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ORIGINAL RESEARCH

TLR Signaling Pathway Gene Polymorphisms, Gene–Gene and Gene–Environment Interactions in Allergic Rhinitis

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Background: Allergic rhinitis (AR) is a nasal inflammatory disease resulting from a complex interplay between genetic and environmental factors. The association between Toll-like receptor (TLR) signaling pathway and environmental factors in AR pathogenesis remains to be explored. This study aims to assess the genetic association of AR with single nucleotide polymorphisms (SNPs) in TLR signaling pathway, and investigate the roles of gene–gene and gene–environment interactions in AR.

Methods: A total of 452 AR patients and 495 healthy controls from eastern China were enrolled in this hospital-based case–control study. We evaluated putatively functional genetic polymorphisms in *TLR2, TLR4* and *CD14* genes for their association with susceptibility to AR and related clinical phenotypes. Interactions between environmental factors (such as traffic pollution, residence, pet keeping) and polymorphisms with AR were examined using logistic regression. Models were stratified by genotype and interaction terms, and tested for the significance of gene–gene and gene–environment interactions.

Results: In the single-locus analysis, two SNPs in *CD14*, rs2563298 (A/C) and rs2569191 (C/T) were associated with a significantly decreased risk of AR. Compared with the GG genotype, the GT and GT/TT genotypes of *TLR2* rs7656411 (G/T) were associated with a significantly increased risk of AR. Gene–gene interactions (eg, *TLR2* rs7656411, *TLR4* rs1927914, and *CD14* rs2563298) was associated with AR. Gene–environment interactions (eg, *TLR4* or *CD14* polymorphisms and certain environmental exposures) were found in AR cases, but they were not significant after Bonferroni correction.

Conclusion: The genetic polymorphisms of *TLR2* and *CD14* and gene–gene interactions in TLR signaling pathway were associated with susceptibility to AR in this Han Chinese population. However, the present results were limited to support the association between gene–environment interactions and AR.

Keywords: allergic rhinitis, toll-like receptors, CD14, single nucleotide polymorphism, gene–gene interaction, gene–environment interaction

Introduction

Allergic rhinitis (AR) is a nasal inflammatory disease induced by immunoglobulin E (IgE)-mediated immune response, with main symptoms of itchy nose, rhinorrhea, sneezing and nasal congestion.[1] The prevalence of AR keeps increasing worldwide. In China, the AR prevalence in adults increased from 11.1% in 2005 to 17.6% in 2011.[2,3] AR and asthma

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often coexist, both triggered by genetic and environmental factors.[4] The development of AR involves not only allergen exposure but also early-life factors, family history, ethnicity, and environmental factors, such as tobacco smoke, cooking fumes, living floors, lifestyle, air pollution and furniture pollution.

Toll-like receptors (TLRs) are type I transmembrane protein natural immune pattern recognition receptors, which can recognize pathogen-associated molecular patterns (PAMP) in nature, initiate intracellular signaling pathways, and activate innate immune response. In addition, TLRs also induce dendritic cell (DC) maturation and T cell activation, thus skewing the adaptive immune response towards Th1,[5] and participate in the induction and perpetuation of asthma and atopy.[6] Given the mediatory role of TLRs between innate and adaptive immunity, genetic variations of TLR signaling pathway genes may drive the progression of inflammatory and allergic diseases. A number of studies have demonstrated the association between single nucleotide polymorphisms (SNPs) of TLR signaling pathway genes and asthma in diverse populations;[7–14] however, little attention has been given to rhinitis.[15–18]

According to the hygiene hypothesis,[19] the allergic diseases may arise from bacterial or viral infections and exposure to non-infectious microbial agents (such as endotoxin) in the environment.[20] TLR signaling pathway genes may participate in the protective effect of microbial agents on allergy. Many studies that have examined the interplay between genetic susceptibility and environmental factors in allergic conditions only focus on asthma. A gene–environment interaction has been observed between *CD14* rs2569190 and asthma, depending on the endotoxin exposure level in house dust.[21] Moreover, the associations between polymorphisms of *TLR2, TLR4, TLR6, TLR9* and *CD14* genes in asthma were affected by environmental factors, such as house dust endotoxin level and living place.[10,22–24] Gene–environment interactions, which have rarely been analyzed in previous studies of AR, may provide new insight into AR pathogenesis. One study on AR children has shown no significant evidence of gene–environment interactions between traffic-related air pollution and *GSTP1, TNF, TLR2*, and *TLR4* genes.[17] At present, there is no literature reporting gene–gene and gene–environment interactions in AR based on a Chinese population. This study was the first to identify the associations of candidate genes and environmental factors with genetic predisposition to AR in the Chinese Han population.

According to the data about weather, air quality, transportation and lifestyle in East China, we selected several environmental factors that may be related to AR. We screened 10 SNPs of *TLR2, TLR4* and *CD14* genes in the TLR signaling pathway, and conducted a case–control study in East China Han population to explore the association between polymorphisms and AR, as well as gene–gene and gene–environment interactions.

Methods

Subjects

A total of 452 AR cases (302 males and 150 females) and 495 healthy controls (303 males and 192 females) were recruited from the First Affiliated Hospital of Nanjing Medical University, Nanjing, China. All subjects were Han Chinese in Jiangsu and Anhui provinces in eastern China. The diagnosis of AR was established according to the "Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update"[1] and the "Chinese Society of Allergy Guidelines for Diagnosis and Treatment of Allergic Rhinitis".[3] The medical history and clinical data were collected from out-patients' medical records and face-to-face questionnaire surveys. The cases did not use glucocorticoids within 4 weeks, and antihistamines, leukotriene receptor antagonists and other anti-allergic drugs within 2 weeks before blood sampling. The controls were recruited from the hospital seeking health care or routine health examinations, and were frequency-matched with the cases in age (±5 years) and sex. The selection criteria for controls:[25,26] (1) no symptoms and medical history of AR and nasal diseases; (2) no symptoms and medical history of other allergic diseases, such as asthma, eczema and urticaria; (3) negative blood test for serum allergen-specific IgE; (4) no history of AR or other allergic diseases in the immediate family. All the study subjects were divided into < 18-year-old group and ≥ 18-year-old group for further stratification analysis.

