ORIGINAL RESEARCH

GARP Polymorphisms Associated with Susceptibility to House Dust Mite-Sensitized Persistent Allergic Rhinitis in a Chinese Population

Xin-Yuan Chen[1],*, Xin-Jie Zhu[1],*, Min Chen[1], Mei-Ping Lu[1], Mei-Lin Wang[2,3], Min Yin[1,4], Ruo-Xi Chen[1], Zhong-Fei Wu[1], Dong-Yun Bu[1], Zheng-Dong Zhang[2,3], Lei Cheng [1,4]

[1]Department of Otorhinolaryngology & Clinical Allergy Center, The First Affiliated Hospital, Nanjing Medical University, Nanjing, People's Republic of China; [2]Department of Environmental Genomics, Jiangsu Key Laboratory of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Personalized Medicine, School of Public Health, Nanjing Medical University, Nanjing, People's Republic of China; [3]Department of Genetic Toxicology, The Key Laboratory of Modern Toxicology of Ministry of Education, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing, People's Republic of China; [4]International Centre for Allergy Research, Nanjing Medical University, Nanjing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Lei Cheng, Department of Otorhinolaryngology & Clinical Allergy Center, The First Affiliated Hospital, Nanjing Medical University, 300 Guangzhou Road, Nanjing, 210029, People's Republic of China, Email chenglei@jsph.org.cn; Zheng-Dong Zhang, Department of Environmental Genomics, Jiangsu Key Laboratory of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Personalized Medicine, School of Public Health, Nanjing Medical University, 101 Longmian Avenue, Nanjing, 211166, People's Republic of China, Email drzdzhang@njmu.edu.cn

Background: Genetic variants in *GARP* (also known as *LRRC32*) have been reported to have significant associations with asthma and eczema in special populations, but little is known about allergic rhinitis. This study purposes to evaluate the association of single nucleotide polymorphisms (SNPs) in *GARP* with house dust mite (HDM)-sensitized persistent allergic rhinitis (PER) in a population of Han Chinese.
Methods: In this hospital-based case–control study, 534 HDM-sensitized PER patients and 451 healthy controls were recruited from East China. In this population, six SNPs in *GARP* were identified. Serum total and specific IgE levels were measured with ImmunoCAP. Secondary structure and minimum free energy were predicted by RNAfold.
Results: rs79525962 was associated with the risk of HDM-sensitized PER ($P < 0.05$). The individuals with CT+TT genotype demonstrated a higher risk of HDM-sensitized PER than those with CC genotype (adjusted OR=1.393, 95% CI=1.019–1.904). The homozygous genotype CC of rs3781699 rendered a lower risk of HDM-sensitized PER than the wild-type genotype AA (adjusted OR=0.646, 95% CI=0.427–0.976); however, the genotype and allele frequencies of rs3781699 demonstrated no associations with HDM-sensitized PER ($P > 0.05$). rs79525962 increased the risk of HDM-sensitized PER in the subgroup aged ≥16 years (adjusted OR=1.745, 95% CI=1.103–2.760), and this high risk was also found in the females (adjusted OR=1.708, 95% CI=1.021–2.856). The G-C haplotype of rs1320646-rs3781699 rendered a lower risk of HDM-sensitized PER than the common haplotype G-A (adjusted OR=0.819, 95% CI=0.676–0.993). The secondary structure of *GARP* altered in response to different genotypes of rs79525962 and rs3781699.
Conclusion: SNP rs79525962 in the *GARP* gene marks a risk locus of HDM-sensitized PER in Chinese Hans.
Keywords: allergic rhinitis, mites, *GARP*, *LRRC32*, single nucleotide polymorphism, genetic association studies

