

Genetic variations in interleukin-12B in allergic rhinitis

Ping Wei^{1,2,3,4} · Wei Kou^{1,2,3,4} · Cheng Zhang^{1,2,3,4} · Houyong Kang⁵ ·
Hongbing Yao^{1,2,3,4} · Xinye Tang^{1,2,3,4} · Wu Wang^{1,2,3,4}

Published online: 10 December 2015

© Springer Science+Business Media New York 2015

Abstract Interleukin (IL)-12B is a key regulator in the protection against allergic reaction induced by various allergens in patients with allergic rhinitis (AR). This study was performed to investigate the associations between single nucleotide polymorphisms in the *IL-12B* gene and AR in a Chinese Han population. A total of 543 patients with AR and 600 healthy controls were genotyped for *IL-12B*/rs6887695, rs10045431, rs3212227, rs3212217, and rs2082412 using polymerase chain reaction (PCR) restriction fragment length polymorphism and PCR direct sequencing. The expression of *IL-12B* mRNA in peripheral blood mononuclear cells (PBMCs) obtained from normal controls was detected by quantitative real-time PCR. Levels of interferon (IFN)- γ and IL-4 in culture supernatants were measured by enzyme-linked immunosorbent assay. The frequencies of the rs3212227 genotype with the CC and C alleles were higher in patients with AR ($p_c = 8.55 \times 10^{-4}$; $p_c = 4.20 \times 10^{-4}$, respectively) than

in normal controls. After peptidoglycan stimulation in PBMCs, *IL-12* mRNA expression was reduced in healthy carriers of the CC genotype of rs3212227. Additionally, decreased production of IFN- γ was observed in patients having the CC genotype as compared with that in patients having the AC and AA genotypes. In contrast, IL-4 expression was higher in patients with the CC genotype than in patients with the other two genotypes. The *IL-12B* gene was involved in mediating susceptibility to AR.

Keywords Allergic rhinitis · *IL-12B* gene · Interferon- γ · Interleukin-4 · Single nucleotide polymorphism

Abbreviations

AR	Allergic rhinitis
SNPs	Single nucleotide polymorphisms
PCR-RFLP	PCR restriction fragment length polymorphism assay

✉ Wu Wang
entweiweidoctor@sina.cn

Ping Wei
entweiweidoctor@gmail.com

Wei Kou
43878942@qq.com

Cheng Zhang
zhiminqing-doctor@hotmail.com

Houyong Kang
xiake_ent@hotmail.com

Hongbing Yao
cts_yurong@hotmail.com

Xinye Tang
hsl_prof@hotmail.com

¹ Department of Otolaryngology, The Children's Hospital of Chongqing Medical University, Chongqing, 136# Zhongshan Road, Chongqing 400014, China

² Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing, China

³ Key Laboratory of Pediatrics in Chongqing, Chongqing, China

⁴ Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Chongqing, China

⁵ Department of Otorhinolaryngology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Introduction

Allergic rhinitis (AR) is a symptomatic disorder of the nose induced after exposure to allergens, such as pollen, dust, and animal dander, arising from IgE-mediated inflammation of the membranes lining the nose [1, 2]. The clinical traits of AR include sneezing, nasal congestion, nasal itching, and rhinorrhea [2]. Recent studies suggest that T cells play an important role in the pathogenesis of AR. Additionally, the scarcity of Th1 cells and Th1-associated cytokines, i.e., interleukin (IL)-12 and interferon (IFN)- γ , accompanied by a surplus of Th2/Th17 cells and their associated cytokines, i.e., IL-4, IL-17, and IL-23, in nasal mucosa and peripheral blood is crucial to the development of AR [3–5].

To date, the definite etiology and pathogenesis of AR are not fully understood; however, recent studies have shown that an immunogenetic predisposition to AR is important for the development of AR. Various studies have found that genes such as *IL-12R*, *IL-4*, *IL-10*, *IL-17*, and *IL-23R* are associated with AR [6–9], suggesting that genetic factors may play an important role in AR.