Clinical Parameters

The questionnaire survey covered general information, demographic characteristics, disease symptoms and scores, physical signs, medical history, accompanying diseases, and environmental factors, including smoking, residence

(floors), sunlight exposure, in-house chemicals, near-residence pollutants, near-residence traffic network, radiation exposure to computers, oil fume exposure and pet-keeping. A visual analogue scale (VAS) ranging from 0 cm (not bothersome at all) to 10 cm (extremely bothersome) was used to assess the patient's subjective perception of nasal symptoms, including rhinorrhea, sneezing, nasal congestion, itchy nose and eyes, and total nasal symptoms. VAS scores < 5 were diagnosed as mild AR, and VAS scores ≥ 5 as moderate-to-severe AR.[27]

About 5 mL of peripheral venous blood was collected from each subject for in vitro allergen detection. The levels of eosinophil cationic protein (ECP), serum total IgE and specific IgE were measured with ImmunoCAP assays (Phadia, Uppsala, Sweden). Total IgE and ECP were determined in all subjects. Specific IgE antibodies to common inhalant allergens including *Dermatophagoides pteronyssinus* (Der *p*; d1), *Dermatophagoides farinae* (Der *f*; d2), cat dander (e1), dog dander (e5), *Blatella germanica* (i6), *Alternaria alternata* (m6), *Ambrosia elatior* (w1), and *Artemisia vulgaris* (w6) were determined in AR cases. We collected patients allergic to dust mites (d1 and/or d2), one of the most common allergens in eastern China. The positive rates of other aeroallergens (e1, e5, i6, m6, w1 and w6) were low, and they were not the main allergens causing symptoms.

SNPs Selection and Genotyping

A total of 10 SNPs from *TLR2, TLR4* and *CD14* genes in the TLR signaling pathway were obtained: rs7656411 (G/T), rs76112010 (A/G) and rs7682814 (A/G) of *TLR2* gene; rs10983755 (A/G), rs11536889 (G/C), rs1927914 (A/G) and rs7873784 (G/C) of *TLR4* gene; rs2563298 (A/C), rs2569190 (A/G) and rs2569191 (C/T) of *CD14* gene (Table 1). The data of 10 SNPs were selected by using genotype data of Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT) from the 1000 Genomes Project (March 2012) and based on previously published studies.[12–17,28,29] Distributions of all genotypes in the control subjects were consistent with those estimated according to the minor allele frequency (MAF) > 0.05 and *P*-value of Hardy-Weinberg equilibrium (HWE) > 0.05. In addition, web-based tools were used to predict putative functions of genetic variants, including SNPinfo (https://snpinfo.niehs.nih.gov/), HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg_v4.php), RegulomeDB (https://www.regulomedb.org/regulome-search/), MirSNP (http://bioinfo.bjm-u.ed-u.cn/mirsnp/search/), and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/). Secondary structural changes and minimum free energy (MFE) changes caused by different SNP genotypes were predicted by using RNAfold (http://rna.tbi.univie.ac.at/).

Table 1 Gene Frequency Distribution of *TLR2, TLR4* and *CD14* Alleles

No.	SNPs	Gene	Location	Base Change	MAF			HWE *P*[b]
					Database[a]	Case	Control	
1	rs7656411	*TLR2*	3′near gene	G/T	0.419	0.482	0.451	0.168
2	rs76112010	*TLR2*	5′near gene	A/G	0.136	0.203	0.169	0.661
3	rs7682814	*TLR2*	5′near gene	A/G	0.167	0.202	0.172	0.286
4	rs10983755	*TLR4*	5′ near gene	A/G	0.267	0.127	0.154	0.457
5	rs11536889	*TLR4*	3′UTR	G/C	0.261	0.238	0.218	0.065
6	rs1927914	*TLR4*	5′near gene	A/G	0.360	0.434	0.416	0.204
7	rs7873784	*TLR4*	3′UTR	G/C	0.122	0.274	0.259	0.537
8	rs2563298	*CD14*	3′UTR	A/C	0.151	0.123	0.184	0.067
9	rs2569190	*CD14*	5′UTR	A/G	0.500	0.380	0.412	0.988
10	rs2569191	*CD14*	5′near gene	C/T	0.453	0.363	0.426	0.625

Notes: [a]MAF for CHB from the HapMap databases (http://www.hapmap.org) or PubMed (http://www.ncbi.nlm.nih.gov/snp). [b]Two-sided χ^2 test for the allele frequencies of the controls.

Abbreviations: SNPs, single nucleotide polymorphisms; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

Genomic DNA was purified from peripheral blood leukocytes using a commercial kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions, and stored at –70°C until usage. Genotyping was performed with the TaqMan SNP Genotyping Assay using the 384-well ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). More than 15% of the samples were randomly selected for confirmation, and the discordance rate between genotypes was below 0.3%.

Statistical Analysis

The values of total IgE, specific IgE and ECP were transformed by log logarithm. The Student's *t*-test was performed to analyze continuous variables, and the χ^2 test to analyze categorical variables and the genotype distributions of SNPs in two groups. HWE of the genotype distribution in controls was tested by a goodness-of-fit χ^2 test. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis after adjustment for gender and age, to quantify the association between the polymorphisms and risk of AR. Gene–gene interactions were analyzed using multifactor dimensionality reduction (MDR) software (http://sourceforge.net/projects/mdr/). Logistic regression analyses were performed to explore gene–environment interactions, with adjustment for gender and age. Calculations were carried out using Statistical Analysis System software (version 9.1.3; SAS Institute, Cary, NC, USA). A P-value < 0.05 was considered statistically significant, and all statistical tests were two-sided. The Bonferroni correction ($P < 0.05$ divided by the number of SNPs analyzed, $P < 0.005$) was applied to adjust for multiple comparisons.

Results

Characteristics of Subjects

The demographic characteristics of the study subjects are summarized in Table 2. There was no significant difference in the distribution of age ($P = 0.597$) and sex ($P = 0.073$). Apparently, serum levels of total IgE (264.0 [120.6–593.5] kU/L)

Table 2 Distribution of Selected Variables Among Cases and Controls

Variables	Case (n = 452)		Control (n = 495)		P^a
	N	%	N	%	
Age (years), mean ± SD	19.70 ± 12.60		20.10 ± 12.61		0.597
Gender					
Male	302	66.8	303	61.2	0.073
Female	150	33.2	192	38.8	
Duration of rhinitis (year), mean ± SD	6.62 ± 5.87				
Total nasal symptoms (VAS), mean ± SD	5.25 ± 2.39				
Serum total IgE (kU/L), median (IQR)[b]	264.0 (120.6–593.5)		26.8 (11.1–52.2)		< 0.001*
Serum specific IgE (kU_A/L), median (IQR)[b]					
Dermatophagoides pteronyssinus	29.0 (4.6–72.5)				
Dermatophagoides farinae	24.1 (5.0–69.9)				
Serum ECP (μg/L), median (IQR)[b]	12.7 (5.2–28.7)		4.6 (3.1–7.5)		< 0.001*
With asthma[c]					
Yes	118	26.1			
No	271	60.0			

Notes: [a]Derived from two-sided χ^2 test for comparison of discrete variables and unpaired Student's *t*-test for continuous variables. [b]Selective variables were transformed into logarithmic model before unpaired Student's *t*-test between cases and controls. [c]Some information of concomitant asthma of allergic diseases was not available in cases. *Statistically significant ($P < 0.05$).

