

RESEARCH ARTICLE

Association between SP-A rs1965708 gene polymorphism and allergic rhinitis risk in Chinese population

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Abstract

Background: Pulmonary surfactant protein A (SP-A) in the respiratory tract plays an important role in host. In the present, we assessed the association between SP-A gene polymorphism and allergic rhinitis.

Methods: Using a case-control design, we compared the genotype frequencies of SP-A rs1965708 between allergic rhinitis patients and healthy control group. Genotyping was performed using real-time quantitative PCR-based molecular identification methods. Univariate and multivariate logistic regression were performed to quantitatively assess the association between rs1965708 polymorphism and allergic rhinitis, and the odds ratio (OR) and 95% confidence interval (CI) were also calculated.

Results: 500 patients with allergic rhinitis and 500 healthy controls were included in the study. Compared with the CC genotype, we found that AA genotype of rs1965708 could increase the allergic rhinitis risk in the univariate analysis (OR = 2.63, 95% CI: 1.56–4.54, $p = 0.000$). For dominant model, we found no significant difference in the dominant model (OR = 1.14, 95% CI: 0.86–1.52, $p = 0.367$). In the recessive model, the CC genotype could elevate the risk of allergic rhinitis compared with CC + AA genotype (OR = 2.70, 95% CI: 1.61–4.54, $p = 0.000$). Similar results were also found in the allele model (OR = 1.28, 95% CI: 1.07–1.54, $p = 0.008$). Interactions between rs1965708 AA or AC and smoking increased the allergic rhinitis risk.

Conclusions: The rs1965708 variants of SP-A gene polymorphism are associated with allergic rhinitis, and the A allele could increase the allergic rhinitis risk. The AA SNP variants that interact with smoking may alter the susceptibility to allergic rhinitis.

KEYWORDS

allergic rhinitis, gene polymorphism, interaction, surfactant protein A

1 | INTRODUCTION

Allergic rhinitis (AR) is an allergic response to allergens that are mediated by IgE, a variety of immunocompetent cells, and cytokines involved in the nasal mucosa.¹ AR is a global health problem, and an

extremely common disease worldwide, affecting 10%–25% of the population.² Although AR is not usually a severe disease, it significantly alters the social life of patients and affects school learning performance as well as work productivity. Moreover, the costs incurred by rhinitis are substantial. In addition, AR is associated with

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asthma, sinusitis, nasal polyposis, otitis media, and conjunctivitis and has been identified as one of the top ten reasons for visiting primary care clinics.³ The incidence of AR has increased recently. Although the etiology of AR is not well understood,⁴ it is established that the occurrence of AR is a consequence of gene-environment interactions.

Surfactant proteins (SPs) are produced and secreted by alveolar type II cells and Clara cells, including hydrophilic SPs A and D (SP-A and SP-D), and the hydrophobic SPs B and C (SP-B and SP-C). The human SP-A and SP-D genes are located on chromosome 10q22-q24.⁵ It is suggested that SP-A consists of one SP-A1 molecule and two SP-A2 molecules. Both SP-A1 and SP-A2 transcripts are expressed in adult human alveolar type II cells. However, SP-A1 and SP-A2 are differentially expressed in different tissues.⁶ Both SP-A1 and SP-A2 transcripts have been expressed in the human small and large intestines, whereas only the SP-A2 genes were predominantly detected in the epithelium of the reproductive system.⁷ SP-A was also detected in the human nasal mucosa. Both SP-A and SP-D belong to the C-type lectin superfamily and prevent lung collapse during expiration. It has been demonstrated that these molecules participate in the innate immune response and regulate inflammatory processes.⁸ Genetic variations in SP-A1, SP-A2, and SP-D genes are related to susceptibility to several infectious diseases, such as respiratory syncytial virus infection and meningitis.^{9,10} SP-A and SP-D directly bind to rough lipopolysaccharides and other components present on the surface of bacteria and other microorganisms. SP-A and SP-D also inhibit the growth of gram-negative bacteria by increasing membrane permeability and the ability to kill bacteria.¹¹ In addition, SP-A and SP-D bind to different receptors on the surface of several types of immune cells and modulate inflammatory processes and innate immune function by influencing MAPK and other signaling pathways.¹² Recently, Malhotra et al¹³ found SP-A-specific binding allergen particles. SP-A can also reduce the proliferation of mononuclear cells in the peripheral blood of asthmatic children caused by house dust mites. The SP-A1 alleles differ at five codons, the amino acids 19, 50, 62, 133, and 219, while the SP-A2 alleles differ at four codons, the amino acids 9, 91, 140, and 223. Previous studies have reported that several allele variants of SP-A1 and SP-A2 genes are related to respiratory distress syndrome, severe respiratory syncytial virus bronchiolitis, and pulmonary tuberculosis. A study also found that genetic polymorphism of SFTPA and risk of *Aspergillus* allergy in Caucasian populations, as well as polymorphism of the SFTPA gene, were also strongly associated with susceptibility to asthma in children.¹³ In the present study, we assumed that gene polymorphisms of SP-A may be associated with the overall genetic predisposition to allergic airway diseases instead of affecting the occurrence of pulmonary diseases. Previous studies with small sample sizes found a positive association between SP-A rs1965708 and AR.¹⁴ In the present study, we validated the association in different population settings and explored the potential interaction between rs1965708 polymorphism and other factors.