ILs are a group of cytokine signaling molecules that function in the immune system and regulate the activities of lymphocytes, which are critical for immune responses. The *IL-12B* gene encodes the key subunit IL-12p40, which is a common component of IL-12 and IL-23. The bioactive subunit is produced mostly by activated dendritic cells and macrophages in response to inflammatory factors [10, 11]. A deficiency in IL-12p40 results in weakened migration of dendritic cells and decreased ability to activate naïve T cells [12–14]. IL-12p40 also has key roles in the function of Th1, Th2, and Th17 cell subsets. Previous studies have shown that functional polymorphisms in the *IL-12B* gene are associated with several immune-related disorders, such as ankylosing spondylitis, psoriasis, and atopic dermatitis [15–17]. Therefore, the *IL-12B* gene may be a promising candidate gene involved in AR susceptibility.

Based on the important role of IL-12B in Th1-, Th2-, and Th17-related immune responses, we examined the potential association of polymorphisms in the *IL-12B* gene with AR and the effects of variants on gene expression and relevant cytokine production. We verified the influence of *IL-12B*/rs6887695, rs10045431, rs3212227, rs3212217, and rs2082412 gene polymorphisms on AR susceptibility in a Chinese Han population.

Materials and methods

Study population: patients and normal controls

The study population comprised 543 patients with AR and 600 healthy controls that were referred to the First

Affiliated Hospital of Chongqing Medical University. All of the participants involved in this study were Chinese Han individuals. Patients with AR fulfilled the Allergic Rhinitis and its Impact on Asthma (ARIA) criteria of appearance of typical nasal symptoms, positive skin prick tests (SPTs; Allergopharma, Germany), and higher than normal serum IgE levels [2]. SPTs were completed according to the guidelines of the European Academy of Allergy and Clinical Immunology [18]. Patients diagnosed with AR and concomitant rhinosinusitis, asthma, or systemic diseases were excluded. Clinical data were collected, including age, sex, occupation, and allergen category. All patients stopped using antileukotrienes, corticosteroids, and antihistamines for at least 4 weeks prior to this study.

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (permit code: 201212). All procedures followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the participants before sample collection. No minors were involved in the study.

Single nucleotide polymorphism (SNP) selection and genotyping

Choosing initial candidate SNPs is critical for candidate gene association studies. The SNPs examined in this study were selected according to previously reported *IL-12B* gene associations in various immune-mediated diseases. In this study, we chose the SNPs rs3212227 and rs6887695 in *IL-12B* as candidate SNPs based on the identified associations with psoriasis, psoriatic arthritis, and atopic dermatitis [16, 19, 20]. The SNPs rs10045431, rs3212217, and rs2082412 in *IL-12B* have also been shown to be associated with various immune diseases; therefore, these SNPs were also included as candidate SNPs to explore the possible associations with AR in our study [15–17, 19].

Genomic DNA was extracted from heparinized whole blood of patients with AR and controls using a QIAamp DNA Blood Mini Kit (Qiagen, Germany). The extracted DNA was stored at -20°C until use. The appropriate primers used to amplify the corresponding target DNA sequence by polymerase chain reaction (PCR) and corresponding restriction enzymes are shown in Table 1. PCR restriction fragment length polymorphism (RFLP) was used for genotyping these SNPs. The formed digestion products were separated on 5 % agarose gels and stained with GoldView (SBS Genetech, China). The genotyping success rate ranged from 96.7 to 98.3 % for the various groups. We randomly selected 20 % of the samples to undergo direct sequencing for validation of the results of genotyping by PCR–RFLP and PCR (Sangon Biotech Company, China).

Cell isolation and culture

Peripheral blood mononuclear cells (PBMCs) were segregated from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. PBMCs were cultured in RPMI medium 1640 supplemented with 10 % fetal bovine serum (FBS; Gibco, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin at a density of 1×10^6 cells/mL at 37 °C in an atmosphere containing 5 % CO₂. In order to measure IL-4 and IFN-γ production, PBMCs were cultured in the presence of anti-CD3 antibody (5 µg/mL; eBioscience, USA), anti-CD28 antibody (1 µg/mL; eBioscience), and peptidoglycan (PGN; 5 µg/mL; Fluka, Switzerland) for 3 days.