Introduction

Immunoglobulin E (IgE) mediates inflammation to evoke allergic rhinitis (AR), featuring nasal symptoms such as nasal itching, sneezing, rhinorrhea, and nasal congestion.[1,2] The prevalence of allergic diseases, such as AR and asthma, has kept increasing markedly worldwide over the past 50 years.[1] Currently, AR prevalence ranges from 9.8% to 23% in China adults.[3] Epidemiologic studies have consistently shown that asthma and rhinitis often prevail in the same population throughout the world.[4–6] As the upper and lower airway inflammatory responses present physiological similarities, rhinitis is considered as a risk factor for asthma at the pediatric age.[7,8]

AR involves an intricate cooperation between genetic predisposition and environmental factors, with implicated genes being intensely studied.[9] Genomic searches have verified the roles of chromosomes 2, 3, 4, and 9[9]. Some AR-related single nucleotide polymorphisms (SNPs) have also been discovered[10–13]. Genome-wide association studies have found that through biological pathways involved in the immunosuppression of regulatory T lymphocytes (Tregs),[14] a common variant on chromosome 11q13.5 near the glycoprotein A repetitions predominant gene (*GARP*, also known as leucine-rich repeat containing 32, *LRRC32*) increases the susceptibility to asthma,[15] AR,[16] and atopic dermatitis.[17] Disease-associated genetic polymorphisms within 11q13.5 can regulate GARP expression on human Treg cells.[18] In a recent study, by using a model of conditional Garp-deficient mice, Lehmkuhl et al[19] confirmed that the decreased GARP expression on Tregs increased susceptibility to inflammatory diseases. They also found that as Foxp3 protein acetylation diminished, Garp deficiency led to an unstable Treg phenotype, suggesting that GARP plays a central role in maintaining immune stability.

Genetic variants in *GARP* have been reported to have significant associations with asthma and eczema in special populations,[20] but little is known about AR. Here, we genotyped and analyzed six *GARP* SNPs in a Chinese Han population to manifest the role of *GARP* polymorphisms in AR induced by house dust mites (HDM), and its clinical phenotypes.

Materials and Methods
Subjects
This is a hospital-based, case–control study of the association between genetic variations in *GARP* and susceptibility to AR. This study included 534 unrelated cases (356 males, 178 females) with persistent allergic rhinitis (PER) ages 2 to 66 years (median age [quartiles] of 16.0 [10.0–27.0] years) and 451 unrelated healthy controls (278 males, 173 females) ages 3 to 63 years (median age [quartiles] of 16.0 [10.0–29.0] years). All these subjects were enrolled from Chinese Hans in Jiangsu and Anhui provinces in East China, who had received medical care in the First Affiliated Hospital of Nanjing Medical University between 2008 and 2014. PER was diagnosed according to the criteria described in Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update.[1] A questionnaire was used to obtain data about disease history, family history, symptoms and concurrent diseases. 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:[12,13,21] (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 in the phadiatop assay (Phadia, Uppsala, Sweden); (4) no history of AR or other allergic diseases in the immediate family. The exclusion criteria for all subjects: (1) complicated with acute upper respiratory tract infection, severe deviation of nasal septum, rhinosinusitis with or without nasal polyps, neoplasms in paranasal sinuses and nasal cavity; (2) combined with other immune diseases and systemic diseases; (3) use of corticosteroids during previous 4 weeks and antihistamines/ antileukotrienes during previous 2 weeks. The research protocol complying with the Declaration of Helsinki was approved by the Ethics Committee of Nanjing Medical University (20080305), and written informed consent was obtained from all participants.

Clinical Parameters
A visual analogue scale (VAS) was used to assess the severity of nasal symptoms. 0 was a score meaning "not at all bothersome", and 10 meaning "extremely bothersome". VAS score ≤5 indicated mild AR and VAS score >5 indicated moderate-to-severe AR.[22]

After interview, about 5 mL of peripheral blood was collected from each subject for in vitro allergy testing. The ImmunoCAP assays (Phadia, Uppsala, Sweden) were carried out to measure the levels of serum total IgE and 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). Specific IgE ≥0.35 kU_A/L indicated positivity. AR cases were sensitized with HDM (*Der p* and/or *Der f*), and 139 of them (26.0%) were further sensitized with other aeroallergens.

