08)
	CG	81 (54.4)	127 (50.4)	0.59	0.005	0.30 (0.13–0.69)	0.87 (0.52–1.47)
rs1234315	G	151 (50.7)	221 (43.8)			Reference	
	C	147 (49.3)	283 (56.2)	3.50	0.002	0.44 (0.26–0.73)	
	TT	21 (14.1)	59 (23.4)			Reference	Reference
	CC	38 (25.5)	83 (32.9)	2.46	0.03	4.41 (1.18–16.56)	1.34 (0.71–2.52)
rs12039904	CT	90 (60.4)	110 (43.7)	10.51	5.8×10^{-4}	6.82 (2.29–20.36)	2.37 (1.33–4.21)
	T	132 (44.3)	228 (45.2)			Reference	
	C	166 (55.7)	276 (54.8)	0.07	0.029	1.81 (1.06–3.09)	
	TT	10 (6.7)	21 (8.3)			Reference	Reference
rs844648	CC	61 (40.9)	125 (49.6)	2.83	0.19	0.44 (0.13–1.53)	1.03 (0.46–2.33)
	CT	78 (52.3)	106 (42.1)	3.99	0.95	0.97 (0.31–3.00)	1.54 (0.68–3.46)
	T	98 (32.9)	148 (29.4)			Reference	
	C	200 (67.1)	356 (70.6)	1.09	0.019	0.53 (0.32–0.90)	
rs10912580	GG	47 (31.5)	72 (28.6)			Reference	Reference
	AA	30 (20.1)	56 (22.2)	0.24	0.91	1.06 (0.40–2.77)	0.80 (0.45–1.43)
	AG	72 (48.3)	124 (49.2)	0.03	0.06	0.56 (0.30–1.02)	0.86 (0.54–1.38)
	G	166 (55.7)	268 (53.2)			Reference	
rs10912580	A	132 (44.3)	236 (46.8)	0.48	0.42	0.89 (0.66–1.91)	
	GG	13 (8.7)	26 (10.3)			Reference	Reference
	AA	81 (54.4)	127 (50.4)	0.59	0.78	1.16 (0.41–3.28)	1.30 (0.63–2.69)
	AG	55 (36.9)	99 (39.3)	0.22	0.48	0.70 (0.26–1.87)	1.15 (0.54–2.42)
rs2254546	G	81 (27.2)	151 (30.0)			Reference	
	A	217 (72.8)	353 (70.0)	0.70	0.12	1.48 (0.89–2.22)	
	GG	109 (73.2)	153 (60.7)			Reference	Reference
	AA	11 (7.4)	19 (7.5)	0.003	0.03	0.28 (0.08–0.92)	0.79 (0.36–1.75)
rs13277113	AG	29 (19.5)	80 (31.7)	7.14	7.4×10^{-5}	0.30 (0.17–0.55)	0.51 (0.31–0.84)
	G	247 (82.9)	386 (76.6)			Reference	
	A	51 (17.1)	118 (23.4)	4.47	0.03	0.67 (0.46–0.97)	
	GG	15 (10.1)	26 (10.3)			Reference	Reference
rs1600249	AA	57 (38.3)	120 (47.6)	3.33	0.75	0.84 (0.27–2.50)	0.84 (0.41–1.71)
	AG	77 (51.7)	106 (42.1)	3.49	0.21	1.92 (0.69–5.32)	1.28 (0.63–2.59)
	G	107 (35.9)	158 (31.3)			Reference	
	A	191 (64.1)	346 (68.7)	1.76	0.97	1.01 (0.70–1.45)	
rs1600249	CC	38 (25.5)	32 (12.7)			Reference	Reference
	AA	44 (29.5)	89 (35.3)	1.42	0.001	0.25 (0.11–0.57)	0.41 (0.23–0.75)
	AC	67 (45.0)	131 (52.0)	1.85	0.001	0.28 (0.13–0.61)	0.45 (0.26–0.79)
	C	143 (48.0)	195 (38.7)			Reference	
	A	155 (52.0)	309 (61.3)	6.64	0.024	0.67 (0.47–0.95)	

*Asthma comorbid AR patients.

