Association between TNFSF4 and BLK gene polymorphisms and susceptibility to allergic rhinitis

YANG SHEN[1*], YUN LIU[1,2*], XIAO-QIANG WANG[1], XIA KE[1], HOU-YONG KANG[1] and SU-LING HONG[1]

[1]Department of Otorhinolaryngology, The First Affiliated Hospital of Chongqing Medical University and [2]Chongqing Key Laboratory of Ophthalmology, Chongqing Medical University, Chongqing 400016, P.R. China

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Abstract. Allergic rhinitis (AR) is a common inflammatory disease of the upper airway. Recent evidence suggests that gene-gene interactions between tumor necrosis factor receptor superfamily 4 (TNFSF4) and B cell lymphocyte kinase (BLK) may have a synergistic effect on T and B cells in determining immunologic aberration, via the nuclear factor-κB pathway. The present study was performed to evaluate the potential association between specific single nucleotide polymorphisms (SNPs) in the TNFSF4 and BKL genes with susceptibility to AR in Chinese subjects. A population-based case-control study was performed in 600 Chinese AR patients and 700 controls. Blood was drawn for DNA extraction, and 9 SNPs (6 in TNFSF4 and 3 in BKL genes) were selected and genotyped. The TNFSF4 SNPs rs1234314 and rs1234315, and the BLK SNPs rs13277113 and rs1600249 were observed to occur in different frequencies between the AR patients and the controls. The CC (rs1234314, rs1234315) and AA (rs1600249, rs13277113) genotypes provided protective effects against AR, whereas the AG (rs13277113) genotype presented a risk factor for AR. The haplotypes ACC in the rs1234313-rs1234314-rs1234315 block and GA in the rs2254546-rs13277113 block significantly decreased the risk of AR, whereas the GGT and AG haplotypes served protective roles. SNP interaction analysis further indicated that there may be synergistic effects among the selected sets of polymorphisms. The present study suggests a novel association between specific TNFSF4 and BLK gene polymorphisms and AR risk, highlighting their potential utility as genetic biomarkers for AR susceptibility in a Chinese Han population.

Correspondence to: Professor Su-Ling Hong, Department of Otorhinolaryngology, The First Affiliated Hospital of Chongqing Medical University, 1 Yixueyuan Road, Chongqing 400016, P.R. China
E-mail: hsl_prof@sina.cn

*Contributed equally

Abbreviations: AR, allergic rhinitis; BLK, B cell lymphocyte kinase; TNFSF4, tumor necrosis factor receptor superfamily 4; SNP, single nucleotide polymorphism; SLE, Systemic Lupus Erythematosus; SPT, skin prick test; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HWE, Hardy-Weinberg equilibrium; OR, odds ratios; CI, confidence interval; LD, linkage disequilibrium

Key words: tumor necrosis factor receptor superfamily 4, B cell lymphocyte kinase, polymorphism, allergic rhinitis, susceptibility

Introduction

Allergic rhinitis (AR) is a common inflammatory disorder of the upper airway, which has an estimated worldwide incidence rate of 10-20% (1). Over the last two decades the pathogenesis of AR has been widely studied, and genetic factors are considered to be major players affecting the development, severity and treatment of AR (2). The single nucleotide polymorphisms (SNPs) of important cytokines or genes may predict susceptibility to or clinical features of AR. Several loci and candidate genes have been reported to be associated with AR (3-5). Our recent studies demonstrated associations between polymorphisms in interleukin (IL)-23R, Fc receptor-like 3 gene and IL-27 with AR risk in Chinese subjects (6-8). However, the details of AR pathogenesis currently remain unclear.

Tumor necrosis factor receptor superfamily 4 (TNFSF4, also known as OX4OL) belongs to the TNF superfamily, and is expressed on dendritic cells, macrophages, cluster of differentiation (CD)4^+/$CD8^+$ T cells, activated NK cells and other cells (9,10). Interaction between TNFSF4 and its binding partner OX40 provides a costimulatory signal, resulting in T cell proliferation, differentiation and cytokine production (11,12). Recent studies have indicated that TNFSF4 and OX40 interaction may promote the T-helper (Th)2 response, depress IL-17 production and inhibit the differentiation of regulatory T cells (13-15). Therefore, TNFSF4 is regarded as an important cytokine in the pathogenic mechanisms of immune-related disorders.

