

Histamine H₄ receptor gene polymorphisms: a potential predictor of oral H₁ antihistamine efficacy for allergic rhinitis

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Background: Our study aimed to investigate the associations between *Histamine H₄ receptor (HRH4)* gene polymorphisms (rs77485247, rs74604924, and rs77041280) and oral H₁ antihistamine efficacy for the treatment of allergic rhinitis (AR) patients.

Methods: A total of 142 AR patients were selected as a case group and 160 healthy individuals were recruited as a control group. Single nucleotide polymorphisms (SNPs) in the *HRH4* gene were detected using direct sequencing. Serum immunoglobulin E (IgE), specific IgE, and eosinophil cationic protein (ECP) levels were measured by enzyme-linked immunosorbent assay (ELISA). Clinical efficacy was evaluated by the visual analogue scale (VAS). The occurrence of adverse reaction was recorded.

Results: There were significant differences in the distribution frequencies of mutant genotype (TA + AA) and A allele of rs77485247, mutant genotype (AT + TT) and T allele of rs74604924, and mutant genotype (AT + TT) and T allele of rs77041280 between the case and control groups. AR patients with mutant genotype (TA + AA) of rs77485247 and AR patients with mutant genotype (AT + TT) of rs77041280 had higher specific

IgE, ECP levels, and VAS scores after treatment and lower incidence of adverse reactions and total effective rate than those with TT genotype and those with AA genotype, respectively. However, for rs74604924, there were no differences was found between AR patients with mutant genotype (AT + TT) and those with AA genotype.

Conclusion: Our findings provide evidence that *HRH4* rs77485247 and rs77041280 polymorphisms may be associated with the risk of AR and the efficacy of H₁ antihistamines for the treatment of AR patients. © 2016 ARS-AAOA, LLC.

Key Words:

histamine H₄ receptor; single nucleotide polymorphism; allergic rhinitis; desloratadine; H₁ antihistamines; efficacy

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Allergic rhinitis (AR) is an inflammatory disorder of the nasal mucosa that is characterized by sneezing, nasal congestion, pruritus, and anterior or posterior rhinorrhea.¹ It is a health problem in all countries, ethnic groups, and ages and causes major illness and disability.² AR

adversely affects individuals' normal social life, sleep, social interactions, school performance, and work productivity, particularly in patients with severe disease.^{3,4} In recent decades, the incidence of AR has been increasing continuously and at present affects approximately 60 million people worldwide.^{5,6} Evidence suggests that AR is influenced by many factors, including family history, changes in lifestyle, genetic factors, such as the neurotrophic factor (*BDNF*) gene and the histamine *H1*, *H2*, *H3*, and *H4* receptor genes, and environmental factors, such as air pollution and bacterial and viral infections.^{7–9} According to Allergic Rhinitis and Its Impact on Asthma (ARIA), H₁ antihistamines (H1A) are typically used as the first-line therapy in the treatment of AR.¹⁰

The Histamine H₄ receptor (*HRH4*) was discovered at the beginning of the 21st century and belongs to the class of G-protein coupled receptors.^{11,12} It is found on hematopoietic cells, including eosinophils, dendritic cells, CD8 + T cells, mast cells, and T cells, which prompted

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us to re-evaluate the role of the histamine gene in allergic diseases.¹³ Cowden et al.¹⁴ have shown that HRH4 profoundly influences immune cell function, including cytokine production, chemotaxis and chemokine expression, and mediating T helper type 2 (Th2) responses in vivo and in vitro. Shiraishi et al.¹⁵ also revealed that in AR patients, the HRH4 was upregulated in nasal tissue and was correlated with allergic inflammatory responses, which suggests that HRH4 may be a new therapeutic target for AR. Presently, although H1 antihistamines are available and effective for most AR patients, 1 out of 5 patients still remains unsatisfied.¹⁶ Based on a previous study, polymorphisms of ss142022671, ss142022677, and ss142022679 in HRH4 were associated with the occurrence and development of allergic dermatitis.¹⁷ Moreover, a role of HRH4 in allergy, inflammation, and immune reactions has also been reported.¹⁸ However, the roles of HRH4 gene polymorphisms in AR have not yet been reported. Thus, in our study, we hypothesized that the efficacy of H1 antihistamines can be associated with HRH4 gene polymorphisms. We selected these 3 SNPs (rs77485247, rs74604924, and rs77041280) and investigated their correlations with the efficacy of oral H1 antihistamine in AR patients.