**OR values from logistic regression analyses, adjusted for age and gender.
OR, odds ratio.

production in various immune cells. Furthermore, the intracellular region of TNFRSF4 associates with TNFR-associated (TRAFs) factors, which can form a complex with inhibitor of NF- κ B, thereby allowing activation of both canonical and non-canonical NF- κ B signaling pathways [35]. Salman et al. [36] revealed that OX40 and OX40L expression was increased in bronchial submucosal tissue in mild asthmatic patients. Our results indicated that three SNPs in TNFSF4 (rs1234313, rs1234314 and rs1234315) were associated with asthma in a population of Han Chinese children. The SNP rs1234313, located in intron 1 from the promoter 5 kb upstream of the 5' end to 5 kb downstream of the 3' end of TNFSF4, is an important variant leading to susceptibility to SLE in Asians and Europeans [37]. In the current study, the AA genotype and the A allele of rs1234313 increased the risk of both asthma and AR, while the AG genotype decreased the risk of asthma without

allergic rhinitis but increased the risk of asthma with comorbid AR. Kong et al. [24] reported that the TNFSF4 tag SNP rs1234313 is not associated with increased susceptibility to pSS in Han Chinese populations. With regard to rs1234314, the CG genotype and the C allele are protective factors for asthma without allergic rhinitis, while the CC genotype and the C allele provide protection for asthma with comorbid AR. Similarly, Bossini-Castillo et al. [38] reported that SNPs in rs1234314 were associated with systemic sclerosis in a European cohort. The variant rs1234314 is 931 bp away from exon 1 b, which falls within a transcription factor-binding site, closest to the start of the exon in the upstream region of TNFSF4. Further, we found significant association between the CT genotype and the C allele of rs1234315 and susceptibility to both asthma and AR, while the CC genotype decreased the risk only for asthma with comorbid AR. The rs1234315 SNP is a T/C variation in a region

Table 4. Associations between common haplotypes and asthma risk.

Block	Asthma (%)	Control (%)	χ^2	<i>p</i>	Odds Ratio (95% CI)
Block1: (TNFSF4) rs1234313–rs1234314–rs1234315					
A C C	124.45 (44.1)	191.35 (38.0)	2.860	0.091	1.291 (0.960–1.735)
A C T	11.45 (4.1)	13.32 (2.6)	1.190	0.275	1.559 (0.698–3.483)
A G C	15.14 (5.4)	22.83 (4.5)	0.277	0.599	1.196 (0.614–2.329)
A G T	39.96 (14.2)	63.50 (12.6)	0.390	0.532	1.145 (0.748–1.753)
G C C	9.14 (3.2)	58.95 (11.7)	16.342	0.036	0.253 (0.124–0.516)
G C T	1.96 (0.7)	19.38 (3.8)	6.790	0.009	0.175 (0.040–0.766)
G G C	11.27 (4.0)	2.86 (0.6)	12.031	5.3×10^{-4}	7.284 (1.974–26.876)
G G T	68.63 (24.3)	131.80 (26.2)	0.313	0.576	0.908 (0.649–1.272)
Block2: (TNFSF4) rs12039904–rs844648–rs10912580					
C A A	40.19 (14.3)	95.09 (18.9)	2.704	0.100	0.715 (0.478–1.068)
C A G	14.22 (5.0)	7.11 (1.4)	9.037	0.003	3.712 (1.490–9.246)
C G A	114.99 (40.8)	239.07 (47.4)	3.238	0.072	0.763 (0.568–1.025)
C G G	15.60 (5.5)	14.73 (2.9)	3.319	0.069	1.945 (0.940–4.026)
T A A	11.03 (3.9)	11.79 (2.3)	1.587	0.207	1.700 (0.738–3.916)
T A G	64.56 (22.9)	122.02 (24.2)	0.173	0.677	0.930 (0.659–1.312)
T G A	10.79 (3.8)	7.05 (1.4)	4.802	0.028	2.803 (1.073–7.325)
T G G	10.61 (3.8)	7.14 (1.4)	4.510	0.033	2.721 (1.042–7.107)
Block3: (FAM167A-BLK) rs2254546–rs13277113–1600249					
A A A	5.59 (2.0)	1.12 (0.2)	—	—	—
A A C	10.34 (3.7)	3.18 (0.6)	10.119	0.001	6.115 (1.724–21.689)
A G A	3.39 (1.2)	17.81 (3.5)	3.619	0.0571	0.338 (0.105–1.090)
A G C	65.68 (23.3)	95.89 (19.0)	2.392	0.122	1.323 (0.927–1.887)
G A A	120.95 (42.9)	267.71 (53.1)	6.410	0.011	0.683 (0.509–0.918)
G A C	28.12 (10.0)	74.00 (14.7)	3.229	0.072	0.656 (0.414–1.041)
G G A	13.08 (4.6)	22.36 (4.4)	0.033	0.855	1.067 (0.531–2.146)
G G C	34.85 (12.4)	21.93 (4.4)	17.957	0.015	3.164 (1.814–5.517)

approximately 15 kb distant from the 5'-untranslated region of the TNFSF4 gene. A recent meta-analysis [39] showed significant association between SLE and the SNP rs1234315 in Korean subjects.