Quantitative real-time PCR

PBMCs collected from healthy individuals were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. Total RNA was extracted from PBMCs using TRIzol (Beyotime, China), followed by reverse transcription using a transcriptase kit (TaKaRa, China). The quantity of IL-12 was detected using SYBR Real-time PCR Premixture kit (Bioteke, China). The level of *IL-12* mRNA was normalized to the expression of *β-actin* mRNA. Quantitative RT-PCR was performed with the following primers: *IL-12* forward, 5'-CCTTCACTCCCAAAAC-3', and reverse, 5'-CCAGGCAACTCCCATAGT-3'; *β-actin* forward, 5'-TGACGTGGACATCCGAAAG-3', and reverse, 5'-CTGGAAGGTGGACAGCGAGG-3'. Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

ELISA

The concentrations of IFN-γ and IL-4 in the cell culture supernatants of PBMCs were detected using a human

DuoSet ELISA Development kit (R&D Systems, USA) according to the manufacturer's protocols.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was tested using the χ^2 test. Allele and genotype frequencies were also compared between patient with AR and normal controls using χ^2 tests. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated using statistical software to evaluate the risk of illness. Bonferroni correction was used to account for multiple testing. Analysis of variance (ANOVA) and nonparametric Mann–Whitney tests were used to compare IL-12 expression and cytokine levels among the three genotype groups. All the statistical analyses were performed using SPSS 20.0 (Fig. 1).

Results

Clinical features

The clinical features of AR disorders are summarized in Table 2. Age, sex, and workplace distribution in patients and healthy controls are also presented in Table 2. There were no statistically significant differences in mean age, sex, and workplace ratios.

Allele and genotype frequencies of SNPs in patients and controls

The distributions of genotype frequencies for the five SNPs did not deviate from the HWE in both patients with AR and healthy controls (Table 3). The five SNPs were successfully genotyped for patients with AR and controls. Select PCR–RFLP gels and their corresponding sequencing results are

Table 1 Primers and restriction enzymes used in RFLP analysis

SNP	Primers	Tm (°C)	Restriction enzymes
rs6887695	5'-GAGAGAAGCAGTGTAGTGTAGTGAT-3' 5'-GAACAGAGAGGGGAAGCAAC-3'	60	BclI
rs10045431	5'-CAGGGTAGTGGGTATTGTCCTAAGTAT-3' 5'-GGGTAGTGGGTATTGTCCTAAGTATGC-3'	60	HinfI
rs3212227	5'-GACACAACGGAATAGACCCAAAAAG-3' 5'-GGCAACTTGAGAGCTGGAAAATCT-3'	64	TaqI
rs3212217	5'-TAAAGTACATAGCAAGGTCCGCCTCG-3' 5'-AGCACCCATGGTCTGGAGGAAA-3'	64	XhoI
rs2082412	5'-TCTCCTCTCCAGAGTGTTCAGTAA-3' 5'-CGAGTGAAATTGTACAAATGCAAAGTG-3'	60	SduI

RFLP restriction fragment length polymorphism, SNP single nucleotide polymorphism