2 | METHODS

2.1 | Study populations

Using a case-control study design, we enrolled patients with AR who were from the Department of Otolaryngology—Head & Neck Surgery, Fuyang People's Hospital from May 2016 to June 2019. The healthy control group was from the health examination center over the same period. The diagnosis of AR refers to the Chinese Society of Allergy Guidelines for Diagnosis and Treatment of Allergic Rhinitis¹⁵: (1) Two or more of the following symptoms must appear: sneezing, rhinorrhea, nasal itching, nasal obstruction that can be accompanied by itchy eyes, tears, and red eyes, and other symptoms lasting for at least 1 h every day; (2) sign: pale nasal mucosa, edema, and watery nasal secretion; (3) allergen test: at least one allergen SPT and/or serum IgE should be positive. The inclusion criteria for the control group are as follows: no acute, chronic diseases, no history of allergic diseases or asthma, the routine examinations, and blood biochemical tests were within the normal range, and the serum specific IgE test was negative. Criteria for the cases and control: All the patients with AR had no history of asthma, and systemic diseases, such as lung, liver, and kidney diseases, were excluded, and infectious diseases such as hepatitis and tuberculosis were excluded.

We used the Quanto 1.2.4 software (used for calculation of genes and diseases, https://preventivemedicine.usc.edu/software/Quanto1_2_4.zip) to calculate the sample size using the following parameters: Type I error was 0.05, type II error 0.10, and expected OR = 1.8. The sample size was at least 264 cases and controls, and the present study consisted of 500 cases and 500 controls that met the statistical requirements. This study was approved by the Medical Ethics Committee of the Fuyang People's Hospital. The study was conducted in accordance with the World Medical Association Declaration of Helsinki, and all subjects provided written informed consent.

2.2 | Data collection and genotyping

The general characteristics and clinical information were collected from the medical records, including age, sex, and body mass index (BMI) calculated using the formula weight (kg)/height (m)²; BMI > 24 was defined as overweight. Smoking was defined as current smoking or smoking previously daily, and drinking was defined as two to four times a month.¹⁶ A history of diseases was also collected. Two 5 ml venous blood samples were extracted in the morning before meals using test tubes containing ethylenediaminetetraacetic acid (EDTA). One sample was used to detect IgE, interleukin-6 (IL-6), IL-8, and IL-10 using enzyme-linked immunosorbent assays. The remaining blood sample was stored at -80°C for genotyping.