Patients and methods

Study subjects

From January 2013 to January 2015, a total of 142 AR patients were selected from Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine as a case group. All the patients completed the following tests and questionnaires based on the World Health Organization (WHO) diagnostic criterion of ARIA 2007: anterior rhinoscopy, allergen skin test, history of allergic rhinitis, family history of allergy, and history of food and drug allergies.¹⁹ The inclusion criteria were as follows: (1) age from 15 to 60 years; (2) AR confirmed through skin prick or sensitogen (fungi, mites and food-allergens like fish, shrimp and crab) screening test; and (3) no antihistamine agent used in the 2 weeks prior to treatment. The exclusion criteria were as follows: (1) AR patients who used any type of steroid hormone within 2 weeks prior to treatment; (2) had a history of allergy to H1 receptor antagonists; (3) rhinitis medicamentosa; (4) severe nasal polyps, nasosinusitis, and nasal septal deviation; (5) being treated with desensitization treatment; (6) suffered from viral upper respiratory infection in the week prior to treatment; (7) antibiotics were used in the 2 weeks prior to treatment; (8) lactating and pregnant women; and (9) the presence other serious illnesses. An additional 160 healthy people were recruited as the control group during the same period, and the inclusion criteria were as follows: anterior rhinoscopy, allergen skin test, history of allergic rhinitis, family history of allergy, and history of food and drug allergies all showed negative results. This study was approved by the Ethics

Committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, and all the study subjects provided signed informed consent.

Treatment regimen

All patients were treated with oral H1 antihistamines (5 mg desloratadine; Shanghai Schering-Plough Pharmaceutical Co., Ltd., Shanghai, China; product with code number J200801) once at 8:00 PM daily for 2 weeks. In our study, we selected desloratadine as a therapeutic drug, a novel oral second-generation antihistamine that has no sedative effect and no interaction with cytochrome P450 system. Therefore, it is strongly recommended by ARIA 2010.²⁰ Changes in symptoms were recorded every day, and telephone follow-up was performed in the first and second weeks after the start of treatment. No patients were lost to follow-up, and all the interviews were recorded in appropriate case reports.

Efficacy evaluation and adverse reactions

Changes in symptoms and adverse reactions were recorded by drug-delivery nurses via telephone follow-up. For patients who were lost to telephone follow-up, home follow-up was carried out. In addition, a timely treatment plan was provided for patients with adverse reactions, and abnormal clinical manifestations related to the drug were recorded in detail. Efficacy was evaluated according to the Guidelines for Diagnosis and Treatment of Allergic Rhinitis (2009).²¹ Clinical effect evaluation of nasal assorted symptoms before and after treatment was conducted using a visual analogue scale (VAS). The VAS was a scale ranging from 0 to 10 cm, with 0 representing not plagued and 10 representing seriously plagued. According to subjective symptoms, patients marked their score of nasal obstruction, rhinitis, sneezing, and runny nose on the VAS scale, with 0 to 10 points per dimension (a total of 40 points). The data were then converted to a score by the doctors, as follows: score of efficacy (%) = (total score before treatment – total score at 2 weeks after treatment)/total score before treatment × 100% (≥66% meant significantly effective, 65-26% effective, and ≤25% ineffective).