B cell lymphocyte kinase (BLK) is a tyrosine kinase of the src family with highly restricted B lymphocyte expression. BLK participates in signal transduction downstream of the B-cell receptor; therefore, it may influence the proliferation and differentiation of B cells (16). B cells serve critical roles in the pathogenesis of immune-related disorders via antigen presentation to T cells, antibody production and cytokine secretion. Therefore, it may be hypothesized that the BLK

protein may have an impact on the immune mechanisms of B cells, and participate in the adaptive immune response.

Although the pathogenic mechanism of AR is not completely understood, it is known to be associated with a dysfunctional immune system, and involves T and B cell responses. Recent research indicated that gene-level interaction between BLK and TNFSF4 may have a synergistic effect on T cells and B cells via the nuclear factor (NF)-κB pathway, and this may have a role in determining immunologic aberration (17). Furthermore, previous studies have reported that TNFSF4 and BLK polymorphisms may contribute to the pathogenesis of further immune-related diseases, including primary Sjogren's syndrome (18,19) and Systemic Lupus Erythematosus (SLE) (20).

The present study hypothesized that TNFSF4 and BLK genes may participate in NF-κB pathway regulation, and may contain SNPs that are associated with AR risk. Therefore, the association between TNFSF4 and BLK polymorphisms and AR susceptibility were examined in a Han Chinese population.

Materials and methods

Ethics statement. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). All participants were from Chongqing and were of the Han Chinese ethnic origin. Informed consent was obtained from the next of kin, caretakers or guardians of minors and children participating in the study.

Subjects. A total of 600 patients (296 men, 304 women; age range, 6-81 years) were recruited from April 2013 to June 2014. All patients were enrolled and treated at the outpatient clinic of the Department of Otolaryngology Head and Neck Surgery at the First Affiliated Hospital of Chongqing Medical University. AR diagnoses were based on medical history, symptoms and positive skin prick test (SPT; Allergopharma GmbH & Co., KG, Reinbek, Germany) according to ARIA 2008 guidelines (21). A total of 18 inhaled allergens were tested, including house dust, pollen, grass, tree, mold, food, cat and dog dander, cockroaches, feathers, cotton, cigarettes, penicillin, milk, shrimp, egg, soybean and peanut. SPT results were diagnosed in accordance with the recommendations of the Subcommittee on Allergen Standardization and Skin Tests of the European Academy of Allergy and Clinical Immunology (22). AR patients with chronic sinusitis, asthma, hypertension, diabetes or any other systemic disease were excluded from the study. A total of 700 healthy volunteers of the same ethnicity as the patients were recruited as the control group from the Department of Physical Examination at the First Affiliated Hospital of Chongqing Medical University (Chongqing, China), from April 2013 to October 2013. The selection criteria for healthy volunteers were as follows: No chronic pathology, in particular, no history of allergy or respiratory pathology, no other systemic diseases and no family history of allergy. The clinical features of the study cohort are described in Table I.

SNP selection and DNA extraction. Nine SNPs in the TNFSF4 (rs1234313, rs1234314, rs1234315, rs12039904, rs844648, rs10912580) and BLK (rs1600249, rs13277113, rs2254546) genes were analyzed as candidate sites (Table II). SNPs were selected on the basis of recent reports, which demonstrated their possible functional effect in immune-related diseases (23-27). Genomic DNA was isolated from EDTA-anticoagulated peripheral blood leukocytes, using a Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. Briefly, 300 μl blood was mixed with cell lysis solution. Leucocytes were spun down at 11,100 x g for 1 min at room temperature and lysed with the nuclei lysis solution, and the pellet was separated using the protein precipitation solution. Precipitated proteins were removed by centrifugation at 16,000 x g for 30 sec at room temperature. Two-filar DNA was subsequently separated out by methyl alcohol. The DNA on the EP tube was dissolved in 100 μl DNA rehydration solution.

Genotyping. SNPs were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. Amplification was performed using initial denaturation at 95˚C for 4 min, followed by 37 cycles of 95˚C for

Table I. Clinical features and demographic characteristics of the study population.

Characteristic	Value
Allergic rhinitis (n=600)	
Gender (male/female)	296/304
Age [mean (range)] years	33.06 (6.5-81)
Allergen	
House dust mite	377
Pollen	94
Multiple allergens	129
Control (n=700)	
Gender (male/female)	343/357
Age [mean (range)] years	31.28 (9-78)

Table II. Characteristics of the studied SNPs in TNFSF4 and BLK genes.