Using the DNA Purification Kit, the "salting-out" method was used to extract genomic DNA, which was stored at -80°C. The genotypes of SP-A rs1965708 loci were analyzed from the DNA samples of each participant using the SNPscan™ genotyping method. To

determine whether the obtained genotypes of SP-A SNPs could not be influenced by the technology, we randomly selected 110 DNA samples for checking, and no inconformity was found. DNA concentration and purity were measured using a UV spectrophotometer (Pharmacia Biotech). PCR amplification primers and single-base extension primers (forward: 5'-AAGAAAGCAAGTCTCTGCCTG-3'; reverse: 5'-CTGTGTACATCTCCACACACTT-3') for the rs1800544 site were designed using Sequenom Genotyping. Amplification was performed in a 5 μ L reaction system using multiplex PCR. The PCR assay conditions were as follows: 94°C for 4 min, 45 cycles of 94°C for 20 s, 56°C for 30 s, 72°C for 1 min, and 72°C for 3 min, and the amplicons were maintained at 4°C.

2.3 | Statistical analysis

Continuous variables (age, BMI, IgE, IL6, IL8, and IL10) were expressed as the mean \pm standard deviation, and Student's *t* test was used for comparison between the case and control groups. Categorical variables (sex, smoking, drinking, family history, overweight, and genotype frequency) were expressed using count and percentage, and the chi-square test was used for comparisons between the two groups. The Hardy-Weinberg equilibrium (HWE) was assessed using SP-A genotype frequencies. The following gene models were used to assess the association between gene polymorphism and allergic rhinitis: heterozygote (AC vs. AA), homozygote (AA vs. CC), dominant (AC + AA vs. CC), recessive (CC + AC vs. AA), additive model (AA/AC/CC), and allele model (A vs. C). Multiple comparisons were corrected using the Bonferroni method. Multivariate logistic

regression was performed to adjust for the role of environmental factors such as smoking, drinking, sex, age, BMI, and inflammatory factors. We calculated the Akaike information criterion (AIC) to select the best genetic model. The smaller the AIC, the better the model. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Subgroup analysis was performed for categorical variables (sex, age, overweight, family history, drinking, and smoking) to compare the relationship between SP-A gene polymorphisms and AR. Furthermore, we also analyzed the association between gene polymorphisms and inflammation factors (IgE, IL6, IL8, and IL10). All the analyses were computed using SPSS 23.0, and two-sided $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | General characteristics between the case and control groups

This study included 500 patients with AR and 500 healthy controls. Table 1 summarizes the demographic information and the rs1965708 gene frequency distributions in the study subjects. There were no significant differences in sex ratio ($p = 0.746$), age ($p = 0.291$), BMI ($p = 0.233$), or overweight ratio ($p = 0.139$) between the case and control groups. However, the case group had a higher smoking ratio than the control group (36.0% vs. 22.8%, $p < 0.001$). The drinking ratio in the case group was 24.8%, with a rate of 30.0% in the control group, but no significant difference was observed between the two groups ($p = 0.076$). There was no significant difference in

TABLE 1 Comparisons of general characteristics between case and control group

Parameters	Level	Control group	Case group	<i>p</i>
Sex (%)	Male	198 (39.6)	192 (38.4)	0.746
	Female	302 (60.4)	308 (61.6)	
Age (mean (SD))		49.8 (17.7)	48.6 (18.1)	0.291
BMI (mean (SD))		22.9 (2.9)	23.2 (3.4)	0.233
Overweight (%)	No	347 (69.4)	324 (64.8)	0.139
	Yes	153 (30.6)	176 (35.2)	
Smoking (%)	No	386 (77.2)	320 (64.0)	<0.001
	Yes	114 (22.8)	180 (36.0)	
Drinking (%)	No	350 (70.0)	376 (75.2)	0.076
	Yes	150 (30.0)	124 (24.8)	
Family history (%)	No	237 (47.4)	269 (53.8)	0.050
	Yes	263 (52.6)	231 (46.2)	
IgE (mean (SD))		32.7 (12.1)	136.0 (19.9)	<0.001
IL6 (mean (SD))		117.3 (20.3)	142.09 (22.7)	<0.001
IL8 (mean (SD))		103.8 (21.0)	153.7 (27.5)	<0.001
IL10 (mean (SD))		9.3 (23.0)	18.8 (4.0)	<0.001
rs1965708 (%)	AA	27 (5.4)	69 (13.8)	<0.001
	AC	263 (52.6)	235 (47.0)	
	CC	210 (42.0)	196 (39.2)	

the family history of allergic rhinitis between the case and control groups ($p = 0.050$). Furthermore, the IgE level was higher in the case group than in the control group ($p < 0.0001$). The levels of inflammatory factors IL-6, IL-8, and IL-10 were also higher in the case group than in the control group ($p < 0.001$ for all).