Abbreviations: IQR, interquartile range; ECP, eosinophil cationic protein.

and ECP (12.7 [5.2–28.7] μg/L) in AR cases were significantly higher ($P < 0.001$) than those in controls (26.8 [11.1–52.2] kU/L and 4.6 [3.1–7.5] μg/L, respectively). In AR cases, the serum levels of allergen-specific IgE against *Der p* and *Der f* were 29.0 (4.6–72.5) kUA/L and 24.1 (5.0–69.9) kUA/L, respectively. A total of 247 (54.6%) cases presented mild (VAS < 5) and 194 (42.9%) presented moderate-to-severe (VAS ≥ 5) AR, with a mean VAS score of 5.25 ± 2.39; 118 (26.1%) cases reported to have concomitant asthma. According to the questionnaire, asthma information missed in 63 (13.9%) patients, for the related questions in their questionnaires were not answered.

Association Between SNPs and AR Risk in Single-Locus Analyses

The genotype and allele distributions of the 10 SNPs and their associations with AR risk are presented in Table 3. The single-locus analyses revealed that the genotype frequencies of two SNPs rs2563298 (A/C) and rs2569191 (C/T) in *CD14* were significantly different between the cases and the controls (rs2563298: $P = 0.003$; rs2569191: $P = 0.008$). Multivariate logistic regression analysis indicated that the variant CA and AA genotypes of *CD14* rs2563298 were associated with a significantly decreased risk of AR, compared with the wild-type CC genotype (adjusted OR = 0.65, 95% CI = 0.48–0.89 for CA and adjusted OR = 0.40, 95% CI = 0.18–0.89 for AA). For the *CD14* rs2569191, compared with the CC genotype, the CT and TT genotypes were associated with a significantly decreased risk of AR (adjusted OR = 0.65, 95% CI = 0.48–0.87 for CT and adjusted OR = 0.64, 95% CI = 0.44–0.95 for TT). We also found that the dominant models of *CD14* rs2563298 (CA/AA vs CC) and rs2569191 (CT/TT vs CC) were significantly associated with AR risk (adjusted OR = 0.62, 95% CI = 0.46–0.83 for rs2563298 and 0.65, 0.49–0.85 for rs2569191). The genotype and allele frequencies of *TLR2* rs7656411 showed no significant difference between cases and controls; however, compared with the GG genotype, the GT and GT/TT genotypes were associated with a significantly increased risk of AR (adjusted OR = 1.41, 95% CI = 1.04–1.91 for GT and adjusted OR = 1.35, 95% CI = 1.01–1.79 for GT/TT). No significant difference was found between the associations of genotype and allele frequencies with AR susceptibility in the other seven SNPs ($P > 0.05$).

Association of Stratification Analysis Between the SNPs and AR

In the stratification analysis (Table 4), compared with the controls, the dominant model of *TLR2* rs7656411 (GT/TT vs GG) exhibited a significantly increased risk of AR in the subgroups of males (adjusted OR = 1.60, 95% CI = 1.12–2.30) and concomitant asthma (adjusted OR = 1.61, 95% CI = 1.13–2.28). Furthermore, a significantly decreased risk of AR was found in the dominant model of *CD14* rs2563298 (CA/AA vs CC) in the subgroups of < 18-year-old, females, lower total IgE, mild symptoms, moderate-to-severe symptoms, and asthma (with/without). This decreased risk was also more pronounced in the dominant model of *CD14* rs2569191 (CT/TT vs CC) among all the subgroups of age, gender, asthma, VAS, and total IgE, compared with that in the controls. However, only the associative significance of *CD14* rs2563298 in the subgroup of < 18-year-old, females, without asthma and lower total IgE, and that of *CD14* rs2569191 in the subgroup of without asthma, moderate-to-severe symptoms and lower total IgE remained statistically evident after Bonferroni correction.

Locus–Locus Interactions of SNPs in AR

We investigated the locus–locus interactions of 10 SNPs in *TLR2, TLR4* and *CD14* genes using MDR analysis. As shown in Table 5, *CD14* rs2569191 polymorphism was a significant single-locus model, with a cross-validation consistency (CVC) of 8/10 and a test accuracy of 53.52% ($P = 0.0036$), and the best interaction model was the three-factor model (*TLR2* rs7656411, *TLR4* rs1927914 and *CD14* rs2563298), with a testing accuracy to 53.08% and a CVC of 6/10 ($P < 0.0001$), as determined empirically by the permutation testing.

Besides, we applied the interaction dendrogram to determine whether there was a synergistic relationship among these polymorphisms in the best model (Figure 1). The closer distance between *TLR2* rs7656411 and *TLR4* rs1927914 in the diagram indicated a stronger synergistic interaction, which showed that there may be a gene–gene synergistic interaction between *TLR2* and *TLR4*.

Table 3 Genotype and Allele Frequencies in *TLR2, TLR4* and *CD14* Polymorphisms Among Cases and Controls