Chromosome	SNP ID	Location	Alleles
TNFSF4			
1	rs1234313	Intron region	A/G
1	rs1234314	Upstream region	C/G
1	rs1234315	Upstream region	C/T
1	rs12039904	Intron region	C/T
1	rs844648	Intron region	A/G
1	rs10912580	Intron region	A/G
BLK			
8	rs1600249	Intron region	A/C
8	rs13277113	Promoter region	A/G
8	rs2254546	Intergenic region	A/G

TNFSF4, tumor necrosis factor receptor superfamily 4; BLK, B cell lymphocyte kinase; SNP, single nucleotide polymorphism.

Table III. Primer sequences, PCR conditions and restriction enzymes used for PCR-restriction fragment length polymorphism analysis of the tumor necrosis factor receptor superfamily 4 and B-cell lymphocyte kinase polymorphisms.

SNP reference	Primer sequence	Annealing temperature (˚C)	Restriction enzyme
rs1234313	F: 5'CCTACCATGTCTCAAACATAATGGCAC-3' R: 5'TGTCTTCCACAGTCCTCTACAATGGTT-3'	60	*Tai*I
rs1234315	F: 5'CACCAGGCTGGAAGTTTCAGGC-3' R: 5'TAGCCAGACCTGGTGTTGCGTG-3'	62	*Tsp*RI
rs1234314	F: 5'ACCAGGTACCCTTTACCACTAAAATAAAC-3' R: 5'TCTCCCTCCTTTCTTTACATATCTGCT-3'	60	*Mva*I
rs12039904	F: 5'TGCTCATAGTTGCTTAATGC-3' R: 5'AATAATCAGGCTGTGGAAAC-3'	60	*Mn1*I
rs844648	F: 5'AGTTACACTATGTGGCGTTTA-3' R: 5'CAGGCAGTTCCTCTTTGAT-3'	54	*Ase*I
rs10912580	F: 5'GCAAGACCCTGACTCAAAAATAAAATAA-3' R: 5'TTGCCTTAGTAGATATCACTGAACGC-3'	60	*Acc*I
rs1600249	F: 5'ACAACTAAATGGATTCTATTAAAACAAAGG-3' R: 5'GTTCCTGTTGTATAAATACTCCCACC-3'	60	*Dra*I
rs13277113	F: 5'ACCATTCCCATTAGGTAACCT-3' R: 5'CAATAAAGTAAGTGGAAGAAACATAAA-3'	58	*Bsa*BI
rs2254546	F: 5'GAAAATGGCTCTGAGAGAACTCCAAAGT-3' R: 5'ATTGGCGAAGACTCTGTGGTATTCAGT-3'	60	*Eco*NI

PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; F, forward; R, reverse.

40 sec, 56-60˚C for 40 sec and 72˚C for 40 sec, followed by a final extension at 72˚C for 4 min. The primer sequences and reaction conditions used in the present study are provided in Table III. The PCR products were incubated with restriction enzymes for ≥4 h. The selected SNP genotyping was performed using the Sequenom MassARRAY iPLEX Gold platform (Sequenom Laboratories, San Diego, CA, USA) according to the manufacturer's instructions. To verify the genotyping results, PCR-amplified DNA samples were examined by direct sequencing (20% of all the blood samples). The sequencing PCR reaction system included 30 μl Taq enzyme (Go Taq® Green Master Mix; Promega Corporation), 20 μl enzyme free water and 5 μl primer pairs (forward, 2.5 μl; reverse, 2.5 μl). Amplification of the target fragments by PCR and the results were read by Chromas 2.1.1 software (Technelysium Pty Ltd., South Brisbane, Australia). The results of RFLP and direct sequencing were 100% concordant.

Statistical analysis. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate statistical significance. To evaluate the quality of the genotyping data, the Hardy-Weinberg equilibrium for SNP genotype frequencies was tested using a Chi-square test (χ^2 test). Allelic and genotypic frequencies between patients with AR and the control patients 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 and probability values. The association between genotypes/alleles and AR risk was estimated by calculating the odds ratios (OR) and 95% confidence intervals (CI). Additionally, pairwise linkage disequilibrium (LD) among the SNPs was calculated according to the genotype correlation coefficient (r^2). The r^2 values were calculated using Haploview v4.2, with default settings (CI for a strong LD was minimal for upper 0.98 and low 0.7, and maximal for a strong recombination of 0.9, a fraction of strong LD in informative comparisons was ≥0.95) (28).