3.2 | SP-A gene rs1965708 polymorphism and allergic rhinitis risk

We identified three genotypes (CC, AC, and AA) from the samples. The chi-square test indicated that genotype frequencies were in accordance with the Hardy-Weinberg equilibrium ($\chi^2 = 0.012$, $p = 0.914$). The frequencies of the three genotypes were 39.2% (CC: $n = 196$), 47.0% (AC: $n = 235$), and 13.8% (AA: $n = 69$) in the case group. The frequencies were 42.0% (CC: $n = 210$), 52.6% (AC: $n = 263$), and 13.8% (AA: $n = 27$) in the control group. The A allele frequency was significantly lower in the case group than in the control group ($p < 0.001$). Table 2 presents the associations between different gene models and the risk of allergic rhinitis. For rs1965708 variants, compared with the CC genotype, we found that the AA genotype of rs1965708 could increase the risk of AR in the univariate analysis (AA vs. CC: $p = 0.000$, AA vs. AC: $p = 0.000$). For the dominant model, we found no significant difference in the dominant model (AC + AA vs. CC: $p = 0.367$). In the recessive model, the AA genotype could elevate the risk of AR compared to the CC + AA genotype ($p = 0.000$). Similar results were also found in the allele model (OR = 1.28, 95% CI: 1.07–1.54, $p = 0.008$). After adjusting for some potential confounding factors, it also indicated that the recessive model (OR = 2.70, 95% CI: 1.61–4.54, $p = 0.000$) and homozygote model (AA vs. CC: OR = 2.63, 95% CI: 1.56–4.54, $p = 0.000$; AA vs. AC: OR = 2.70, 95% CI: 1.61–4.54, $p = 0.000$) had an elevated risk of AR in the case group than in the control group. According to the AIC, the recessive model may be the best genetic model for rs1965708 polymorphism in AR.

3.3 | Subgroup analyses between rs1965708 polymorphism and allergic rhinitis risk

Table 3 summarizes the results of subgroup analyses between the SP-A rs1965708 gene polymorphism and AR. Regarding sex, the co-dominant model (AA vs. AC: $p = 0.036$) and recessive model (AA vs. AC + CC: $p = 0.045$) increased the risk of AR for males and the co-dominant model (AA vs. CC: $p = 0.000$; AA vs. AC: $p = 0.0001$), recessive model (AA vs. AC+CC: $p = 0.001$), and allele model (A vs. C: $p = 0.007$) for females. For smoking, drinking, and age components, the co-dominant, recessive, and allele models showed significant associations in different subgroups, except for the dominant model. For overweight, compared with the CC genotype, we found that AA was associated with an increased risk of AR (AA vs. CC: $p = 0.000$, CC vs. AC: $p = 0.000$). The AA genotype also increased the risk of AR compared to the AC + CC genotype ($p = 0.000$). The allele model also showed a significant association (A vs. C: $p = 0.004$). However, the rs1965708 variants were not associated with the risk of AR in the overweight population in all genotype models ($p > 0.05$).

3.4 | Interactions between genes and environment components

To explore the interaction effects between environmental components and genes on the risk of AR, we performed an interaction analysis. Interaction analysis was performed for two factors: smoking and overweight. Table 4 presents the interaction between the rs1965708 variants and other factors for AR. We found that participants with the AA genotype and without smoking had an increased risk of allergic rhinitis (OR = 2.50, 95% CI: 1.43–4.38, $p = 0.001$), and the AC genotype combined with smoking increased the risk of allergic rhinitis (OR = 1.97, 95%

TABLE 2 Logistic regression analysis of associations between rs1965708 polymorphism and allergic rhinitis