Blood sampling and DNA extraction

Fasting peripheral venous blood samples (4 mL) were collected from the patients in the case and control groups before treatment. Ethylenediamine tetraacetic acid (EDTA) was added to the samples. A total of 2 mL of blood was used to measure total serum IgE level, specific IgE level, and ECP level at clinical research center, whereas the other 2 mL of blood was used for DNA extraction using genomic DNA extraction kits (column centrifugation method; Tiangen Biotech Co., LTD., Beijing, China; DP318-03) according to the manufacturer's instructions. The concentration and purity of the recovered DNA fragment were assessed using an ultraviolet spectrophotometer. The optical diameter at nanometer value (OD260)/OD280

TABLE 1. Primer sequences of *HRH4* gene polymorphisms for PCR

SNP	Sequence (5' to 3')	Product size (bp)
rs77485247 T>A (ss142022671)	F: TGGAGATGGATGCATCACAG	566
	R: TCCCCAGTTGTATACAGTC	
rs74604924 A>T (ss142022677)	F: GCTTGATGAAATACCGTG	513
	R: ATGGTGCATAGGTGAGTG	
rs77041280 A>T (ss142022679)	F: GTCCACATCGTGATACCCA	853
	R: GGATACAAAAGAGGATTGAC	

bp = base pair; F = forward; *HRH4* = histamine H4 receptor; PCR = polymerase chain reaction; R = reverse; SNP = single nucleotide polymorphism.

ratio of the samples were required to be between 1.7 and 1.9, and the extracts were kept at -20°C

Polymerase chain reaction amplification, sequencing, and genotyping

The primers for rs77485247, rs74604924, and rs77041280 in *HRH4* were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Table 1). The polymerase chain reaction (PCR) was as follows: genomic DNA 0.1 μg , buffer solution: 10 mmol/L Tris-HCl (pH = 8.0) (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China), 50 mmol/L KCl, 2.5 mmol/L MgCl_2 , 200 $\mu\text{mol/L}$ dNTPs, 1 U Taq DNA polymerase (Sino-American Biotechnology Co., Ltd., Luoyang, China), and 0.4 $\mu\text{mol/L}$ oligonucleotide primer (Beijing JenKem Technology Co., Ltd., Beijing, China). The amplification conditions were as follows: predenaturation for 3 minutes at 95°C ; 35 cycles (denaturation for 30 seconds at 95°C ; annealing for 30 seconds at 60°C and another 30 seconds at 72°C), and extension for 10 minutes at 72°C gel analysis and purification of the PCR amplification products was performed using a DNA gel analysis system (TLN Technology Co., Ltd., Beijing, China), and the purified products were sent directly to Invitrogen Trading Co., Ltd. (Shanghai, China) for sequencing. The results were compared with the National Center for Biotechnology Information (NCBI) NT-010966.13 sequence using NCBI Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and the genotypes of the different individuals were determined based on the map and sum of sequencing.

Measurement of total serum IgE, specific IgE, and ECP levels

Venous blood (2 mL) that was collected from each patient in the 2 groups was allowed to rest for 30 minutes and was then centrifuged for 10 minutes at 3000 rpm (1509 g). Immuno CAP 250 (Amersham Pharmacia Biotech Europe

GmbH., Freiburg, Germany) was used to perform quantitative detection of total serum IgE and ECP levels in both groups and specific IgE level (aeroallergen-specific IgE, mainly including 20 items, such as grass combination, tree combination, birch, elm, Platanaceae, willow, poplar, mulberry, pollen assemblage, animal hair combination, *Dermatophagoides pteronyssinus* [DP], *Dermatophagoides culinae*, cockroach, mold combination, etc. in the case group.