To evaluate the synergistic relationships between the TNFSF4 and BLK polymorphisms and the risk of AR, the multifactor dimensionality reduction (MDR) method was used, to detect and characterize locus-locus and gene-gene interaction models (27). Each best model was tested for accuracy, cross-validation consistency and significance level, determined using permutation testing, testing accuracy and testing OR (95% CI). Cross-validation consistency (CVC) was defined as the number of cross-validation replicates (partitions) for which the same n-locus model was chosen as the best model (i.e., the number of replicates within which the classification error was minimized).

Results

Clinical features of the participants. The clinical characteristics of the subjects are presented in Table I. The AR patients and the controls were similar in terms of gender distribution ($P>0.05$) and mean age ($P>0.05$). A total of 377 (62.8%) patients were recorded as sensitive to house dust mite, 94 (15.5%) were sensitive to tree pollen and 129 (21.5%) were sensitive to multiple allergens.

Genotype distribution of the TNFSF4 polymorphisms. The genotype distribution of all 9 analyzed SNPs in the AR group

Table IV. The genotype and allele frequencies of tumor necrosis factor receptor superfamily 4 polymorphisms in AR patients and controls.

Genotype allele	AR (%)	Control (%)	χ^2	P-value (unadjusted)	OR (95% CI)
rs1234313					
AA	222 (37.0)	294 (42.0)	3.37	1.26	0.81 (0.65-1.01)
AG	319 (53.2)	306 (43.7)	11.56	0.02	1.46 (1.17-1.82)
GG	59 (9.8)	100 (14.3)	5.97	0.27	0.65 (0.47-0.92)
A	763 (63.6)	894 (63.9)	0.02	5.34	0.99 (0.84-1.16)
G	437 (36.4)	506 (36.1)	0.02	5.34	1.01 (0.86-1.19)
rs1234315					
CC	135 (22.5)	233 (33.3)	18.52	$3.06x10^{-4}$	0.58 (0.45-0.75)
CT	334 (55.7)	330 (47.1)	9.39	0.036	1.41 (1.13-1.75)
TT	131 (21.8)	137 (19.6)	1.01	5.67	1.15 (0.88-1.50)
C	604 (50.3)	796 (56.9)	11.07	$5.51x10^{-3}$	0.77 (0.66-0.90)
T	596 (49.7)	604 (43.1)	11.07	$5.51x10^{-3}$	1.30 (1.11-1.52)
rs1234314					
CC	106 (17.7)	192 (27.4)	17.43	$5.40x10^{-4}$	0.57 (0.43-0.74)
CG	302 (50.3)	328 (46.9)	1.56	3.79	1.15 (0.92-1.43)
GG	192 (32.0)	180 (25.7)	6.25	0.22	1.36 (1.07-1.73)
C	514 (42.8)	712 (50.9)	16.69	$2.64x10^{-4}$	0.72 (0.62-0.84)
G	686 (57.2)	688 (49.1)	16.69	$2.64x10^{-4}$	1.38 (1.18-1.61)
rs12039904					
CC	282 (47.0)	380 (54.3)	6.86	0.16	0.75 (0.60-0.93)
CT	275 (45.8)	276 (39.4)	5.43	0.36	1.30 (1.04-1.62)
TT	43 (7.2)	44 (6.3)	0.4	9.47	1.15 (0.75-1.78)
C	839 (69.9)	1036 (74.0)	5.36	0.12	0.82 (0.69-0.97)
T	361 (30.1)	364 (26.0)	5.36	0.12	1.23 (1.03-1.45)
rs844648					
AA	110 (18.3)	152 (21.7)	2.3	2.34	0.81 (0.62-1.06)
AG	302 (50.3)	345 (49.3)	0.14	12.71	1.04 (0.84-1.30)
GG	188 (31.3)	203 (29.0)	0.84	6.48	1.12 (0.88-1.42)
A	522 (43.5)	649 (46.4)	2.13	0.86	0.89 (0.76-1.04)
G	678 (56.5)	751 (53.6)	2.13	0.86	1.12 (0.96-1.31)
rs10912580					
AA	354 (59.0)	383 (54.7)	2.42	2.16	1.19 (0.96-1.49)
AG	222 (37.0)	280 (40.0)	1.23	4.82	0.88 (0.70-1.10)
GG	24 (4.0)	37 (5.3)	1.19	4.93	0.75 (0.44-1.26)
A	930 (77.5)	1046 (74.7)	2.75	0.58	1.17 (0.97-1.40)
G	270 (22.5)	354 (25.3)	2.75	0.58	0.86 (0.72-1.03)