Models	Genotype	Controls (n)	Cases (n)	OR (95% CI)	<i>p</i>	OR (95% CI) [*]	<i>p</i> [*]	AIC
rs1965708								
Co-dominant	CC	210 (42.0%)	196 (39.2%)	1.00		1.00		1387.2
Heterozygote	AC	263 (52.6%)	235 (47.0%)	0.96 (0.74–1.24)	0.745	0.98 (0.73–1.30)	0.877	
Homozygote	AA	27 (5.4%)	69 (13.8%)	2.74 (1.68–4.45)	0.000	2.63 (1.56–4.54)	0.000	
	AA vs. AC	27 (5.4%)	69 (13.8%)	2.86 (1.77–4.61)	0.000	2.70 (1.61–4.54)	0.000	
Dominant	CC	210 (42.0%)	196 (39.2%)	1.00		1.00		1389.5
	AC + AA	290 (58.0%)	304 (51.2%)	1.12 (0.87–1.45)	0.367	1.14 (0.86–1.52)	0.356	
Recessive	CC + AC	473 (94.6%)	431 (86.2%)	1.00		1.00		1369.3
	AA	27 (5.4%)	69 (13.8%)	2.80 (1.76–4.46)	0.000	2.70 (1.61–4.54)	0.000	
Allele	C	683 (68.3%)	627 (62.7%)	1.00		–	–	
	A	317 (31.7%)	373 (37.3%)	1.28 (1.07–1.54)	0.008	–	–	
Additive	AA/AC/CC	–	–					1382.5

Abbreviations: AIC, Akaike information criterion.

^{*}Adjusted *p* value.

TABLE 3 Subgroup analyses between SP-A rs1965708 gene polymorphism and allergic rhinitis

Factors	Control (n)/Case (n)		AA vs. CC		AA vs. AC		AA/AC vs. CC		AA vs. AC/CC		A vs. C												
	CC	AC	AA	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p										
Sex																							
Male	79/82	101/92	12/24	1.92	0.90	4.17	0.087	2.17	1.04	4.55	0.036	0.99	0.66	1.47	0.957	2.08	1.00	4.35	0.045	1.14	0.84	1.52	0.409
Female	131/114	162/143	15/45	3.45	1.82	6.67	0.000	3.45	1.82	6.25	0.000	1.22	0.88	1.69	0.228	3.45	1.85	6.25	0.000	1.39	1.10	1.75	0.007
Smoking																							
Yes	76/46	97/55	7/13	3.03	1.14	8.33	0.022	3.23	1.23	9.09	0.013	1.08	0.67	1.75	0.751	3.23	1.23	8.33	0.013	1.23	0.87	1.75	0.237
No	134/150	166/180	20/56	2.50	1.43	4.35	0.001	2.56	1.49	4.55	0.001	1.14	0.84	1.54	0.416	2.56	1.49	4.35	0.000	1.28	1.03	1.59	0.027
Drinking																							
Yes	57/61	61/68	6/21	3.23	1.23	8.33	0.014	3.13	1.19	8.33	0.017	1.23	0.77	2.00	0.378	3.23	1.25	8.33	0.011	1.39	0.97	2.00	0.074
No	153/135	202/167	21/48	2.56	1.47	4.55	0.001	2.78	1.59	4.76	0.000	1.09	0.81	1.47	0.560	2.70	1.56	4.55	0.000	1.25	1.01	1.56	0.041
Age																							
<60	139/137	190/157	18/46	2.56	1.43	4.76	0.001	3.13	1.72	5.56	0.000	0.99	0.73	1.35	0.950	2.86	1.61	5.00	0.000	1.19	0.96	1.49	0.114
≥60	71/59	73/78	9/23	3.03	1.32	7.14	0.007	2.38	1.04	5.56	0.037	1.49	0.94	2.33	0.087	2.70	1.20	5.88	0.013	1.49	1.08	2.08	0.018
Overweight																							
Yes	69/60	94/76	13/17	1.52	0.68	3.33	0.316	1.61	0.74	3.57	0.226	1.00	0.64	1.56	0.998	1.56	0.74	3.33	0.242	1.09	0.79	1.49	0.618
No	141/136	169/159	14/52	3.85	2.04	7.14	0.000	4.00	2.13	7.14	0.000	1.19	0.88	1.64	0.255	3.85	2.13	7.14	0.000	1.39	1.11	1.75	0.004