Statistical methods

The SPSS 21.0 statistical package (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The measured data are expressed as the mean $\pm$ standard deviation, and the comparison between the 2 groups of normal distribution was conducted using a *t* test. If multiple groups met the requirement of homogeneity of variance and normality, the comparison among them was performed by single-factor variance analysis. If not, the comparison was performed using nonparametric rank sum test (Kruskal-Wallis test). Enumeration data are presented in percentage and rate, and Hardy-Weinberg equilibrium among the patients was tested using chi-square test or Fisher's exact test. The relative risk of genotypes and alleles was expressed as the odds ratio (OR) and 95% confidence interval (CI). Linkage disequilibrium analysis for rs77485247, rs74604924, and rs77041280 was conducted using Haploview software (Broad Institute, Cambridge, MA; <https://www.broadinstitute.org/haploview/haploview>). A value of α of 0.05 was used as a standard test.

Results

Comparisons of baseline characteristics between the case and control groups

The case group included 83 males and 59 females with a mean age of 38.6 ± 13.3 years old (range, 5 to 73 years) and a mean duration of disease of 5.29 ± 2.0 years (range, 1 to 9 years). The control group comprised 89 males and 71 females with a mean age of 39.2 ± 12.7 years old (range, 16 to 58 years). There was no difference in gender or age between the case and control groups (both $p > 0.05$). Compared with the control group, total serum IgE and ECP levels were significantly higher in the case group (both $p < 0.05$) (Table 2).

Linkage disequilibrium analysis

The results of linkage disequilibrium analysis showed that there was a significant linkage disequilibrium between rs74604924 and rs77041280 ($D' = 0.98$; $r^2 = 0.92$), and a weak linkage disequilibrium between rs77485247 and rs74604924 ($D' = 0.80$; $r^2 = 0.51$), as well as between rs77485247 and rs77041280 ($D' = 0.81$; $r^2 = 0.54$), suggesting linkage disequilibrium among these 3 sites (Fig. 1). Therefore, we selected these 3 sites simultaneously for our study.

TABLE 2. Baseline characteristics of study subjects

Characteristics	Case group (n = 142)	Control group (n = 160)	p
Age (years)	38.6 ± 13.3	39.2 ± 12.7	0.689
Gender (male/female)	83/59	89/71	0.621
Total serum IgE (kU/L)	4.38 ± 1.06	1.39 ± 0.35	<0.001
ECP (μg/L)	19.27 ± 3.05	5.43 ± 1.30	<0.001
Duration of disease (years)	5.29 ± 2.15	–	–
Total VAS score	5.18 ± 2.04	–	–
Concomitant asthma (yes/no)	39/103	–	–
Family history (yes/no)	45/97	–	–
Specific IgE-induced by DP	34.65 ± 9.63	–	–
Specific IgE-induced by DC	31.57 ± 7.46	–	–

DC = *Dermatophagoides culinae*; DP = *Dermatophagoides pteronyssinus*; ECP = eosinophilic cationic protein; IgE = immunoglobulin E; VAS = visual analogue scale.

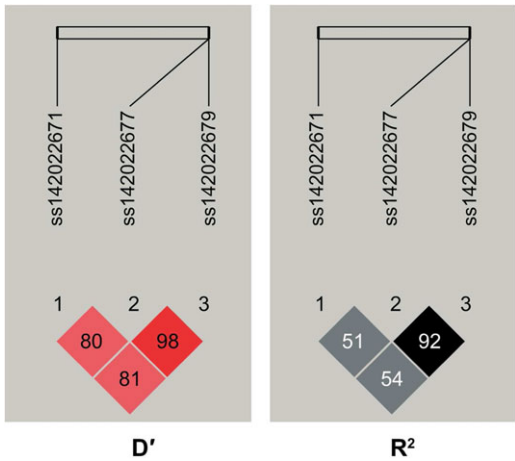


FIGURE 1. Linkage disequilibrium map for single nucleotide polymorphisms in rs77485247, rs74604924, and rs77041280 of the Histamine H4 receptor gene.

Distribution of genotype and allele frequencies of each SNP in HRH4 gene

The distribution of genotype and allele frequencies was consistent with Hardy-Weinberg balance in the case and control groups, indicating that the selected groups were representative (all $p > 0.05$). There were significant differences in the distribution frequencies of mutant genotype (TA + AA) and A allele of rs77485247, mutant genotype (AT + TT) and T allele of rs74604924, and mutant genotype (AT + TT) and T allele of rs77041280 between the case and control groups (all $p < 0.05$) (Table 3).