SNPs	Genotype	Case		Control		Crude OR (95% CI)	Adjusted OR (95% CI)[a]	*P*[b]
		N	%	N	%			
TLR2								
rs7656411		n = 441		n = 493				
	GG	112	25.4	156	31.6	1.00 (reference)	1.00 (reference)	0.079
	GT	233	52.8	229	46.5	1.42 (1.05–1.92)	1.41 (1.04–1.91)	
	TT	96	21.8	108	21.9	1.23 (0.86–1.79)	1.22 (0.84–1.76)	
	GT/TT	329	74.6	337	68.4	1.36 (1.02–1.81)	1.35 (1.01–1.79)	0.035*
	T allele	0.482		0.451				0.186
rs76112010		n = 446		n = 485				
	GG	287	64.4	331	68.3	1.00 (reference)	1.00 (reference)	0.144
	GA	137	30.7	141	29.1	1.12 (0.84–1.49)	1.15 (0.86–1.53)	
	AA	22	4.96	13	2.7	1.95 (0.97–3.95)	1.98 (0.98–4.02)	
	GA/AA	159	35.7	154	31.2	1.19 (0.91–1.53)	1.22 (0.93–1.60)	0.209
	A allele	0.203		0.172				0.089
rs7682814		n = 441		n = 486				
	GG	285	64.6	330	67.9	1.00 (reference)	1.00 (reference)	0.074
	GA	134	30.4	145	29.8	1.07 (0.81–1.42)	1.09 (0.82–1.45)	
	AA	22	5.00	11	2.26	2.32 (1.10–4.86)	2.29 (1.09–4.81)	
	GA/AA	42	15.9	38	13.9	1.15 (0.88–1.52)	1.17 (0.90–1.55)	0.292
	A allele	0.202		0.172				0.097
TLR4								
rs10983755		n = 423		n = 455				
	GG	327	77.3	327	71.9	1.00 (reference)	1.00 (reference)	0.157
	GA	84	19.9	115	25.3	0.73 (0.53–1.01)	0.72 (0.52–0.98)	
	AA	12	2.8	13	2.9	0.92 (0.42–2.05)	0.98 (0.44–2.18)	
	GA/AA	96	22.7	128	28.1	0.75 (0.55–1.02)	0.74 (0.54–1.01)	0.065
	A allele	0.128		0.155				0.101
rs11536889		n = 427		n = 482				
	GG	254	59.5	298	61.8	1.00 (reference)	1.00 (reference)	0.765
	GC	143	33.5	153	31.7	1.10 (0.83–1.46)	1.09 (0.82–1.45)	
	CC	30	7.0	31	6.4	1.13 (0.67–1.93)	1.18 (0.69–2.01)	
	GC/CC	173	40.5	184	38.1	1.10 (0.85–1.44)	1.10 (0.84–1.44)	0.471
	C allele	0.238		0.223				0.458

(*Continued*)

Table 3 (Continued).

SNPs	Genotype	Case		Control		Crude OR (95% CI)	Adjusted OR (95% CI)[a]	*P*[b]
		N	%	N	%			
rs1927914		n = 411		n = 453				
	AA	128	31.1	161	35.5	1.00 (reference)	1.00 (reference)	0.286
	AG	209	50.9	207	45.7	1.27 (0.94–1.72)	1.25 (0.92–1.69)	
	GG	74	18.0	85	18.8	1.10 (0.74–1.62)	1.09 (0.73–1.63)	
	AG/GG	283	68.9	292	64.5	1.22 (0.92–1.62)	1.20 (0.90–1.60)	0.171
	G allele	0.434		0.416				0.445
rs7873784		n = 433		n = 453				
	GG	235	54.3	251	55.4	1.00 (reference)	1.00 (reference)	0.644
	GC	159	36.7	169	37.3	1.01 (0.76–1.33)	1.00 (0.76–1.33)	
	CC	39	9.0	33	7.28	1.26 (0.77–2.07)	1.27 (0.77–2.01)	
	GC/CC	198	45.7	202	44.6	1.05 (0.80–1.36)	1.05 (0.80–1.37)	0.734
	C allele	0.274		0.259				0.496
CD14								
rs2563298		n = 434		n = 479				
	CC	336	77.4	326	68.1	1.00 (reference)	1.00 (reference)	0.003*
	CA	89	20.5	131	27.3	0.66 (0.48–0.90)	0.65 (0.48–0.89)	
	AA	9	2.1	22	4.60	0.40 (0.18–0.88)	0.40 (0.18–0.89)	
	CA/AA	98	68.5	153	31.9	0.62(0.46–0.84)	0.62 (0.46–0.83)	0.002*
	A allele	0.123		0.183				<0.001*
rs2569190		n = 450		n = 481				
	AA	174	38.7	166	34.5	1.00 (reference)	1.00 (reference)	0.351
	AG	210	46.7	233	48.4	0.86 (0.65–1.14)	0.86 (0.64–1.15)	
	GG	66	14.7	82	17.1	0.78 (0.88–1.92)	0.76 (0.52–1.94)	
	AG/GG	276	61.3	315	65.5	0.84 (0.52–1.31)	0.84 (0.64–1.09)	0.188
	G allele	0.380		0.413				0.150
rs2569191		n = 441		n = 472				
	CC	187	42.4	153	32.4	1.00 (reference)	1.00 (reference)	0.008*
	CT	188	42.6	236	50.0	0.65 (0.49–0.87)	0.65 (0.48–0.87)	
	TT	66	15.0	83	17.6	0.65 (0.44–0.96)	0.64 (0.44–0.95)	
	CT/TT	254	57.6	319	67.6	0.65 (0.50–0.85)	0.65 (0.49–0.85)	0.002*
	T allele	0.363		0.426				0.006*

Notes: [a]Adjusted for age and sex in logistic regression model. [b]Two-sided χ^2 test for the distributions of genotype and allele frequencies. *Statistically significant ($P < 0.05$).
Abbreviations: SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

Table 4 Stratification Analyses of *TLR2, TLR4* and *CD14* Polymorphisms in the Dominant Model in Cases and Controls

Variables	Subgroups	*TLR2* rs7656411		*P*	*CD14* rs2563298		*P*	*CD14* rs2569191		*P*
		N (Case/ Control)[a]	Adjusted OR (95% CI)[b]		N (Case/ Control)[a]	Adjusted OR (95% CI)[b]		N (Case/ Control)[a]	Adjusted OR (95% CI)[b]	
Age (years)	< 18	247/239	1.33 (0.88–1.99)	0.173	244/239	0.54 (0.36–0.80)	0.003**	247/236	0.65 (0.44–0.94)	0.022*
	≥ 18	194/254	1.36 (0.91–2.06)	0.163	191/240	0.73 (0.47–1.13)	0.164	194/236	0.66 (0.44–0.98)	0.037*
Gender	Male	295/303	1.60 (1.12–2.30)	0.011*	289/296	0.75 (0.52–1.07)	0.115	293/289	0.66 (0.47–0.93)	0.018*
	Female	146/190	0.97 (0.60–1.57)	0.894	146/183	0.42 (0.25–0.72)	0.002**	148/183	0.62 (0.40–0.98)	0.038*
Asthma	No	263/493	1.28 (0.81–2.03)	0.292	261/479	0.45 (0.27–0.75)	0.002**	262/472	0.53 (0.35–0.81)	0.003**
	Yes	116/493	1.61 (1.13–2.28)	0.007*	115/479	0.60 (0.42–0.85)	0.006*	116/472	0.70 (0.51–0.96)	0.027*
VAS	< 5	247/493	1.25 (0.88–1.77)	0.207	241/479	0.65 (0.45–0.92)	0.017*	246/472	0.72 (0.52–0.99)	0.048*
	≥ 5	194/493	1.45 (0.99–2.12)	0.055	193/479	0.60 (0.40–0.88)	0.013*	195/472	0.57 (0.41–0.80)	0.001**
Total IgE[c]	Lower	135/493	1.41 (0.99–2.00)	0.059	234/479	0.45 (0.31–0.67)	<0.0001**	237/472	0.61 (0.44–0.84)	0.002**
	Higher	206/493	1.31 (0.90–1.89)	0.154	200/479	0.82 (0.57–1.19)	0.339	204/472	0.71 (0.50–0.99)	0.047*

Notes: [a]Controls were stratified accordingly when dividing cases into age and gender subgroups, while they were kept as a whole in the situation of the other subgroups. [b]Adjusted for age and gender in logistic regression model. [c]Lower: below the 90th percentile of logarithmic total IgE level; Higher: above the 90th percentile of logarithmic total IgE level. *Nominally significant ($P < 0.05$) but did not withstand Bonferroni correction. **Significant after Bonferroni correction. Dominant model: MW/MM vs WW; MW: heterozygotes; MM: mutation homozygotes; WW: wild homozygotes.