Bold values are just in order to emphasize that these P values <0.05

TABLE 4 Interaction between rs1965708 and others factors for allergic rhinitis

C	E	Controls (n)		Cases (n)		OR	95% CI		χ^2	p
							Lower	Upper		
AA/CC		Smoking								
-	-	134	47.2%	150	52.8%	1.00				
-	+	76	62.3%	46	37.7%	0.54	0.35	0.83	7.805	0.005
+	-	20	26.3%	56	73.7%	2.50	1.43	4.38	10.665	0.001
+	+	7	35.0%	13	65.0%	1.66	0.64	4.28	1.115	0.291
AC/CC		Smoking								
-	-	134	47.2%	150	52.8%	1.00				
-	+	76	62.3%	46	37.7%	1.85	1.20	2.85	7.805	0.005
+	-	166	48.0%	180	52.0%	1.03	0.68	1.58	0.039	0.843
+	+	97	63.8%	55	36.2%	1.97	1.32	2.96	10.99	0.001
AA/CC		Overweight								
-	-	141	50.9%	136	49.1%	1.00				
-	+	69	53.5%	60	46.5%	0.90	0.59	1.37	0.236	0.627
+	-	14	21.2%	52	78.8%	3.85	1.94	7.63	18.970	0.000
+	+	13	43.3%	17	56.7%	1.36	0.63	2.90	0.320	0.431
AC/CC		Overweight								
-	-	169	51.5%	159	48.5%	1.00				
-	+	94	55.3%	76	44.7%	0.86	0.592	1.246	0.86	0.424
+	-	14	21.2%	52	78.8%	3.95	2.03	7.66	3.950	0.000
+	+	13	43.3%	17	56.7%	1.39	0.65	2.95	1.390	0.390

TABLE 5 Comparison between rs1965708 gene polymorphism and inflammation factors

Cases	AA	AC	CC	A	C
IgE > 143.5	125 (38.5)	139 (42.8)	61 (18.8)	389 (59.8)	261 (40.2)
IgE < 143.5	71 (40.6)	96 (54.9)	8 (4.6)	238 (68.0)	112 (32.0)
p		0.326	0.000		0.011
OR (95% CI)	1.00	0.82 (0.56-1.22)	4.33 (1.96-9.57)	1.00	1.43 (1.08-1.88)
IL-6 > 158.3	40 (41.1)	164 (43.2)	60 (15.8)	151 (62.9)	284 (37.4)
IL-6 ≤ 158.3	156 (41.1)	164 (43.2)	9 (7.5)	476 (62.6)	89 (37.1)
p		0.020	0.175		0.936
OR (95% CI)	1.00	1.69 (1.08-2.64)	0.59 (0.27-1.28)	1.00	0.99 (0.73-1.33)
IL-8 > 160.6	114 (37.7)	133 (44.0)	55 (18.2)	361 (59.8)	243 (40.2)
IL-8 ≤ 160.6	82 (41.4)	102 (51.5)	14 (7.1)	266 (67.2)	130 (32.8)
p		0.743	0.001		0.018
OR (95% CI)	1.00	0.94 (0.64-1.38)	2.83 (1.47-5.42)	1.00	1.38 (1.06-1.80)
IL-10 > 16.9	56 (30.1)	70 (37.6)	60 (32.3)	182 (48.9)	190 (51.1)
IL-10 ≤ 16.9	140 (44.6)	165 (52.5)	9 (2.9)	445 (70.9)	183 (29.1)
p		0.990	0.000		0.000
OR (95% CI)	1.00	1.06 (0.70-1.61)	16.67 (7.75-35.86)	1.00	2.54 (1.94-3.31)

Bold values are just in order to emphasize that these P values <0.05

CI: 1.32-296, $p = 0.001$) compared to those with the CC genotype and no smoking. Compared with participants without the CC genotype and non-overweight, those with the AA genotype and non-overweight had an increased risk of AR (OR = 3.85, 95%

CI: 1.94-7.63, $p = 0.000$). The AC genotype combined with non-overweight also increased the risk of AR compared with those with the AA genotype and non-overweight (OR = 3.95, 95% CI: 2.03-7.66, $p = 0.000$).