The associations of HRH4 gene polymorphisms with biochemical parameters of AR patients

For rs77485247, compared with patients with the mutant homozygote AA genotype, patients with the A allele (mutant homozygote (AA + TA) had higher specific IgE and ECP levels (all $p < 0.05$). For rs74604924 and rs77041280, compared with individuals with the wild-type homozygote AA genotype, the levels of specific IgE and ECP were significantly increased in individuals with the T allele (mutant homozygote (TT + TA) (all $p < 0.05$) (Table 4).

The associations of HRH4 gene polymorphisms with adverse reactions of AR patients

As shown in Table 5, the results showed that for rs77485247, compared with individuals with the wild-type homozygote TT genotype, individuals with the mutant heterozygote TA and mutant homozygote AA genotypes had a significantly increased risk of adverse reactions (all $p < 0.05$). For rs77041280, compared with individuals with the wild-type homozygote AA genotype, individuals with the mutant heterozygote TA and mutant homozygote TT genotypes had a greatly increased risk of adverse reactions (all $p < 0.05$). For rs74604924, there was no difference in adverse reactions among the AA, TA, and TT genotypes ($p > 0.05$).

The associations of HRH4 gene polymorphisms with VAS score of AR patients

The VAS scores of the 142 patients before and after treatment are shown in Table 6. Before treatment, there was no significant difference in VAS scores among AR patients with different genotypes (all $p > 0.05$). After treatment, all VAS scores decreased significantly (all $p < 0.05$). For rs77485247, compared with patients carrying the wild genotype, TT patients carrying the mutant genotype (AA + TA) had significantly increased VAS scores after treatment (both $p < 0.05$). For rs74604924 and rs77041280, compared with patients carrying the wild genotype AA, patients carrying mutant genotype (TT + TA) had significantly increased VAS scores after treatment (all $p < 0.05$).

The associations of HRH4 gene polymorphisms with the efficacy of oral H1A for AR

Comparing the efficacy of oral H1A in the 142 patients (Table 7) revealed that for rs77485247, compared with the wild-type homozygote TT genotype, the efficacy of H1 antihistamines in patients with the mutant heterozygote TA and mutant homozygote AA genotypes decreased (all $p < 0.05$). Additionally, compared with T allele, the efficacy of H1 antihistamines in patients with the A allele was significantly less ($p < 0.05$). The results also showed that the A allele might decrease the efficacy of oral H1 antihistamines. For rs77041280, compared with the wild-type homozygote AA genotype, the total efficacy of H1 antihistamines in

TABLE 3. Distribution of genotype and allele frequencies of 3 SNPs in the *HRH4* gene

SNP	Case group (n = 142) (n, [%])	Control group (n = 160) (n, [%])	χ^2	p	OR (95% CI)
rs77485247 T>A					
TT	107 (75.6)	143 (89.4)			1
TA	31 (21.5)	16 (10.2)	8.529	0.004	2.589 (1.347–4.977)
AA	4 (2.9)	1 (0.4)	2.760	0.097	5.346 (0.589–48.540)
TA + AA	35 (24.4)	17 (10.6)	10.380	0.001	2.752 (1.463–5.174)
T	245 (86.3)	302 (94.4)			1
A	39 (13.7)	18 (5.6)	11.570	0.001	2.671 (1.490–4.787)
rs74604924 A>T					
AA	112 (78.9)	145 (90.7)			1
TA	28 (19.7)	15 (9.3)	6.865	0.009	2.417 (1.232–4.742)
TT	2 (1.4)	0 (0)	2.564	0.109	6.467 (0.307–136.1)
TA + TT	30 (21.1)	15 (9.3)	8.194	0.004	2.589 (1.329–5.046)
A	252 (88.7)	305 (95.3)			1
T	32 (11.3)	15 (4.7)	10.710	0.003	2.582 (1.367–4.876)
rs77041280 A>T					
AA	110 (77.6)	146 (91.4)			1
TA	29 (20.6)	14 (8.6)	8.864	0.003	2.749 (1.387–5.450)
TT	3 (1.8)	0 (0)	3.922	0.048	9.281 (0.474–181.700)
TA + TT	32 (22.4)	14 (8.6)	11.07	0.001	3.034 (1.544–5.960)
A	249 (87.7)	306 (95.6)			1
T	35 (12.3)	14 (4.4)	12.750	<0.001	3.072 (1.617–5.839)