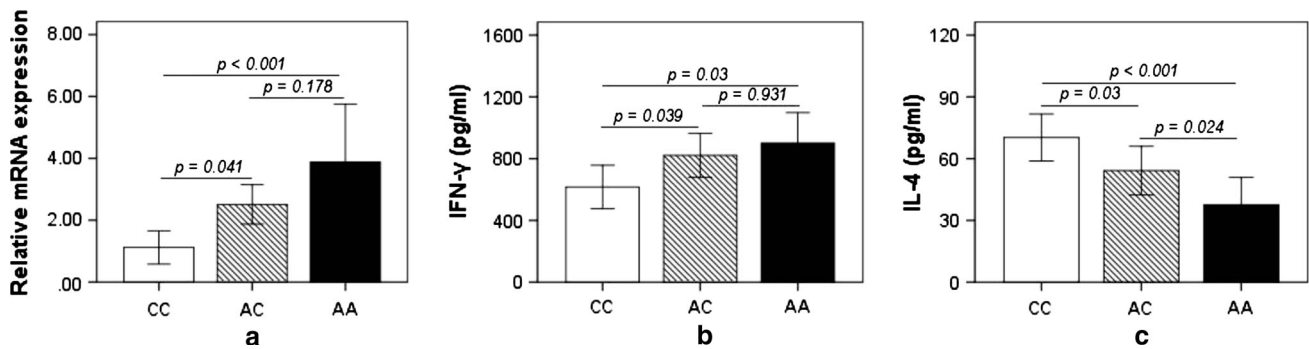


Fig. 1 **a** *IL-12* mRNA expression in individuals with different genotypes of rs3212227/*IL-12B* in PBMCs. *IL-12* expression in carriers of the CC ($n = 8$) genotype was lower than that in individuals carrying the AA ($n = 8$) or AC ($n = 11$) genotypes. Data are expressed as mean $\pm$ SD. **b** IFN- γ and **c** IL-4 levels in supernatants of PBMCs isolated from individuals with different

genotypes at the SNP rs3212227/*IL-12B* were detected by ELISA. IFN- γ production by PBMCs isolated from individuals with the CC genotype ($n = 8$) was lower than that in individuals with the AC ($n = 11$) and AA ($n = 8$) genotypes. IL-4 expression was higher in individuals with the CC genotype ($n = 8$) than that in individuals with the AC ($n = 11$) and AA ($n = 8$) genotypes

Table 2 Clinical features and demographic characteristics in AR patients and healthy controls

Characteristic	AR	Control	<i>p</i> values
Gender (male/female)	282/261	326/274	NS
Age (mean $\pm$ SD)	36.4 $\pm$ 12.7	39.3 $\pm$ 10.2	NS
Occupation (indoor/outdoor)	329/220	359/241	NS
Allergen category (%)			
House dust mite	253 (46.6)	0	<0.01
Pollens	84 (18.5)	0	<0.01
Mixed allergens	206 (37.9)	0	<0.01

AR allergic rhinitis, NS not significant

Table 3 The HWE *p* values of 5 SNPs in AR patients and healthy controls

SNP	HWE <i>p</i> value	
	AR	Control
rs6887695	0.10	0.18
rs10045431	0.06	0.06
rs3212227	0.89	0.14
rs3212217	0.08	0.06
rs2082412	0.07	0.17

AR allergic rhinitis, HWE Hardy–Weinberg equilibrium, SNP single nucleotide polymorphism

shown in Fig. 2. The genotypic and allelic frequencies of the five tested polymorphisms are presented in Table 4. Our results showed that the frequency of rs3212227 differed significantly between patients with AR and healthy controls. The frequencies of the CC genotype (CC genotype vs. AA and AC genotypes) and C allele (C allele vs. A allele) of rs3212227 were increased in patients with AR compared

with that in healthy controls ($p = 5.70 \times 10^{-5}$, $p_c = 8.55 \times 10^{-4}$, OR 1.616, 95 % CI 1.275–2.048; $p = 2.80 \times 10^{-5}$, $p_c = 4.20 \times 10^{-4}$, OR 1.212, 95 % CI 1.108–1.327, respectively). The frequency of the rs3212227 AA genotype (AA genotype vs. AC and AA genotypes) was decreased in patients with AR compared with that in healthy controls ($p = 0.003$, $p_c = 0.045$, OR 0.761, 95 % CI 0.633–0.914). There were no statistically significant differences in the allele and genotype frequencies of the other four SNPs between the AR cohort and healthy controls.

We further investigated whether the *IL-12B* SNPs were associated with certain allergen categories, such as house dust mites, pollens, and other allergens. The analysis failed to find any association between allergen categories and the tested *IL-12B* SNPs.