3.5 | Association between the rs1965708 variants and clinical parameters

We also analyzed the association between the rs1965708 variants and IgE, IL-6, IL-8, and IL10 in participants with AR. As shown in Table 5, we found that IgE ($p < 0.05$ for AA vs. CC, A vs. C), IL-6 ($p < 0.05$ for AC vs. CC), IL-8 ($p < 0.05$ for AA vs. CC, A vs. C), and IL-10 ($p < 0.05$ for AA vs. CC, A vs. C) were associated with the rs1965708 variants. The A allele frequencies of the rs1965708 variant were positively associated with IgE (AA vs. CC: OR = 4.33, 95% CI: 1.96–9.57; A vs. C: OR = 1.43, 95% CI: 1.08–1.88), IL6 (AC vs. CC: OR = 1.69, 95% CI: 1.08–2.64), IL-8 (AA vs. CC: OR = 2.83, 95% CI: 1.47–5.42; A vs. C: OR = 1.38, 95% CI: 1.06–1.80), and IL-10 (AA vs. CC: OR = 16.67, 95% CI: 7.75–35.86; A vs. C: OR = 2.54, 95% CI: 1.94–3.31).

4 | DISCUSSION

The nasal mucosa is exposed to the outside air for a long time. Pollutants, microorganisms, and allergens in the external environment can stimulate the nasal mucosa. In this case, innate host defense plays a key role in resisting damage to the nasal mucosa.¹⁷ Surface-active protein A (SP-A) is the most abundant protein expressed on the surface of alveoli. A growing body of evidence has revealed its important role in innate immune responses, such as bacterial clearance, pollen-specific binding, host defense, allergic response regulation, and suppression of inflammation.^{18,19} Recent studies have shown that in addition to being mainly expressed in the lungs, SP-A proteins are expressed in the lower respiratory tract and nasopharyngeal membranes.²⁰ Previous studies have found that SP-A and SP-D are highly expressed in the AR and nasal polyp groups, while only a small number of positive expressions were found in the control group.²¹ SP-A and SP-D are mainly expressed in the mucosal epithelial cells and glandular epithelial cells. The expression intensity in the mucosal epithelial cells of the AR and nasal polyp groups was higher than that of the control group, and the difference was significant.²² As the first-line defense molecule of the nasal mucosa, SP-A gene polymorphism may cause structural and functional changes that may lead to the development of allergic diseases. The present study found that (1) there were significant differences in terms of allele and/or genotype frequency in rs1965708, and the A allele increased the risk of AR; (2) overweight weakened the effect of rs1965708 on the risk of AR, while the environmental components, including sex, age, smoking, and drinking, had no effect on the association between the rs1965708 variants and increased risk of AR; (3) interactions between rs1965708 AA or AC and smoking increased the risk of AR; (4) increased AR caused by the rs1965708 variants could be associated with IgE and some inflammatory factors. Our results provide evidence of this assumption.

In the present study, the frequencies of CC, AC, and AA were 42.0%, 52.6%, and 5.4% in the control group and 39.2%, 47.0%, and