Abbreviations: OR, odds ratio; CI, confidence interval; VAS, visual analogue scale.

Table 5 Multifactor Dimensionality Reduction Models for Locus–Locus Interactions

Model[a]	CV Training	CV Testing	CV Consistency	OR (95% CI)	*P*
A10	0.5544	0.5352	8/10	1.5555 (1.1468–2.1099)	0.0036*
A3, A10	0.5759	0.5298	6/10	1.8436 (1.3591–2.5009)	< 0.0001*
A1, A6, A8	0.6074	0.5308	6/10	2.4043 (1.7642–3.2768)	< 0.0001*

Notes: [a]A1: rs7656411; A3; rs7682814; A6: rs1927914; A8: rs2563298; A10: rs2569191. *Statistically significant ($P < 0.05$).
Abbreviations: CV, cross-validation; OR, odds ratio; CI, confidence interval.

Gene–Environment Interactions in AR Cases

As shown in Table 6, a total of 237 patients in the case group were asked to answer questionnaires covering environmental factors, including smoking, living or working floors, sunlight exposure, recently renovated or purchased furniture, polluting enterprises nearby, distance to main road, time of using computer, cooking fumes and pet keeping. Since these environmental data are difficult to be obtained from the controls, we only questionnaire-surveyed the cases, and explored gene–environment interactions through the logistic regression in a case-only study.[30]

The correlations between environmental factors and polymorphisms of *TLR4* and *CD14* were evident in AR patients (Table 7). Compared with homozygous wild type GG, the proportion of AR patients with *TLR4* rs10983755 GA/AA genotype living or working on the ≥ 4 floor was significantly less than that on the < 4 floor (OR = 0.51, 95% CI = 0.27–0.99), indicating a negative correlation between *TLR4* rs10983755 and floors ($P = 0.048$). *TLR4* rs11536889 had also a negative correlation with pet keeping ($P = 0.043$), while AR cases in the pet group were significantly less than those in the non-pet group (OR = 0.12, 95% CI = 0.02–0.93) under the dominant model (GC/CC vs GG). In addition, sunlight exposure exhibited significantly positive correlation with three SNPs (OR = 2.66, 95% CI = 1.12–6.34 for *TLR4* rs7873784; 2.22, 1.00–4.90 for *CD14* rs2569190 and 2.56, 1.15–5.73 for *CD14* rs2569191). However, none of these interactions withstood Bonferroni correction. No significant interactions were observed between genotypes of other SNPs and environmental factors (data not shown).

Functional Assessment of SNPs

As shown in Table 8, using SNPinfo, HaploReg, RegulomeDB and MirSNP, we performed a functional annotation analysis of these 10 SNPs and estimated that *TLR2* rs7656411, *CD14* rs2563298 and *CD14* rs2569191 all possessed promoter histone marks, enhancer histone marks, changed motifs, and selected expression quantitative trait locus (eQTL) hits. Moreover, for *CD14* rs2563298 located in the 3′UTR region, using MirSNP and RNAhybrid, we predicted that miR-451 can be bound to rs2563298 A-allele but not to rs2563298 C-allele. RNAfold predicted that rs2563298 A to C substitution led to alteration of *CD14* secondary structure, with the MFE decreasing from −6.8 kcal/mol to −8.0 kcal/mol (Figure 2).

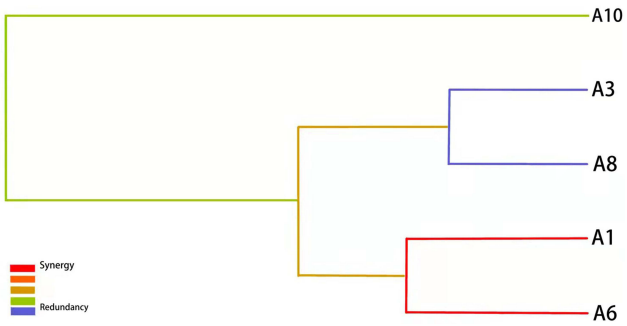

Figure 1 Tree diagram of the best genotype models. The distance between single nucleotide polymorphisms (SNPs) indicates the intensity of the interactions. The color indicates the type of interactions. Red, orange, and green denote strong, moderate and weak interactions, respectively. A1: rs7656411; A3: rs7682814; A6: rs1927914; A8: rs2563298; A10: rs2569191.

Table 6 Statistics of Environmental Factors in AR Case Group

Variables	N	%
Smoke		
No	212	89.5
Yes	25	10.5
Living or working floors		
< 4 floor	125	59.2
≥ 4 floor	86	40.8
Daytime sunshine		
< 4 hours	30	13.4
≥ 4 hours	194	86.6
Recently renovated or purchased furniture		
No	110	46.8
Yes	125	53.2
Polluting enterprises nearby		
No	176	81.5
Yes	40	18.5
Distance to main road		
< 1000 meters	170	78.3
≥ 1000 meters	47	21.7
Time of using computer per day		
< 3 hours	143	72.2
≥ 3 hours	55	27.8
Cooking fumes		
No	125	54.3
Yes	105	45.7
Pet-keeping		
No	219	93.6
Yes	15	6.4

Abbreviation: AR, allergic rhinitis.