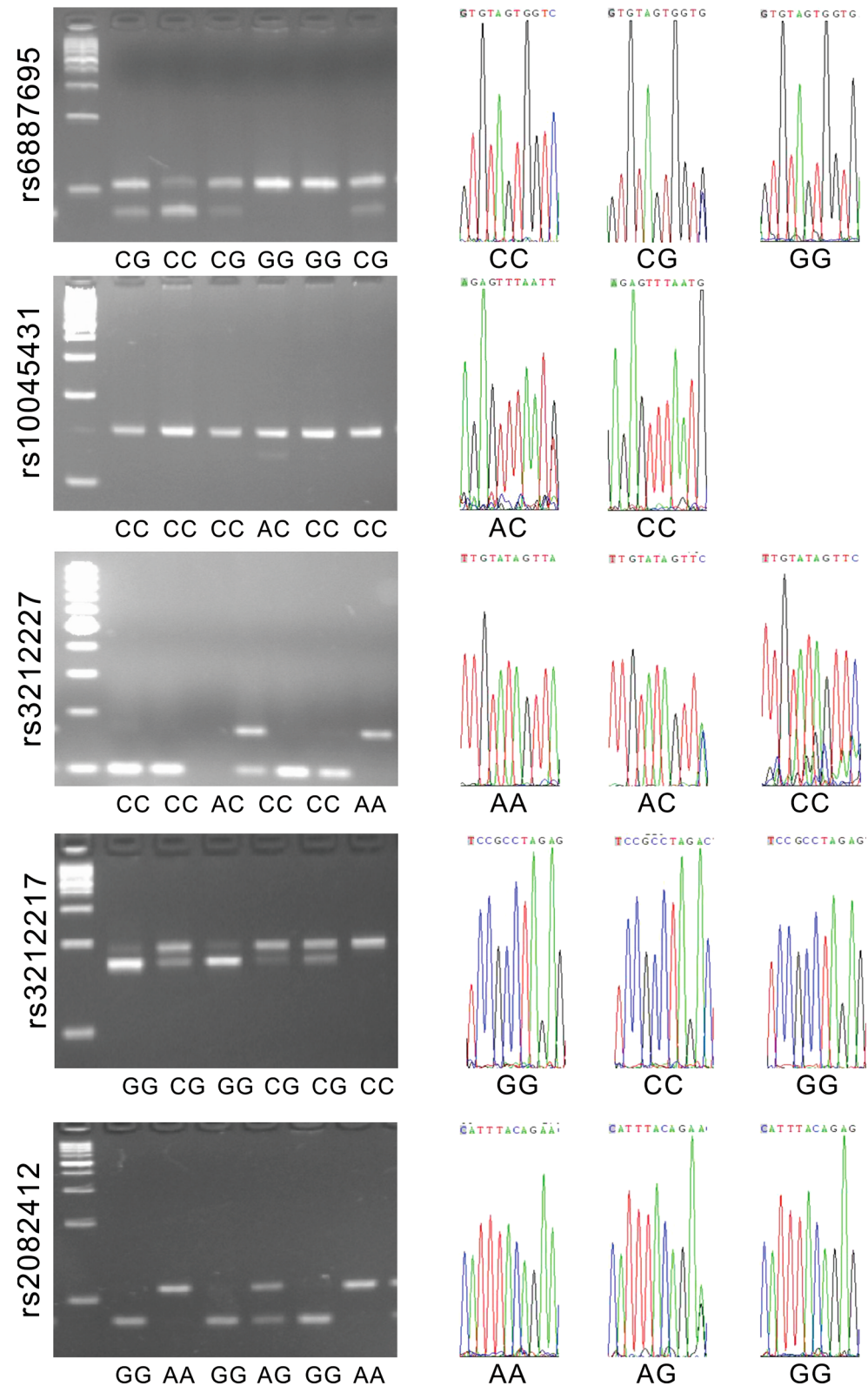
The influence of rs3212227 on IL-12 expression

Since the aforementioned results showed a strong association between the SNP rs3212227 and AR, we further evaluated whether the different genotypes examined herein affected the expression of *IL-12* in PBMCs derived from healthy or inflammatory conditions. *IL-12* mRNA expression levels were determined by quantitative real-time PCR. Carriers with the CC genotype in the SNP rs3212227 had lower *IL-12* mRNA expression compared with that in patients carrying the AC ($p = 0.041$) or AA ($p < 0.001$) genotype, respectively (Fig. 1a).