The function of FAM167A, also known as C8orf13, remains unknown. BLK is known to encode a nonreceptor tyrosine

kinase of the src family that is mainly involved in cell proliferation and differentiation. The protein plays a role in B-cell receptor signaling and B-cell development by activating NF- κ B [40], which shows high-level expression in the airways of asthmatic patients [41]. SNPs rs13277113 and rs2254546 lie in the intergenic region between FAM167A and BLK

Table 5. Associations between common haplotypes and asthma with AR risk.

Block	Asthma-AR (%)	Control (%)	χ^2	<i>p</i>	Odds ratio (95% CI)
Block1: (TNFSF4) rs1234313–rs1234314–rs1234315					
A C C	114.89 (38.6)	191.35 (38.0)	0.003	0.956	0.992 (0.738–1.333)
A C T	1.89 (0.6)	13.32 (2.6)	—	—	—
A G C	13.63 (4.6)	22.83 (4.5)	0.001	0.974	0.989 (0.498–1.965)
A G T	69.58 (23.4)	63.50 (12.6)	14.647	1.3×10^{-4}	2.066 (1.418–3.011)
G C C	27.75 (9.3)	58.95 (11.7)	1.315	0.251	0.757 (0.470–1.219)
G C T	2.46 (0.8)	19.38 (3.8)	6.648	0.010	0.204 (0.054–0.774)
G G C	9.72 (3.3)	2.86 (0.6)	8.551	0.003	5.783 (1.536–21.779)
G G T	58.06 (19.5)	131.80 (26.2)	5.306	0.021	0.664 (0.468–0.942)
Block2: (TNFSF4) rs12039904–rs844648–rs10912580					
C A A	56.27 (18.9)	95.09 (18.9)	0.008	0.927	1.017 (0.705–1.468)
C A G	7.98 (2.7)	7.11 (1.4)	—	—	—
C G A	125.17 (42.0)	V239.07 (47.4)	1.809	0.179	0.819 (0.612–1.096)
C G G	10.59 (3.6)	14.73 (2.9)	0.276	0.599	1.240 (0.555–2.769)
T A A	19.34 (6.5)	11.79 (2.3)	8.896	0.002	2.940 (1.404–6.159)
T A G	48.41 (16.2)	122.02 (24.2)	6.672	0.009	0.616 (0.425–0.891)
T G A	16.23 (5.4)	7.05 (1.4)	11.125	0.001	4.116 (1.680–10.087)
T G G	14.02 (4.7)	7.14 (1.4)	8.073	0.005	3.483 (1.398–8.680)
Block3: (FAM167A-BLK) rs2254546–rs13277113–rs1600249					
A A A	5.70 (1.9)	1.12 (0.2)	—	—	—
A A C	6.17 (2.1)	3.18 (0.6)	—	—	—
A G A	5.61 (1.9)	17.81 (3.5)	1.622	0.203	0.541 (0.207–1.413)
A G C	33.52 (11.2)	95.89 (19.0)	7.387	0.007	0.559 (0.366–0.854)
G A A	128.07 (43.0)	267.71 (53.1)	5.653	0.017	0.702 (0.524–0.940)
G A C	51.06 (17.1)	74.00 (14.7)	1.254	0.263	1.250 (0.846–1.847)
G G A	15.62 (5.2)	22.36 (4.4)	0.383	0.536	1.233 (0.635–2.393)
G G C	52.25 (17.5)	21.93 (4.4)	40.949	7.8×10^{-5}	4.866 (2.885–8.206)

AR, allergic rhinitis.

Table 6. MDR analysis summary.

Best candidate model	Testing balanced accuracy (%)	Permutation <i>p</i> value	Cross-validation consistency
Simple asthma versus HC			
rs1600249	53.47%	0.526	6/10
rs2254546 rs13277113	63.78%	0.002	9/10
rs2254546 rs13277113 rs1600249	64.95%	0.001	8/10
rs1234315 rs2254546 rs13277113 rs1600249	64.14%	0.002	9/10
Asthma-AR versus HC			
rs1234315	55.1%	0.333	9/10
rs 2254546 rs 13277113	65.02%	0.001	10/10
rs2254546 rs13277113 rs1600249	62.43%	0.005	7/10
rs12039904 rs844648 rs2254546 rs13277113	62.59%	0.004	5/10

HC, healthy control; AR, allergic rhinitis; MDR, multifactor dimensionality reduction.