AR, allergic rhinitis; OR, odds ratio; CI, confidence interval; P, probability.

and the controls were revealed to be in Hardy-Weinberg equilibrium (P>0.05). The TNFSF4 genotype and allele frequencies are presented in Table IV. The rs1234315T allele demonstrated a significantly increased prevalence in AR cases (49.7%) compared with the controls (43.1%), demonstrating a statistical association between the rs1234315T allele and AR susceptibility ($P=5.51x10^{-4}$, OR=1.30, 95% CI=1.11-1.52). Similarly, the rs1234314G allele was associated with a higher risk of AR ($P=2.64x10^{-4}$, OR=1.38, 95% CI=1.18-1.61). However, the C allele and CC genotypes of rs1234315 and rs123431 demonstrated lower prevalence in AR patients, compared with the controls ($P=5.51x10^{-3}$, OR=0.77, 95% CI=0.66-0.90; $P=3.06x10^{-4}$, OR=0.58, 95% CI=0.45-0.75; $P=2.64x10^{-4}$, OR=0.72, 95% CI=0.62-0.84; $P=5.40x10^{-4}$, OR=0.57, 95% CI=0.43-0.74; respectively).

Genotype distribution of the BLK polymorphisms. The BLK genotype and allele frequencies are presented in Table V.

Table V. The genotype and allele frequencies of B-cell lymphocyte kinase polymorphisms in patients with AR and controls.

Genotype allele	AR (%)	Control (%)	χ^2 value	P-value (unadjusted)	OR (95% CI)
rs1600249					
AA	198 (33.0)	288 (41.1)	9.15	0.02	0.71 (0.56-0.88)
AC	330 (55.0)	338 (48.3)	5.53	0.17	1.30 (1.04-1.62)
CC	72 (12.0)	74 (10.6)	0.66	3.74	1.15 (0.82-1.63)
A	726 (60.5)	914 (65.3)	6.35	0.04	0.81 (0.69-0.96)
C	474 (39.5)	486 (34.7)	6.35	0.04	1.23 (1.05-1.44)
rs13277113					
AA	157 (26.2)	259 (37.0)	17.43	$2.7x10^{-4}$	0.60 (0.48-0.77)
AG	342 (57.0)	327 (46.7)	13.68	$1.8x10^{-3}$	1.51 (1.21-1.88)
GG	101 (16.8)	114 (16.3)	0.07	7.12	1.04 (0.78-1.40)
A	656 (54.7)	845 (60.4)	8.57	$9x10^{-3}$	0.79 (0.68-0.93)
G	544 (45.3)	555 (39.6)	8.57	$9x10^{-3}$	1.26 (1.08-1.48)
rs2254546					
AA	19 (3.2)	29 (4.1)	0.87	3.17	0.76 (0.42-1.36)
AG	225 (37.5)	225 (32.1)	4.1	0.39	1.27 (1.01-1.59)
GG	356 (59.3)	446 (63.7)	2.62	0.95	0.83 (0.66-1.04)
A	263 (21.9)	283 (20.2)	1.13	0.86	1.11 (0.92-1.34)
G	937 (78.1)	1117 (79.8)	1.13	0.86	0.90 (0.75-1.09)

AR, allergic rhinitis; OR, odds ratio; CI, confidence interval; χ^2, Chi-squared; P, probability.

The frequencies of rs13277113 and rs1600249 were significantly different between the AR cases and the controls. An increased prevalence of the rs13277113G allele and the AG genotype was observed in the AR group, compared with the controls ($P=9.0x10^{-3}$, OR=1.26, 95% CI=1.08-1.48; $P=1.8x10^{-3}$, OR=1.51, 95% CI=1.21-1.88; respectively). However, the frequencies of the A allele and the AA genotype were significantly lower in the AR patients ($P=9x10^{-3}$, OR=0.79, 95% CI=0.68-0.93; $P=2.7x10^{-4}$, OR=0.60, 95% CI=0.48-0.77). A higher frequency of the rs1600249C allele (P=0.04, OR=1.23, 95% CI=1.05-1.44), and a lower frequency of the A allele and the AA genotype (P=0.04, OR=0.04, 95% CI=0.69-0.96; P=0.02, OR=0.71, 95% CI=0.56-0.88) were observed in AR patients, compared with the controls.