CI = confidence interval; *HRH4* = histamine H4 receptor; OR = odds ratio; SNP = single nucleotide polymorphism.

TABLE 4. The associations between *HRH4* gene polymorphisms and biochemical parameters of AR patients

SNP	Genotype	n	Total serum IgE (logU/L) (mean ± SD)	Specific IgE (kUA/L) (mean ± SD)	ECP (μg/L) (mean ± SD)
rs77485247 T>A	TT	107	4.34 ± 1.05	9.24 ± 2.30	18.28 ± 2.17
	TA	31	4.54 ± 1.15	20.70 ± 8.86 ^a	21.99 ± 3.47 ^a
	AA	4	4.36 ± 0.52	29.74 ± 4.80 ^a	24.64 ± 1.03 ^a
rs74604924 A>T	AA	112	4.35 ± 1.05	9.30 ± 2.43	18.29 ± 2.32
	AT	28	4.44 ± 1.15	22.13 ± 8.53 ^b	22.38 ± 3.00 ^b
	TT	2	4.98 ± 0.14	31.15 ± 6.75 ^b	26.43 ± 2.84 ^b
rs77041280 A>T	AA	110	4.33 ± 1.02	9.14 ± 2.15	18.16 ± 2.24
	AT	29	4.56 ± 1.24	22.06 ± 6.99 ^c	22.75 ± 1.67 ^c
	TT	3	4.53 ± 0.64	34.48 ± 1.96 ^c	26.44 ± 1.79 ^c

^aMutant genotypes AA and TA were compared with wild genotype TT in ss142022671, $p < 0.05$.

^bMutant genotypes TT and TA were compared with wild genotype AA in ss142022677, $p < 0.05$.

^cMutant genotypes TT and TA were compared with wild genotype AA in ss142022679, $p < 0.05$.

AR = allergic rhinitis; ECP = eosinophilic cationic protein; *HRH4* = histamine H4 receptor; IgE = immunoglobulin E; SD = standard deviation; SNP = single nucleotide polymorphism.

TABLE 5. The associations between *HRH4* gene polymorphisms and adverse reactions of AR patients after oral H1 antihistamines treatment

SNP/genotype	Drowsiness	Hypodynamic	Nausea	Xerostomia	Headache	Total	<i>p</i>
rs77485247 T>A							
TT	3	5	4	1	1	14	
TA	4	1	2	1	1	9	0.036
AA	1	1	1	0	0	3	0.001
rs74604924 A>T							
AA	4	5	5	1	2	17	
AT	4	2	1	1	0	8	0.098
TT	0	0	1	0	0	1	0.181
rs77041280 A>T							
AA	3	5	4	1	2	15	
AT	4	1	3	1	0	9	0.027
TT	1	1	0	0	0	2	0.011

AR = allergic rhinitis; *HRH4* = histamine H4 receptor; SNP = single nucleotide polymorphism.