at 8p22-23, a region harboring SNPs associated with several autoimmune diseases. Chen et al. [42] suggested that the SNP rs13277113, but not rs2254546, in FAM167A-BLK is associated with polymyositis/dermatomyositis. Another study has shown that the rs2254546 genetic variation was significantly associated with Kawasaki disease susceptibility [43]. The variant rs1600249 is located in an intron region and might serve as a cis-effect regulator of rheumatoid arthritis (RA)-associated genes. Deng et al. [44] have provided functional evidence for contribution of SNP rs1600249 to susceptibility to RA. In the current study, only rs2254546 had a weak relationship with asthma without allergic rhinitis. However, the SNPs rs2254546 and rs1600249 had an association with asthma with comorbid AR. With regard to rs2254546, the AA and AG genotypes and the A allele decreased the risk of asthma with comorbid AR. For rs1600249, the AA and AC genotypes and the A allele were protective factors for asthma with comorbid AR. The SNP rs13277113 did not exhibit significant correlation with asthma and AR in our population.

Haplotype analysis of the TNFSF4 gene revealed that the GCC and GCT haplotypes in block 1 conferred decreased risk of asthma without allergic rhinitis, whereas the haplotype GGC in block 1 and the CAG, TGA and TGG haplotypes in block 2 were associated with susceptibility to asthma without allergic rhinitis. We found that the AGT and GGC haplotypes in block 1 and TAA, TGA and TGG in block 2 increased the risk of asthma with comorbid AR, while the GCT and GGT haplotypes in block 1 and TAG in block 2 were protective factors for asthma with comorbid AR. Haplotype analysis of the FAM167A-BLK gene suggested that GAA conferred reduced risk for asthma without allergic rhinitis, whereas the haplotypes AAC and GGC from block 1' were associated with susceptibility to asthma without allergic rhinitis. Further, we found that the AGC and GAA haplotypes provided protection for asthma with comorbid AR, while GGC increased the risk of asthma with comorbid AR.

As is well known, asthma and AR are polygenic inheritance diseases. The identification of gene-gene interactions is important because these may more accurately predict the disease risk than a single gene. In our study, MDR analysis was used to predict potential gene-gene interactions that may partly determine the complex pathogenesis of asthma and AR. Our results showed that rs1234315, rs2254546, rs13277113 and rs1600249 were associated with asthma without allergic rhinitis, whereas rs2254546 and rs13277113 were associated with asthma with comorbid AR. We also found unique sets of

polymorphisms that had synergistic effects between TNFSF4 and FAM167A-BLK. The development and progression of all atopic diseases are complicated and the temporal pattern described in the atopic march [45,46] may not be a simple progression from atopic dermatitis to AR to asthma. Therefore, more research in this field is needed, including analysis of the gene-gene and gene-environment interactions in asthma and AR cohorts.

In the current study, we made many efforts to decrease the impact of possible confounding factors on the results. We selected all patients and controls using strict guidelines and confirmed the genotyping results by direct sequencing. We revealed novel relationships between gene polymorphisms of TNFSF4 (rs1234313, rs1234314 and rs1234315) and FAM167A-BLK (rs2254546 and rs 1600249) with asthma and AR in a Han Chinese population. However, our study has some limitations. First, our study population was restricted to a Han Chinese cohort. Research on multiethnic populations may be useful to confirm our findings on the association of TNFSF4 and FAM167A-BLK with asthma and AR. Second, we neither investigated the protein levels of TNFSF4 and FAM167A-BLK in the peripheral blood nor did we perform a functional analysis study. Third, we did not study the SNPs associations among various forms of asthma (atopic, non-atopic, controlled and uncontrolled) and the possible relationship between the degree of asthma control and even therapeutic strategy. Therefore, we cannot draw a definitive conclusion on the influence of the studied polymorphisms on the cytokine levels.

Conclusions/key findings

In summary, our results suggested that SNPs in TNFSF4 (rs1234313, rs1234314 and rs1234315) and FAM167A-BLK (rs2254546 and rs 1600249) are risk factors for developing asthma and AR in Han Chinese. To the best of our knowledge, this is the first study to investigate the interactive associations of TNFSF4 and FAM167A-BLK gene polymorphisms with asthma and AR. However, more advanced techniques and analysis methods such as MassArray, genome-wide association studies and gene-environment interaction analysis are required to fully explain the underlying molecular mechanisms. Our study provides a first step in understanding the relation between genetic variability in the TNFSF4 and FAM167A-BLK genes and offers incentive for further research on asthma and AR.

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Declaration of interest

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Supplementary material available online

Supplementary Table S1–S2