Frequencies of haplotypes between AR cases and controls. Plots of the pair-wise LD [r^2 and coefficient of linkage disequilibrium (D')] values for the tag SNPs and the LD structures for the selected region of the chromosome are presented in Fig. 1. The relationships between AR risk and the haplotype frequencies in blocks rs1234313-rs1234314-rs1234315 and rs2254546-rs13277113 are summarized in Table VI. The results indicated that the distribution rate of the GGT haplotype was markedly increased in AR patients (11.4%), compared with the controls (6.2%) ($P=2.87x10^{-8}$, OR=1.940, 95% CI=1.465-2.570). However, the distribution rate of the ACC haplotype was significantly reduced in AR cases (12.2%), compared with controls (17.7%) (P=0.0001, OR=0.650, 95% CI=0.521-0.810). The AG haplotype demonstrated a significantly higher frequency in AR patients (9.3%) compared with controls (6.7%) (P=0.013, OR=1.436, 95% CI=1.079-1.912), whereas the distribution rate of the GA haplotype was significantly reduced (42.1% in cases and 46.8% in controls; P=0.015, OR=0.825, 95% CI=0.706-0.964).

Locus-locus and gene-gene interactions. The best interaction models determined by the MDR analysis for all 9 SNPs analyzed in TNFSF4 and BLK are presented in Table VII. Following cross-validation and permutation tests of the gene-gene interactions in relation to AR, 3 best models were revealed, and these demonstrated interactive effects. The best models included a two-marker model (rs13277113-rs1600249, testing accuracy=60.63%; CVC=9/10; P=0.037), a three-marker model (rs1234314-rs13277113-rs1600249, testing accuracy=61.65%; CVC=6/10; P=0.022), and a four-marker model (rs1234314-rs10912580-rs13277113-rs1600249, testing accuracy=62.69%; CVC=10/10; P=0.013). A dendogram of the markers in the four-marker model is presented in Fig. 2. The presence of the blue line indicates that the four-locus model may have a redundancy interaction effect on modulating the risk of AR.

Discussion

The present study demonstrated a novel contribution of TNFSF4 and BLK polymorphisms towards the risk of AR in a Han Chinese population. The results demonstrated that the CC (rs1234314, rs1234315) and AA (rs1600249, rs13277113) genotypes were statistically associated with protective effects against AR. However, the AG genotype (rs13277113) presented a risk factor for AR. The ACC haplotype in block rs1234313-rs1234314-rs1234315 and the GA haplotype in

Table VI. Frequencies of the haplotypes formed by the rs1234313-rs1234314-rs1234315 and the rs2254546-rs13277113 SNPs in patients with AR and healthy controls.

Haplotype	AR (%)	Control (%)	χ^2 value	P value	OR (95% CI)
ACC	146.72 (0.122)	247.19 (0.177)	14.819	0.0001	0.650 (0.521-0.810)
ACT	171.01 (0.143)	193.91 (0.139)	0.086	0.7694	1.034 (0.828-1.290)
AGC	245.69 (0.205)	241.68 (0.173)	4.375	0.0365	1.234 (1.013-1.503)
AGT	199.58 (0.166)	211.23 (0.151)	1.158	0.2819	1.123 (0.909-1.386)
GCC	107.09 (0.089)	158.72 (0.113)	4.1	0.0429	0.766 (0.592-0.992)
GCT	89.18 (0.074)	112.18 (0.080)	0.306	0.5804	0.922 (0.690-1.231)
GGC	104.50 (0.087)	148.41 (0.106)	2.635	0.1046	0.804 (0.618-1.047)
GGT	136.23 (0.114)	86.69 (0.062)	21.953	$2.87x10^{-8}$	1.940 (1.465-2.570)
AA	150.99 (12.6)	189.36 (13.5)	0.505	0.477	0.920 (0.732-1.157)
AG	112.01 (9.3)	93.64 (6.7)	6.209	0.013	1.436 (1.079-1.912)
GA	505.01 (42.1)	655.64 (46.8)	5.892	0.015	0.825 (0.706-0.964)
GG	431.99 (36.0)	461.36 (33.0)	2.656	0.103	1.144 (0.973-1.346)

AR, allergic rhinitis; OR, odds ratio; CI, confidence interval; χ^2, Chi-squared; P, probability.