The influence of rs3212227 on cytokine production

An additional experiment was performed to investigate different genotypes of the SNP rs3212227 on cytokines crucial to the resistance to or development of AR, such as IFN- γ and IL-4. The results showed that IFN- γ production by PBMCs

Fig. 2 Select PCR–RFLP gels and their corresponding sequencing results



from individuals with the CC genotype was lower than that in individuals with the AC ($p = 0.039$) and AA ($p = 0.030$) genotypes, respectively (Fig. 1b). Conversely, IL-4

expression was found to be higher in individuals with the CC genotype than that in individuals with the other two genotypes (AC: $p = 0.030$; CC: $p < 0.001$; Fig. 1c).

Table 4 Frequencies of alleles and genotypes of *IL-12B* polymorphisms in AR patients and healthy controls

SNP	Genotype allele	AR (N = 543)	Control (N = 600)	χ^2	p value	P_c value	OR (95 % CI)
rs6887695	CC	71 (0.131)	87 (0.145)	0.652	0.420	6.300	0.887 (0.663–1.187)
	CG	278 (0.512)	303 (0.505)	0.055	0.814	12.210	1.014 (0.904–1.136)
	GG	194 (0.357)	210 (0.350)	0.066	0.797	14.685	1.021 (0.873–1.194)
	C	420 (0.387)	477 (0.398)	0.277	0.599	8.985	0.973 (0.878–1.078)
	G	666 (0.613)	723 (0.603)	0.277	0.599	8.985	1.018 (0.953–1.087)
rs10045431	AC	89 (0.164)	89 (0.148)	0.526	0.468	4.680	1.105 (0.844–1.447)
	CC	454 (0.836)	511 (0.852)	0.526	0.468	4.680	0.982 (0.934–1.032)
	AA	0 (0)	0 (0)	–	–	–	–
	A	89 (0.082)	89 (0.074)	0.481	0.488	4.880	1.105 (0.833–1.465)
rs3212227	C	997 (0.918)	1111 (0.926)	0.481	0.488	4.880	0.992 (0.968–1.016)
	AA	137 (0.252)	199 (0.332)	8.650	0.003	0.045	0.761 (0.633–0.914)
	AC	270 (0.494)	308 (0.513)	0.295	0.587	8.805	0.969 (0.863–1.087)
	CC	136 (0.251)	93 (0.155)	16.213	5.70×10^{-5}	8.55×10^{-4}	1.616 (1.275–2.048)
	A	544 (0.501)	706 (0.588)	17.578	2.80×10^{-5}	4.20×10^{-4}	0.851 (0.789–0.919)
rs3212217	C	542 (0.499)	494 (0.412)	17.578	2.80×10^{-5}	4.20×10^{-4}	1.212 (1.108–1.327)
	CC	86 (0.158)	101 (0.168)	0.206	0.650	9.750	0.941 (0.723–1.224)
	CG	286 (0.527)	317 (0.528)	0.003	0.956	14.340	0.997 (0.893–1.113)
	GG	171 (0.315)	182 (0.303)	0.179	0.672	10.080	1.038 (0.873–1.235)
	C	458 (0.422)	519 (0.432)	0.270	0.603	9.045	0.975 (0.887–1.072)
rs2082412	G	628 (0.578)	681 (0.568)	0.270	0.603	9.045	1.019 (0.949–1.094)
	AA	105 (0.193)	104 (0.173)	0.766	0.381	5.715	1.116 (0.873–1.425)
	AG	293 (0.540)	311 (0.518)	0.517	0.472	7.080	1.041 (0.933–1.161)
	GG	145 (0.267)	185 (0.308)	2.367	0.124	1.860	0.866 (0.721–1.041)
	A	503 (0.463)	519 (0.432)	2.169	1.141	17.115	1.097 (0.978–1.173)
	G	581 (0.537)	681 (0.568)	2.169	1.141	17.115	0.946 (0.878–1.019)

AR allergic rhinitis, OR odds ratio, SNP single nucleotide polymorphism

Discussion

In the present study, we explored the association between gene polymorphisms in *IL-12B* and the risk of developing AR in a Chinese Han population and found that the CC genotype of the *IL-12B* gene rs3212227 conferred a risk of developing AR. Based on the association of AR with *IL-12B*, we carried out additional analyses to determine the possible functional association. This approach revealed that healthy carriers with the CC genotype in the SNP rs3212227 exhibited lower expression of *IL-12* mRNA than individuals carrying the other genotypes. Additionally, these individuals had higher expression of IL-4 and lower expression of IFN- γ .