TABLE 6. The associations between *HRH4* gene polymorphisms and VAS score of AR patients before and after treatment

SNP	Genotype	n	VAS before treatment (mean ± SD)	VAS after treatment (mean ± SD)	<i>p</i>
rs77485247 T>A	TT	107	23.81 ± 1.36	9.64 ± 2.87	<0.001
	TA	31	23.62 ± 1.40	11.96 ± 4.28 ^a	<0.001
	AA	4	24.35 ± 0.46	15.69 ± 3.67 ^a	0.003
rs74604924 A>T	AA	112	23.86 ± 1.41	9.71 ± 2.93	<0.001
	TA	28	23.51 ± 1.11	12.03 ± 4.20 ^b	<0.001
	TT	2	23.73 ± 1.24	18.34 ± 0.81 ^b	0.036
rs77041280 A>T	AA	110	23.92 ± 1.39	9.74 ± 2.98	<0.001
	TA	29	23.25 ± 1.12	11.83 ± 4.14 ^c	<0.001
	TT	3	23.98 ± 1.04	16.90 ± 3.64 ^c	0.032

^aMutant genotypes AA and TA were compared with wild genotype TT in rs77485247, *p* < 0.05.

^bMutant genotypes TT and TA were compared with wild genotype AA in rs74604924, *p* < 0.05.

^cMutant genotypes TT and TA were compared with wild genotype AA in rs77041280, *p* < 0.05.

AR = allergic rhinitis; *HRH4* = histamine H4 receptor; SD = standard deviation; SNP = single nucleotide polymorphism; VAS = visual analogue scale.

patients with the mutant heterozygote TA and the mutant homozygote TT genotypes significantly decreased (all *p* < 0.05). Additionally, compared with the A allele, the efficacy of H1 antihistamines in patients with the T allele was significantly reduced (*p* < 0.05), which indicates that the T allele might decrease the efficacy of oral H1 antihistamines. For rs74604924, there was no significant difference in total drug efficacy in patients with different genotypes and alleles (*p* > 0.05), showing that rs74604924 was not associated with the efficacy of oral H1 antihistamines.

Discussion

In this study, the results showed that the rs77485247 and rs77041280 SNPs affected drug efficacy, but rs74604924 was not associated with drug efficacy in AR. According to our study, for rs77485247, patients with mutant genotype (TA + AA) had an increased risk of adverse reactions and reduced drug efficacy. For rs77041280, patients with mutant genotype (TA + TT) had a higher risk of adverse reactions and lower efficacy. For rs74604924, no significant

TABLE 7. The associations between *HRH4* gene polymorphisms and the efficacy of oral H1 antihistamines of AR patients after treatment

SNP	Ineffective n (%)	Effective n (%)	χ^2	<i>p</i>
rs77485247 T>A				
TT	7 (6.5)	100 (93.5)		
TA	7 (22.6)	24 (77.4)	6.783	0.009
AA	2 (50.0)	2 (50.0)	9.771	0.002
T	21 (8.6)	224 (91.4)	10.880	0.001
A	11 (28.2)	28 (71.8)		
rs74604924 A>T				
AA	8 (7.1)	102 (92.9)		
TA	7 (25.0)	23 (75.0)	1.867	0.172
TT	1 (50.0)	1 (50.0)	3.802	0.051
A	23 (9.1)	227 (90.9)	2.558	0.110
T	9 (28.1)	25 (71.9)		
rs77041280 A>T				
AA	8 (7.3)	102 (92.7)		
TA	6 (20.7)	23 (79.3)	4.561	0.033
TT	2 (66.7)	1 (33.3)	12.770	0.001
A	22 (8.8)	227 (91.2)	10.770	0.001
T	10 (28.6)	25 (71.4)		

AR = allergic rhinitis; *HRH4* = histamine H4 receptor; SNP = single nucleotide polymorphism.