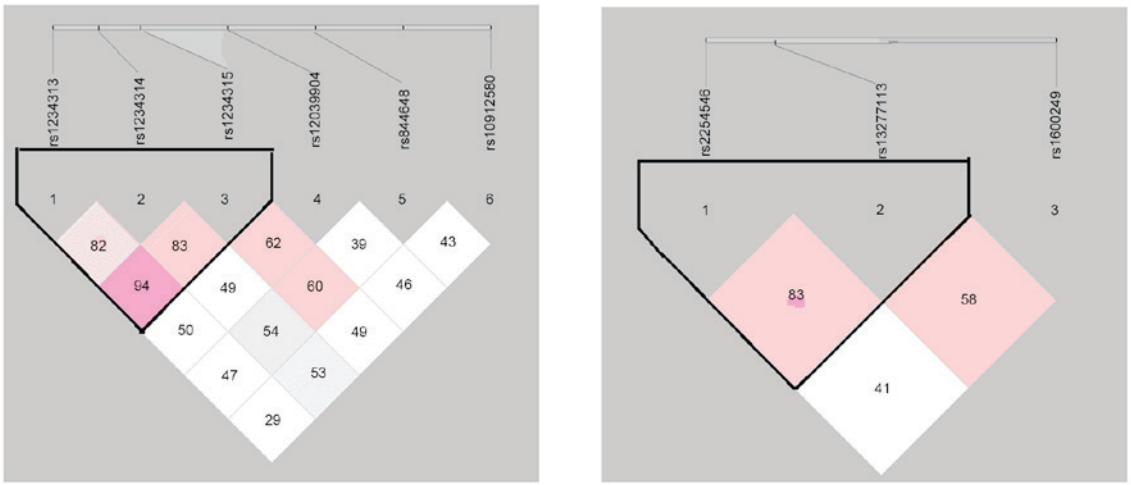

Figure 1. The Haplotype-block of the single nucleotide polymorphisms in tumor necrosis factor receptor superfamily 4 and B cell lymphocyte kinase gene linkage disequilibrium tests. The color of the box represents the D' value and the number in the box represents the r^2 value; the darker the color the greater the strength of the linkage disequilibrium.

block rs2254546-rs13277113 significantly decreased the risk of AR, whereas the GGT and AG haplotypes served protective roles. Our results suggest that specific SNPs in the TNFSF4 and BLK genes may modify the risk of suffering from AR.

TNFSF4 regulates the differentiation and proliferation of Th cells in different cytokine microenvironments (29). The interaction between TNFSF4 and OX40 serves an important role at critical immunoregulatory checkpoints, which are likely involved in the development of immunorelated diseases, such as inflammatory and autoimmune diseases and tumors. Previous studies have demonstrated that TNFSF4 gene polymorphisms are associated with SLE, systemic sclerosis, breast cancer and myocardial infarction (30-34). The present study demonstrated an association between the rs1234314 and rs1234315 SNPs and AR susceptibility. These results are mostly congruent with previous studies concerning immune-related diseases. In Caucasian populations, the rs1234315 and rs1234314 SNPs in the 5'untranslated region of TNFSF4 were correlated with susceptibility to primary Sjögren's syndrome (35). Furthermore, an association between the rs1234315 allele of TNFSF4 and SLE in Asians was revealed in a meta-analysis (4). In Caucasian patients, the strongest associated SLE variants were rs844648 and rs12039904 (36,37), whereas the present results demonstrated no significant associations between AR and rs844648 and rs12039904. Our study indicates that the genetic background of AR may be partially similar to those of other immune-related diseases. However, the disparities of these findings suggest that the risk of developing AR is determined by a complex interaction amongst several genes. Furthermore, there may be genetic heterogeneity of AR amongst different populations.

BLK serves a role in the signal transduction of B cells. The present study demonstrated a relationship between SNPs rs13277113 and rs1600249 in the BLK gene and AR

Table VII. MDR analysis of gene-gene interactions in relation to allergic rhinitis.

Best candidate model	Testing balanced accuracy (%)	Testing OR (95% CI)	Testing χ^2 value	P-value	Cross-validation Consistency
rs13277113	54.84	1.51 (0.659-3.440)	0.944	0.331	7/10
rs13277113 + rs1600249	60.63	2.37 (1.046-5.387)	4.345	0.037	9/10
rs1234314 + rs13277113 + rs1600249	61.65	2.59 (1.136-5.884)	5.227	0.022	6/10
rs1234314 + rs10912580 + rs13277113 + rs1600249	62.69	2.83 (1.236-6.457)	6.203	0.013	10/10

MDR, multifactor dimensionality reduction; OR, odds ratio; CI, confidence interval; χ^2, Chi-squared; P, probability.