Recently, we reported the association between genes involved in various immune responses and susceptibility to AR, including involvement of IL-23R, IL-12R β 1/ β 2, and IL-27 [8, 9, 21]. Many studies have demonstrated the presence of a hypersensitive response in which the *IL-12B* gene, as a potential candidate gene, may play a key role in

the pathogenesis of allergic disease [19]. To the best of our knowledge, the possible association of *IL-12B* gene polymorphisms with AR has not yet been reported. Thus, this is the first report demonstrating an association between the SNP rs3212227 of *IL-12B* and noninfectious nasal inflammatory disease. Our study showed that individuals carrying the CC genotype of *IL-12B* rs3212227 had a higher risk of developing AR. However, an individual having the AA genotype of rs3212227 showed resistance to AR, suggesting that *IL-12B* may be a susceptibility factor in this disease.

The aforementioned results identified a strong association between rs3212227 in the *IL-12B* gene and AR. An additional study was designed to investigate whether this SNP influenced IL-12 expression and the production of cytokines involved in AR development. Our results showed that the rs3212227 CC genotype could downregulate *IL-12* mRNA expression. Our data also revealed that IL-4 expression in individuals carrying the rs3212227 CC genotype was markedly enhanced compared with that in

individuals having the other two genotypes. The lower level of IFN- γ observed in individuals with the CC genotype was consistent with the promotion of the Th2-like profile in patients with AR having this genotype. Collectively, these results demonstrated that the SNP rs3212227 not only functionally regulated the expression level of IL-12, but also affected inflammatory cytokine production and potentially influenced the Th1/Th2 imbalance.

Reducing the effects of influencing factors, such as regional disparity, systemic diseases, and rhinosinusitis, was important in this study. AR patients were carefully selected according to ARIA criteria. Controls were also strictly selected to ensure that they were from the same geographical regions as the patients. Additionally, the controls were age- and sex-matched healthy individuals. Finally, 20 % of the samples were randomly chosen, and direct sequencing was performed to verify the method used in our study, with similar results.

There were several limitations to our study. The environment is a key factor affecting AR; it is therefore important that further studies analyze gene–environment interactions in AR. We detected an association between rs3212227/*IL-12B* and AR in a Chinese Han population; however, our data cannot yet be extrapolated to other ethnic groups. Therefore, analysis of patients from other ethnic groups is necessary to rule out patient subgroup bias. Our data were confined to patients with AR with other allergic diseases, such as asthma; studies should be carried out to investigate whether IL-12B is also involved in the pathogenesis of other allergic diseases.

In summary, our study illustrated the strong association of rs3212227 in the *IL-12B* gene with AR. The CC genotype was confirmed to promote the development to AR, while the AA genotype was confirmed as a protective factor against AR. Additionally, our study demonstrated that SNPs within this region may affect the genetic predisposition to AR, probably through regulation of the expression of IL-12 and by affecting inflammatory cytokine production.

Acknowledgments The authors would like to thank very much for all the donors contributed to the present study.

Author Contributions In this study, Ping Wei, Wei Kou, and Wu Wang conceived and designed the experiments. Ping Wei and Wei Kou performed the experiment in the laboratory. Houyong Kang, Xinye Tang, and Cheng Zhang collected the specimen. Ping Wei and Wei Kou analyzed the data and wrote the manuscript. Hongbing Yao and Wu Wang modified the thesis.

Compliance with ethical standards

Conflict of interest We would simply emphasize that after the discussion and decision of all authors, we recommend Ping Wei and

Wei Kou as the co-first authors, as the equal contribution to this study. The authors declare that they have no competing interests.