difference was found in drug efficacy and risk of adverse reactions. Recently, a study on *HRH4* polymorphisms and hypersensitivity to nonsteroidal anti-inflammatory drug (NSAID) showed that *HRH4* SNPs (rs11665084 and rs11662595) did not modify the risk of clinical presentation of NSAID-induced hypersensitivity reactions, indicating that *HRH4* polymorphisms did not reduce adverse reactions after NSAID.²² Another study revealed that the *HRH4* variant rs4483927 was significantly associated with risperidone efficacy and that its homozygote TT genotype predicted poor therapeutic response, both on the positive, negative, and general subscales.²³ The mechanism by which *HRH4* influences drug efficacy might be similar to that reported in a previous study in which it was revealed that p21^{Cip1} and p27^{Kip1}, when induced by *HRH4*, could, by inhibiting cyclin D1/Cdk4 or Cdk6 kinase activity, increase the percentage of cells in the G1 phase following clozapine treatment.²⁴

Our study also found that for rs77485247, the mutant heterozygote genotype TA, the mutant genotype (TA + AA), or the A allele was associated with an increased risk of AR. For rs74604924, the mutant heterozygote genotype TA, the mutant genotype (TA + TT), and the T allele

were significantly associated with an increased risk of AR. For rs77041280, individuals with the mutant homozygote genotype TT, the mutant genotype (TA + TT), or the T allele were more likely to have AR. Similar to our study, a previous study revealed that the TT haplotype and T allele of rs77485247 were significantly associated with atopic dermatitis (AD).¹⁷ It has also been shown that the *HRH4* variants rs623590 and rs1421125 are significantly associated with breast cancer risk, according to both genotype and allele association analysis.²⁵ Another study revealed that *HRH4* activation could promote the 5-Fu-mediated cell apoptosis in colon cancer cells, which may provide new clues for histamine receptor-targeted therapies in colorectal carcinoma (CRC).²⁴ Previous research has also demonstrated that the AA genotype and A allele at rs657132 of *HRH4* were higher in the case group, indicating that the AA genotype and A allele were associated with an increased susceptibility to ankylosing spondylitis (AS).²⁶ The mechanism might be as follows: activated *HRH4* could promote the accumulation of inflammatory mast cells and particularly, eosinophils, which occur via the $\beta \gamma$ subunit acting at phospholipase C to cause actin polymerization and eventually chemotaxis.¹⁸

According to our results, for rs77485247, individuals with the AA and TT genotypes and A allele had higher specific IgE and ECP levels. For rs74604924 and rs77041280, specific IgE and ECP levels were significantly increased in individuals with the TT or AA genotype and T allele. A previous study showed that IgE levels were associated with *HRH4* amplifications in AD, indicating that the strong expression of the *HRH4* gene might regulate the expression of IgE.²⁷ On the contrary, another study showed that the distribution of 21 selected *HRH4* SNPs were not correlated with the severity of eosinophil count, asthma, or IgE level.²⁸ These differences could be due to differences in the age and gender of the study subjects, which could have affected the results. The mechanism by which *HRH4* polymorphisms affect specific IgE and ECP levels is not clearly understood. However, an underlying mechanism might be that low concentrations of histamines are chemotactic to eosinophils, whereas high histamine concentrations inhibit eosinophil chemotaxis, which might influence the levels of eosinophil.²⁹

Conclusion

Our findings provide evidence that *HRH4* rs77485247 and rs77041280 polymorphisms may be associated with the risk of AR and the efficacy of H1 antihistamines for the treatment of AR patients. In *HRH4* gene, the rs77485247 mutant genotype (TA + AA) and rs77041280 mutant genotype (TA + TT) might reduce drug efficacy and increase the incidence of adverse reactions after taking oral H1A. This study provides a reference for the treatment of AR. Our study is the first to explore the correlations between *HRH4* polymorphisms and H1 antihistamines in AR. However, there are still some limitations to our findings. For

example, we did not investigate the influence of *HRH4* polymorphisms on other antihistamines. Additionally, due to a lack of related materials and time limitations, the sample size was small, which might likely lead to a low power for detecting differences among the SNPs. Therefore, in the future, we will extend the time period of our study to

involve more subjects and further study the correlation between *HRH4* and other antihistamines (H2-antihistamines, H3-antihistamines and H4-antihistamines). ④

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