SNP Selection and Genotyping

Six SNPs in the *GARP* gene (rs947998, rs79525962, rs1320646, rs3781699, rs1803627 and rs7685) were selected based on genotype data of Han Chinese in Beijing (CHB) in CSHL-HAPMAP (HapMap Data Rel 27 Phase II+III, Feb09, on NCBI B36 assembly, dbSNP b126), and the allele frequency data from 1000GENOMES (pilot_1_CHB+JPT_mar_2010, b132). For genotyping, the TaqMan SNP Genotyping Assay was performed using the 384-well ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Concordance was 100% in more than 15% of the samples randomly selected. The primers and TaqMan probes are shown in Table 1.

In silico Analysis

HaploReg (http://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and RegulomeDB (https://www.regulomedb.org/regulome-search/) were used to predict putative functions of SNPs.[23] HaploReg could be used to predict the functions of variations, including sequence conservation, regulatory protein binding, expression quantitative trait loci, regulatory motifs, and catalog of variants.[24] RegulomeDB score represented different functions of SNPs.[25] Changes in secondary structure and minimum free energy (MFE) in different SNP genotypes were predicted by using RNAfold (http://rna.tbi.univie.ac.at/). The alterations of secondary structure and MFE might change the binding affinity to microRNAs.[26]

Statistical Analysis

A goodness-of-fit χ^2 test was first performed to measure allele frequencies against departure from the Hardy-Weinberg equilibrium (HWE). Differences in demographic data, selected variables, and frequencies of genotypes and alleles between PER patients and the controls were evaluated. Two-sided χ^2 test was employed for categorical variables, Student's *t*-test for continuous variables in normal distribution, and nonparametric test for continuous variables in non-normal distribution. Data about serum total IgE were normalized with a logarithmic model. Percentiles of total IgE measurements (n=979) were calculated to divide the total serum IgE into "low" (<90th quantile [712.0 kU/L] after logarithmic transformation) and "high" (≥90th quantile [712.0 kU/L] after logarithmic transformation).[27] Analysis of covariance and nonparametric tests were conducted to manifest the associations between *GARP* polymorphisms and clinical phenotypes. Unconditional univariate and multivariate logistic regression models were established to generate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between *GARP* polymorphisms and the risk of HDM-sensitized PER. Multivariate analysis was performed after age and gender adjustment. The SNP indicating a positive association with PER was further submitted to stratification analysis of age, gender, concomitant asthma, family history of allergic diseases, VAS score, and serum total IgE levels. The linkage disequilibrium (LD) between SNPs was computed using the normalized measure of allelic association *D'* and r[2] and illustrated using HaploView 4.2 software.[28] Haplotype analysis was performed by HAPSTAT 3.0 (The University of North Carolina

Table 1 Primers and Probes for Genotyping by TaqMan Assay

NCBI rs No.	Base Change	Primers[a]	Probes[a]
rs947998	G>T	F: GCCTGATCTTTGAAAACACTACACA	G allele: FAM-CCCAGGCCGCAGC-MGB
		R: CCACACTGCTTCTCCAAAATTAGTT	T allele: HEX-CCCAGGCCTCAGC-MGB
rs79525962	C>T	F: GGTTCCCCTGCAGGTTGAG	G allele: FAM-ACACCTTTGCCAATC-MGB
		R: CTTAAGCCACAATGCCCTGGAG	A allele: HEX-ATACACCTTTACCAATCT-MGB
rs1320646	G>A	F: TCTTCTGAGAATGACTTTCAGTCTCTCT	G allele: FAM-TAGGACCGGAAGAGA-MGB
		R: CCAGATCCGAGACACACTCGTA	A allele: HEX-TTAGGACCAGAAGAGAG-MGB
rs3781699	A>C	F: GCCAAGCTGGGTGCAAAA	T allele: FAM-AGAACCAGATATCTAAG-MGB
		R: GCTGCTGAGCCAGGAGCTAA	G allele: HEX-AGAACCAGATAGCTAAGGT-MGB
rs1803627	T>G	F: TCTCTCTGTGCTCTTGCATTCTCT	T allele: FAM-ATTCCCTTTTCCTCTATTGA-MGB
		R: TCATTCTCTTCCTAAGCCTCAGTTTC	G allele: HEX-TTTTCCTCTATGGAGCAGA-MGB
rs7685	T>G	F: GCCTGATCTTTGAAAACACTACACA	G allele: FAM-CCCAGGCCGCAGC-MGB
		R: CCACACTGCTTCTCCAAAATTAGTT	T allele: HEX-CCCAGGCCTCAGC-MGB