Discussion

TLRs modulate Th1/Th2 immune balance via a variety of cells closely related to AR, such as dendritic cells, mast cells and regulatory T-cells (Treg). In this study, we explored the associations of genetic variations and environmental factors with the susceptibility to AR in a Chinese Han population. The selected genes, *TLR2, TLR4* and *CD14*, are implicated in the pathogenesis of allergy. We found that polymorphisms of rs2563298 (A/C) and rs2569191 (C/T) in *CD14*, and

Table 7 Association Between Polymorphisms and Environmental Factors in the Dominant Model Among AR Case Group

SNPs	Genotype	Floors		Adjusted OR (95% CI)[a]	*P*	Sunlight Hours		Adjusted OR (95% CI)[a]	*P*	Pet-Keeping		Adjusted OR (95% CI)[a]	*P*
		< 4F	≥ 4F			< 4h	≥ 4h			No	Yes		
TLR4 rs10983755	GG	111	62	1.00 (reference)	0.048*	20	131	1.00 (reference)	0.730	147	11	1.00 (reference)	0.998
	GA/AA	41	17	0.51 (0.27–0.99)		7	51	1.18 (0.47–2.99)		57	4	1.00 (0.30–3.33)	
TLR4 rs11536889	GG	76	49	1.00 (reference)	0.515	16	121	1.00 (reference)	0.268	128	14	1.00 (reference)	0.043*
	GC/CC	44	34	1.21 (0.68–2.15)		14	65	0.64 (0.29–1.41)		82	1	0.12 (0.02–0.93)	
TLR4 rs7873784	GG	67	48	1.00 (reference)	0.725	22	95	1.00 (reference)	0.027*	118	5	1.00 (reference)	0.128
	GC/CC	55	36	0.90 (0.52–1.59)		8	94	2.66 (1.12–6.34)		96	10	2.38 (0.78–7.27)	
CD14 rs2569190	AA	39	33	1.00 (reference)	0.352	15	64	1.00 (reference)	0.049*	79	5	1.00 (reference)	0.812
	AG/GG	85	53	0.76 (0.42–1.36)		15	129	2.22 (1.00–4.90)		139	10	1.15 (0.37–3.52)	
CD14 rs2569191	CC	45	39	1.00 (reference)	0.186	18	76	1.00 (reference)	0.022*	94	6	1.00 (reference)	0.777
	CT/TT	79	46	0.68 (0.39–1.20)		12	116	2.56 (1.15–5.73)		123	9	1.17 (0.40–3.44)	

Notes: [a]Adjusted for age and gender in logistic regression model. *Nominally significant ($P < 0.05$) but did not withstand Bonferroni correction. Dominant model: MW/MM vs WW; MW: heterozygotes; MM: mutation homozygotes; WW: wild homozygotes.

Abbreviations: AR, allergic rhinitis; SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

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Table 8 Functional Annotation Information of 10 SNPs

SNPs	Promoter Histone Marks	Enhancer Histone Marks	DNAse	Proteins Bound	eQTL Results	Motifs Changed	MicroRNA[a]	Function Annotation	Score[b]
rs7656411	BLD	BLD			12 eQTL results	PU.1, Rad21			5
rs76112010		BLD							4
rs7682814						4 altered motifs			6
rs10983755			BLD		2 eQTL results	AIRE, DMRT4, Tgif1			7
rs11536889		12 tissues	4 tissues	STAT3	4 eQTL results	Ik-1	hsa-miR-1208, hsa-miR-1236	3′UTR	4
rs1927914		BLD			4 eQTL results	6 altered motifs			6
rs7873784		BLD			3 eQTL results		hsa-let-7d-3p, hsa-miR-16-1-3p, hsa-miR-4528	3′UTR	5
rs2563298	13 tissues	7 tissues			41 eQTL results	Myb, SIX5	hsa-miR-451a	3′UTR	6
rs2569190	20 tissues	11 tissues	25 tissues	8 bound proteins	49 eQTL results	5 altered motifs		5′UTR	1b
rs2569191	BLD, LIV	4 tissues			2 eQTL results	Mef2, Pou2f2, TATA			5

Notes: [a]Predicted possible binding microRNA by SNPinfo and MirSNP. [b]Based on RegulomeDB.
Abbreviations: SNPs, single nucleotide polymorphisms; BLD, blood; LIV, liver; eQTL, expression quantitative trait locus; UTR, untranslated region.

rs7656411 (G/T) in *TLR2* were significantly associated with AR. In addition, gene–gene and gene–environment interactions may contribute to the development of AR.

TLR2 has a wide recognition spectrum, which can recognize bacterial peptidoglycans, lipoproteins, viral envelope proteins and other microbial components, activate signal transduction pathways, and induce adaptive immunity. In this study, the GT/TT genotype of rs7656411 in *TLR2* was associated with AR risk ($P = 0.035$). The rs7656411 was located in the 3′near gene region, which may participate in mRNA transcription and affect gene expression. *TLR2* rs7656411 was first reported to be associated with AR, while this rs7656411 has been confirmed to be associated with asthma in Chinese population.[7] Few studies have investigated the role of *TLR2* SNPs in the etiology of AR.[15,17] The results of studies on *TLR2* SNPs and the risk of asthma in different populations are contradictory.[22,31–35] Moreover, meta-analyses analyzed the correlation of four SNPs (rs5743708, rs3804099, rs3804100 and rs4696480) of *TLR2* to susceptibility of asthma, suggesting that rs4696480 and rs3804099 were associated with asthma.[9,11]

TLR4 acts as a surface receptor on macrophages for bacterial endotoxin or lipopolysaccharide (LPS) in a dose-dependent manner, and activates macrophages to produce cytokines that affect Th1/Th2 balance. A recent study reported that polymorphisms of rs4986790 and rs4986791 in *TLR4* were associated with COVID-19 severity, cytokine storm, and mortality,[36] while the association between these two SNPs with asthma was almost negative result.[10,31,32] In this study, four SNPs of *TLR4* (rs10983755, rs11536889, rs1927914 and rs7873784) were not significantly associated with AR risk. A lack of association between *TLR4* SNPs and asthma risk was also reported in a Chinese population with two SNPs (rs10983755 and rs1927914),[12] and a European population with rs11536889;[13] whereas rs10759930 in *TLR4* was associated with AR risk.[37] However, more negative results have demonstrated that *TLR4* gene polymorphisms may not be strongly associated with allergic diseases; this may be explained by the effect of endotoxin level, the race of the population, and the complexity of the allergic mechanism.