13.8%, respectively, in the case group. The frequencies are different from the Spanish population (2.9%, 31.7%, and 65.4% in the general population, and 69.0%, 31.0%, and 0.0% in acute respiratory failure). However, our results are similar to those of a previous study in the Chinese population (64.3%, 34.5%, and 1.2% in the general population and 49.5%, 42.1%, and 8.3%, respectively, in the case group).²³ In another study on the Chinese population, the reported frequencies of the three genotypes were 96.0%, 4.0%, and 0.0%.²⁴ These results indicate that the SP-A variant greatly varied in different populations. A previous study also found that SP-A gene polymorphisms were associated with asthma risk,²⁵ and the 6A allele haplotype of SFTPA1, with an estimated frequency of 6% among our study infants, was associated with an increased risk of persistent cough (OR = 3.69, 95% CI 1.71–7.98) and wheeze (OR = 4.72, 95% CI 2.20–10.11). The 6A/1A haplotype of SFTPA, found in approximately 5% of the infants, was associated with an increased risk of persistent cough (OR = 3.20, 95% CI 1.39–7.36) and wheezing (OR = 3.25, 95% CI 1.43–7.37). To exclude confounding factors, we did not include patients with asthma. However, since AR and asthma are closely related, we hypothesized that SP-A plays a similar role in asthma and allergic rhinitis. We also found that the interactions between the C allele and smoking increased the risk of AR. Smoking can cause allergic rhinitis, and this synergy was identified in the present study.²⁶ IgE plays a central role in the pathogenesis of allergic diseases. Therapeutic administration of SP-A in allergic fungal mice inhibited eosinophil and IgE levels in the peripheral blood of mice, leading to ThZ cytokine migration to the Th1 type.²⁷ SP-A may be directly involved in the regulation of IgE synthesis, but the exact relationship between SP-A and serum IgE levels in patients with AR requires further experimental study with large sample sizes. AR is a systemic inflammatory response, and the cascade of inflammation is an important cause of the increasing inflammatory response.²⁸ The pathogenesis of AR is related to the activation of circulating T lymphocytes and mononuclear macrophages, and its activation is related to cytokines such as IL-1, TNF- α , and IL-6. The activation of these cytokines reflects the acute phase of the inflammatory response and has important consequences for the clinical manifestations of AR.²⁹ Our results also showed that the rs1965708 variants were associated with IgE and inflammation factors.

This could be explained as follows: It is known that the human SP-A gene is located on chromosome 10q22-q23, including two highly homologous functional genes (SP-A1 and SP-A2) and one pseudogene. A total of four single-nucleotide polymorphisms (SNPs) mapped to SP-A1 and five SNPs mapped to SP-A2 have been identified.³⁰ SP-A1 is not associated with the risk of AR, whereas only one SNP of SP-A2 is associated with AR. Allergen inflammatory response is known to play a key role in airway inflammatory disease. Some allergens can be identified and bound to the collagen lectin family. SP-A is a member of the collagen lectin family, which has the same structural characteristics: It is located in a region rich in hemieptide at the N-terminal of the skin chain. The original area of the *Treponema pallidum* structure adhesive alpha-coiled spiral "bottleneck" area plant lectin area (CRD area) at the

C-end of the skin chain. An important biological function of the CRD region is its ability to bind to aspirated allergen particles.³¹ The rs1965708 variants belong to the SP-A2 gene and are located in the CRD region. The rs1965708 variant may affect the ability of SP-A2 to allergens. Previous studies also found that SP-A2 has a greater ability to cytophagy, inhibit the secretion of surface-active substances, and induce the release of tumor necrosis factor.³² This may explain why the rs1965708 variants are associated with allergic rhinitis susceptibility.

The present study had some limitations. First, based on the inherent shortcomings of case-control design, the cause-effect explanation is limited. Second, we only reported one SNP associated with AR because some SNPs have been previously associated with AR, but the interaction with other SNPs should be reported. Third, AR is a disease caused by genes and environments, and more gene-environmental interactions should be explored for AR risk. Finally, we found that the increased risk of AR caused by these SNP variants could be associated with IgE and some inflammatory factors; however, the specific molecular mechanism needs to be investigated in future studies.

In conclusion, the rs1965708 variants of SP-A gene polymorphisms are associated with AR, and the A allele could increase the risk of AR. The AA SNP variants that interact with smoking may alter the susceptibility to AR. Studies with larger sample sizes are required to validate the present findings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

ZXH contributed to this research idea and study design. YXH performed data collection. YXH wrote the manuscript. WB, HLL, and YZQ revised the manuscript. ZXH proofread the final version. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Original data will be provided upon request by correspondence author (zhangxinhaify@foxmail.com).

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