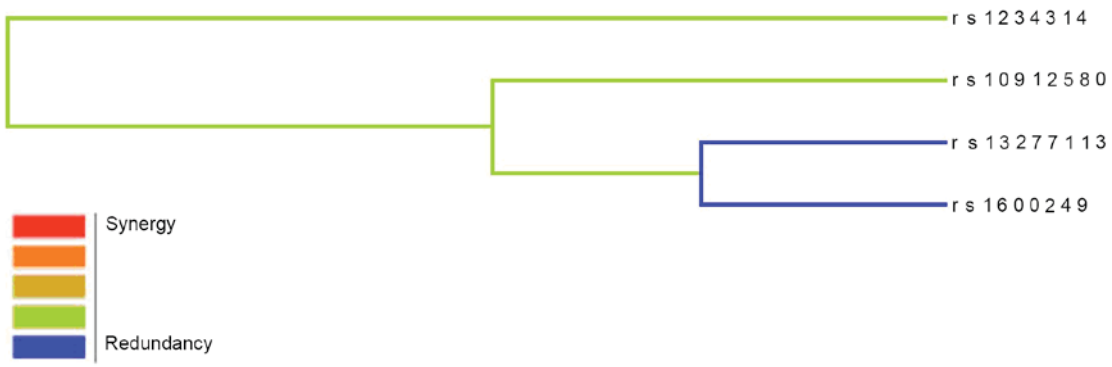

Figure 2. Dendrogram analysis of the four-marker model. The dendogram comprises a spectrum of colours that represent a continuum from synergy to redundancy; orange represents a relatively high degree of synergy (positive information gain) and blue represents redundancy (negative information gain).

susceptibility. The rs13277113 SNP may be directly involved in AR susceptibility. Our data indicated that the AG genotype of rs13277113 increases the risk of AR by 1.51-fold, and the G allele of rs13277113 is related to a 1.26-fold increase in AR risk. However, the AA genotype and the A allele decreased the risk of AR by 0.60- and 0.79-fold, respectively. These results indicate that the G allele is likely to result in AR susceptibility, and individuals with the G allele in the rs13277113 SNP of the BLK gene may be more likely to develop AR. By contrast, the A allele may protect against AR development. Previous studies have indicated that a BLK rs13277113 A/G polymorphism is associated with RA susceptibility (38) and associated with the development of SLE in European (39), Japanese (40) and Chinese (41) populations. The risk allele appears to be involved in decreased BLK mRNA expression (39). Based on these studies and the results of the present study, it may be speculated that the difference between the A and G alleles in rs13277113 influences AR susceptibility. This alteration may impact on gene splicing, transcription factor binding or the non-coding RNA sequence, and might thereby influence the expression of certain proteins.

Haplotype analysis revealed that the GGT haplotype in block rs1234313-rs1234314-rs1234315 and the AG haplotype in block rs2254546-rs13277113 are positively correlated with AR, however, the ACC and GA haplotypes were negatively correlated with AR. It is therefore possible that subjects with the GGT and/or AG haplotypes are at a higher risk of developing AR. By contrast, individuals with the ACC and/or GA haplotypes may be more resistant to AR, suggesting that these two haplotypes may serve a role in protecting against AR.

The present study was carefully designed to minimise the influence of confounding factors on the results. AR patients and controls were selected using strict guidelines and the genotyping results were confirmed by direct sequencing. However, there are a few limitations that need to be considered. The protein levels of TNFSF4 and BLK were not measured and functional experiments were not performed. Furthermore, detailed information about AR severity was not obtained, and this restricted the analyses. Finally, gene-gene interactions and environmental factors are critical for AR development, therefore, more intensive studies investigating the gene-gene or gene-environment interactions are needed to clarify the genetic influence of TNFSF4 and BLK in the pathogenesis of AR.

In conclusion, the present study indicated that polymorphisms in the TNFSF4 and BLK genes may be correlated with

susceptibility to AR in a Han Chinese population. However, further studies are needed to elucidate the complex gene-gene and gene-environment interactions in AR. The results of the present study provide novel biomarkers that may be investigated as predictive factors for AR susceptibility in a Han Chinese population.

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