In this study, three SNPs of *CD14* were located in the functional region: rs2563298 was located in the 3′UTR region, which may participate in the translation of mRNA regulated by microRNA; rs2569190 and rs2569191 were located in the 5′UTR region and 5′near gene region, which may be involved in the expression of promoter region. Our study first explored that the polymorphisms of rs2563298 and rs2569191 in *CD14* significantly decreased the risk of AR in Chinese population. Studies in Egypt[14] and Pakistan[28] confirmed that rs2569191 polymorphism was associated with asthma. No

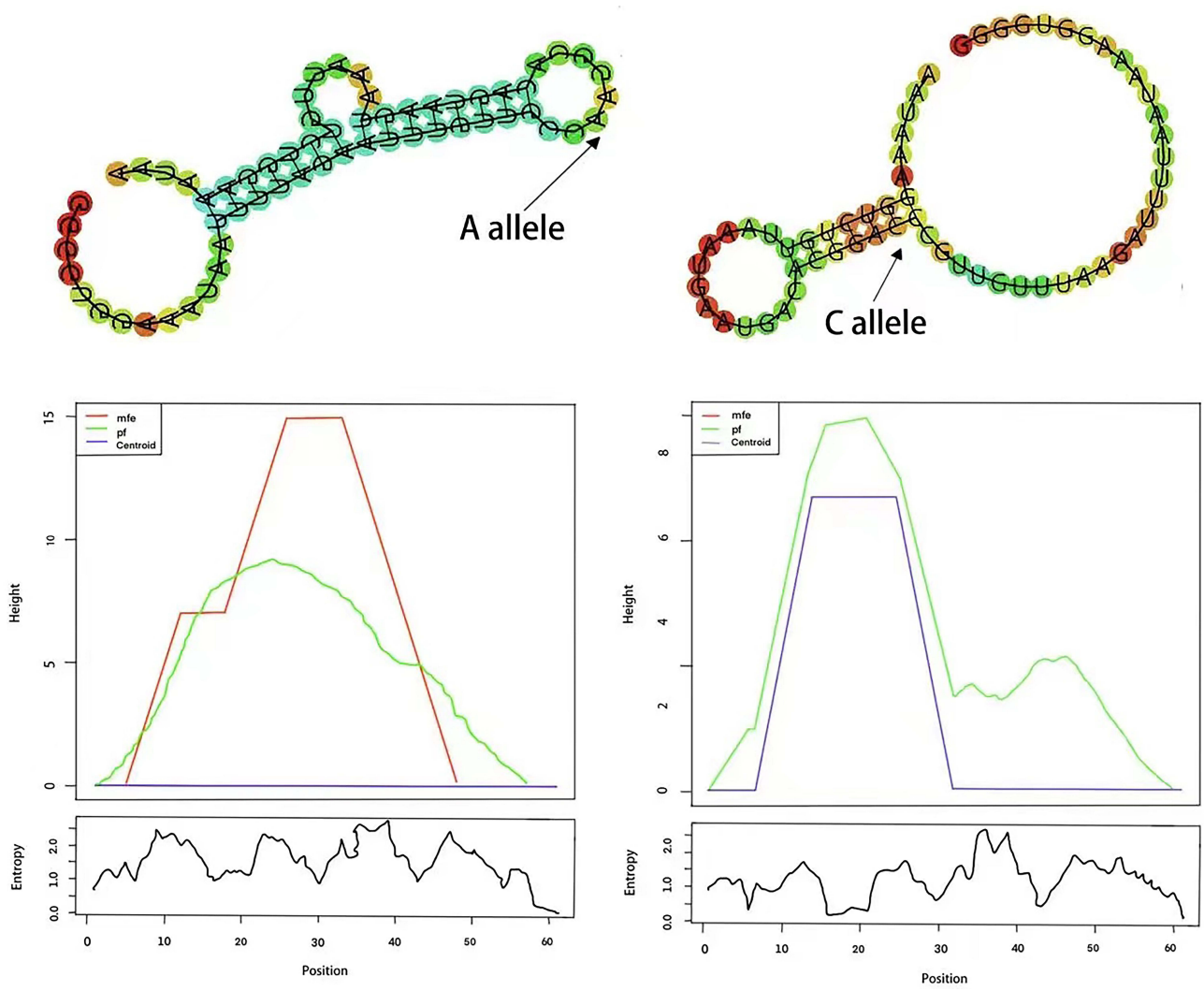

Figure 2 *In silico* prediction of *CD14* folding structures and minimum free energy (MFE) changes corresponding to rs2563298 A to C allele. The arrows indicated the changes in structure caused by rs2563298.

association between rs2569190 polymorphism and AR was also reported in a meta-analysis.[29] However, a study from northern China[16] found that the TT genotype of rs2569190 was associated with AR. This contradiction may arise from the low reliability of the results in that study with a small sample size (92 cases and 72 controls) and the environmental differences in regions between northern and eastern China.

In this study, 118 patients (26.1%) with AR had concomitant asthma. AR and asthma are interrelated in many aspects, including epidemiology, physiology, histology, immunopathology and therapeutic principles. Since upper and lower respiratory tract diseases usually coexist in one patient in an interdependent manner,[38] the concept of "one airway, one disease" has been proposed.[1] In the stratification analysis, the mutation heterozygous/homozygous genotypes of rs2563298 and rs2569191 in *CD14* gene could reduce the risk of AR with and without asthma, and the AR without asthma demonstrated higher associative significance to withstand the Bonferroni correction. We speculate that AR combined with asthma may bring about more serious allergic symptoms than simple AR, suggesting that the genetic polymorphisms do play a protective role in the allergic diseases. A study of French population has suggested that the heterozygous/homozygous genotype of *CD14* rs2563298 can reduce the risk of allergic asthma.[22] Another study in a Chinese population has shown that the frequency of *CD14* rs2569191 A-allele in allergic asthma is significantly lower than that in non-allergic asthma.[39] Moreover, we believe that although AR and asthma share many similarities, the inhibitory effects of genetic variations in TLR pathway

on AR, asthma or other allergic diseases may differ to some extent, as Micheal et al[28] confirmed that in Pakistani adults, the *CD14* rs2569191 is significantly associated with atopic asthma, but not AR.

In the stratification analysis, the mutation heterozygous/homozygous of rs2563298 (CA/AA) and rs2569191 (CT/TT) in *CD14* reduced the risk of AR in patients with lower IgE levels, while rs2569190 was not associated with total IgE levels. However, the association between *CD14* rs2569190 and atopy has been confirmed in studies of diverse populations including French,[40] Australian[41] and Chinese,[42] but not in German[43] and Japanese[44] populations. The controversial results may be explained by the various alleles frequencies among races. Furthermore, Martinez et al[21] have reported that the *CD14* rs2569190 C-allele is a risk factor for allergic phenotypes at low levels of endotoxin exposure, whereas the T-allele is a risk factor at high levels of exposure, suggesting *CD14* polymorphisms may be associated with allergic sensitization and environmental endotoxin exposure. After Bonferroni correction, this study still showed that *CD14* rs2569191 was associated with AR severity evaluated by VAS score. The associations of SNPs with allergic disease severity vary across studies.[33,45] VAS is a tool to evaluate AR severity using subjective indexes which may depend on individual cognition and sensitivity. Therefore, the correlation with disease severity cannot be fully explained from the perspective of SNPs alone, since the severity of AR is related to many other factors, such as quality of life, sleep status, daily activities, workload, course of disease, and so on.