References

1. Dykewicz MS, Hamilos DL. Rhinitis and sinusitis. *J Allergy Clin Immunol*. 2011;125:S103–15.
2. Pawankar R, Bunnag C, Khaltayev N, et al. Allergic rhinitis and its impact on asthma in Asia Pacific and the ARIA Update 2008. *World Allergy Organ J*. 2012;5:S212–7.
3. Ren J, Deng Y, Xiao B, et al. Protective effects of exogenous surfactant protein A in allergic rhinitis: a mouse model. *Ann Otol Rhinol Laryngol*. 2013;122(4):240–6.
4. Sogut A, Yilmaz O, Kirmaz C, et al. Regulatory-T, T-helper 1, and T-helper 2 cell differentiation in nasal mucosa of allergic rhinitis with olive pollen sensitivity. *Int Arch Allergy Immunol*. 2012;157(4):349–53.
5. Yao J, Zhou H, Xu M, et al. Relationship between GATA-3 and IL-12 in patients with allergic rhinitis. *J Clin Otolaryngol Head Neck Surg*. 2007;12(23):1087–9.
6. Gaddam SL, Priya VHS, Babu BMVS, et al. Association of interleukin-10 gene promoter polymorphism in allergic patients. *Genet Test Mol Biomark*. 2012;16:632–5.
7. Movahedi M, Amirzargar AA, Nasiri R, et al. Gene polymorphisms of Interleukin-4 in allergic rhinitis and its association with clinical phenotypes. *Am J Otolaryngol*. 2013;34(6):676–81.
8. Hu D, Hu G, Zhu J, et al. Association between polymorphisms of the IL-23R gene and allergic rhinitis in a Chinese Han population. *PLoS ONE*. 2013;8(5):e63858.
9. Wei P, Kou W, HU G, et al. Association Study between Interleukin-12 Receptor β 1/ β 2 genes and allergic rhinitis in the Chinese Han population. *Eur Arch Otorhinolaryngol*. 2014;. doi:10.1007/s00405-014-3145-9.
10. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol*. 2003;3:133–46.
11. Trinchieri G, Pflanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity*. 2003;19:641–4.
12. Johnston A, Xing X, Swindell WR, Kochkodan J, Riblett M, et al. Susceptibility-associated genetic variation at IL12B enhances Th1 polarization in psoriasis. *Hum Mol Genet*. 2013;22:1807–15.
13. Lim HX, Hong HJ, Jung MY, Cho D, Kim TS. Principal role of IL-12p40 in the decreased Th1 and Th17 responses driven by dendritic cells of mice lacking IL-12 and IL-18. *Cytokine*. 2013;63:179–86.
14. Xinyu Li, Lin Bai, Jing Fang, et al. Genetic variations of IL-12B, IL-12R β 1, IL-12R β 2 in Behcet's disease and VKH syndrome. *PLoS ONE*. 2014;9(5):e98373.
15. Wong RH, Wei JC, Huang CH, et al. Association of IL-12B genetic polymorphism with the susceptibility and disease severity of ankylosing spondylitis. *J Rheumatol*. 2012;39(1):135–40.
16. Lee YH, Song GG. Associations between interleukin-23R and interleukin-12B polymorphisms and psoriasis susceptibility: a meta-analysis. *Immunol Invest*. 2012;42(8):726–36.
17. Tug E, Ozbey U, Tug T, et al. Relationship between the IL-12B promoter polymorphism and allergic rhinitis, familial asthma, serum total IgE, and eosinophil level in asthma patients. *J Investig Allergol Clin Immunol*. 2009;19(1):21–6.
18. Dreborg S, Backman A, Basomba A. Skin tests used in type I allergy testing. Position paper. *Allergy*. 1989;44:1–59.
19. Van wanrooij RL, Zwiers A, Kraal G, et al. Genetic variations in interleukin-12 related genes in immune-mediated diseases. *J Autoimmun*. 2012;39(4):359–68.

20. Namkung JH, Lee JE, Kim E, et al. Association of single nucleotide polymorphisms in the IL-12 (IL-12A and B) and IL-12 receptor (IL-12Rbeta1 and beta2) genes and gene-gene interactions with atopic dermatitis in Koreans. *J Dermatol Sci*. 2010;57:199.
21. Shen Y, Yuan XD, Hu D, et al. Association between Interleukin-27 gene polymorphisms and susceptibility to allergic rhinitis. *Hum Immunol*. 2014;75(9):991–5.