Using MDR model, we discovered that the locus–locus interactions of *TLR2, TLR4* and *CD14* genes might be associated with the susceptibility to AR. In the presence of CD14, LPS can activate TLR2 or TLR4 signaling pathways to enhance Th2 type immune response.[46] The interactions between *CD14* and allergy-related genes were also found in the Philippine allergic population (*CD14, IL4, FCER1B, IL4RA* and *ADRB2*)[47] and in Korean children with asthma (*CD14, IL4RA, IL13, IL13RA1* and *CTLA4*).[48]

A large number of studies[49–52] have confirmed that environmental factors, including rural farm environment, tobacco smoke, traffic pollution, climate change, nutrition and occupational factors, are related to IgE levels, atopic and allergic diseases. At present, there are many studies on the role of gene–environment interaction in the pathogenesis of asthma in TLR pathway,[10,22–24] but few on TLR pathway with AR.[17] In this study, 237 patients providing data about environmental factors were collected in a case-only study.[30] The premise of the application is that genotype and environmental exposure should occur as independent factors in a general population. In this study, we can only evaluate the factor–factor associations in cases but not the main effect between cases and controls. Although none of these interactions withstood Bonferroni correction, the results of the case-only analysis still suggest a possible interaction between gene and environment during the development of AR.

Most of the AR patients were collected from two provinces with a humid climate in East China. Asthma and allergic symptoms are associated with mold and humidity.[53] A study of 400 Swedish children showed that low home ventilation rate in combination with moldy odor from the building structure increased the risk of allergic symptoms in children.[54] Compared with the higher floors, the lower floors are more humid, moldy and ventilated, which may increase the susceptibility to AR. We found that the patients carrying *TLR4* rs10983755 GA/AA genotype living on higher floors (≥ 4 F) were less than those living on lower floors (< 4 F), but this result did not pass the Bonferroni correction. According to the function annotation query (Table 8), *TLR4* rs10983755 is located in the 5′near gene region, which may enrich DNase hypersensitivity sites, eQTL hits and changed motifs and affect the expression of TLR4 protein. In the TLR signaling pathway, the binding of TLR4 to bacterial endotoxin or LPS was affected by the environmental humidity and moldiness in a dose-dependent manner. Therefore, we suspected that the mutation of *TLR4* rs10983755 may have a synergistic effect with environmental humidity and moldiness in the pathogenesis of AR. Similarly, Chen et al[55] have found gene–environment interactions between the polymorphism rs769214 and moldy odor in AR children. However, more functional research is needed to verify this interaction.

Moreover, we deduced a positive interaction between gene polymorphisms and sunlight exposure in AR. We found that AR patients carrying *CD14* rs2569191 CT/TT genotype were more prone to sunshine exposure, whereas *CD14* rs2569191 T-allele decreased the risk of AR in this study. The lack of environmental data in the controls may cause data deviation. Therefore, the interaction between *CD14* rs2569191 and sunlight exposure in AR, and related TLR pathways, needs to be further studied. Ultraviolet radiation (UVR) from sunlight stimulates anti-inflammatory and immunosuppressive pathways in the skin that modulate psoriasis, atopic dermatitis and some systemic diseases (such as multiple

sclerosis, type 1 diabetes and asthma) through the actions of UVR-induced regulatory cells and mediators, including 1,25-dihydroxy vitamin D3, IL-10, and nitric oxide.[56] The potential beneficial effects of UVR in controlling allergic airways disease have been evaluated in murine asthma models showing that UVR on the skin can repress Th2 response to asthma.[57] Rueter et al[58] have reported that exposure to UVR can reduce the risk of eczema in early childhood but has undefined associations with other allergic disease outcomes. Furthermore, it has been discovered that vitamin D, from either sunshine exposure or oral intake, may function in the process of allergic diseases.[59] However, the association between vitamin D level and AR risk is still controversial.[60]

In addition, pet-keeping seems to protect against AR in this study, though this result failed to challenge Bonferroni correction. Opinions differ a lot about whether pet-keeping can prevent allergic diseases.[61,62] It has been established that the *CD14* rs2569190 polymorphism is associated with increased total and specific serum IgE levels in children with frequent contact with pets.[63] Interestingly, another study has found that the sensibility to asthma is different between adults keeping cats and dogs.[64] Thereby, the effects of pet-keeping are influenced by various aspects, like pet species, individual allergic sensitivity and wide environmental exposure to allergen.

Several limitations exist in our study. First, this study only collected AR patients sensitive to dust mites in eastern China, but did not include patients allergic to other allergens (such as pollen, cockroach, and molds), for dust mites were the major allergens in this area. However, other allergens may also arouse various gene–environment interactions. Second, only analysis of gene–environment interactions in AR cases may not be powerful enough to cement the present evidence. A larger sample size and environmental data for healthy controls were required. Finally, functional experiments, especially combined with environmental factors, are required to further explain the correlation between genetic polymorphisms in TLR signaling pathway and AR pathogenesis from the perspective of the mechanism.

Conclusions

The polymorphisms of *TLR2* and *CD14* and gene–gene interactions in TLR signaling pathway were associated with susceptibility to AR in the Han Chinese population. However, the present results were limited to support the association between gene-environment interactions and AR. Moreover, more environmental data in the general population and functional studies are needed to warrant these findings.

Abbreviations

AR, allergic rhinitis; ARIA, allergic rhinitis and its impact on asthma; CHB, Han Chinese in Beijing; CI, confidence intervals; CVC, cross-validation consistency; DC, dendritic cell; ECP, eosinophil cationic protein; eQTL, expression quantitative trait locus; HWE, Hardy-Weinberg equilibrium; JPT, Japanese in Tokyo; LPS, lipopolysaccharide; MAF, minor allele frequency; MDR, multifactor dimensionality reduction; MFE, minimum free energy; OR, odds ratios; PAMP, pathogen-associated molecular patterns; SNPs, single nucleotide polymorphisms; TLR, toll-like receptor; Treg, regulatory T-cells; UTR, untranslated region; UVR, ultraviolet radiation; VAS, visual analogue scale.

Ethics Approval and Informed Consent

The research protocol complying with the Declaration of Helsinki was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (2015-SRFA-122) and written informed consent was obtained from all participants.

Consent for Publication

We have obtained the informed consent from all patients or their legal guardians.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas. All authors took part in drafting, revising, or critically reviewing the article; have agreed on the journal to which the article has been submitted; gave final approval for the version to be published; and agreed to be accountable for the contents of the article.

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Disclosure

The authors report no conflicts of interest in this work.

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