Note: [a]The alleles were arrayed as the location of the primers or probes from 5' to 3'.

at Chapel Hill, NC, USA) with observed genotypes. All statistical analyses were accomplished with SPSS software version 20.0.0 (IBM Corporation, Armonk, NY, USA). *P*-value <0.05 was considered statistically significant.

Results

Basic Demographic Data

In terms of frequency distributions (Table 2), the cases and controls were well matched in age (*P*=0.535) and gender (*P*= 0.101). The median age of all subjects was 16 years. PER cases showed higher serum total IgE (255.1 [118.9–560.5] kU/ L) (*P*<0.001) than controls (25.2 [10.4–47.2] kU/L). All PER cases were sensitized with HDM (*Der p* and/or *Der f*), and 139 of them (26.0%) were further sensitized with other aeroallergens; however, they were not the main allergens causing symptoms. The serum levels of allergen-specific IgE against *Der p* and *Der f* in PER cases were 27.4 (6.1–69.5) kU$_A$/L and 23.1 (6.0–63.0) kU$_A$/L, respectively. In PER cases, 208 (45.6%) had mild and 248 (54.4%) had moderate-to-severe AR according to VAS score; 124 (27.5%) reported concomitant asthma and 154 (34.4%) had family history of allergic diseases. These variables were subjected to the multivariate logistic regression analysis to eliminate residual confounding effect.

Association Analysis of Each SNP

The genotype distributions in controls presented similarity with those in HWE (all *P* > 0.05, Table 3). As shown in Table 4, the genotype (*P*=0.034) and allele (*P*=0.018) frequencies of rs79525962 were significantly different between the cases and the controls. In addition, genotype CT+TT of rs79525962 brought with a higher risk of HDM-sensitized PER than the wild-type genotype CC (adjusted OR=1.393, 95% CI=1.019–1.904). The homozygous genotype CC of rs3781699 brought with a lower risk of HDM-sensitized PER than the wild-type genotype AA (adjusted OR=0.646, 95% CI=0.427–0.976); however, the genotype and allele frequencies of rs3781699 showed no significant association with the risk of HDM-sensitized PER (all *P* > 0.05).

Table 2 Distribution of Selected Variables Among Cases and Controls

Variables	Case (n=534)		Control (n=451)		*P*
	N	**%**	**N**	**%**	
Age (years), median (quartiles)	16.0 (10.0–27.0)		16.0 (10.0–29.0)		0.535[a]
Gender					0.101[a]
Male	356	66.7	278	61.6	
Female	178	33.3	173	38.4	
Serum total IgE (kU/L), median (quartiles)	255.1 (118.9–560.5)		25.2 (10.4–47.2)		< 0.001[b]
Specific IgE (kU$_A$/L), median (quartiles)					
Der p	27.4 (6.1–69.5)				
Der f	23.1 (6.0–63.0)				
Rhinitis severity (VAS score)[c]					
Mild (VAS score ≤ 5)	208	45.6			
Moderate-to-severe (VAS score > 5)	248	54.4			
Concomitant asthma[c]					
Yes	124	27.5			
No	327	72.5			
Family history of allergic rhinitis[c]					
Yes	154	34.4			
No	294	65.6			

Notes: [a]Two-sided χ^2 test for comparison of discrete variables and nonparametric test for continuous variables with non-normal distribution. [b]Selective variables were transformed into logarithmic model before unpaired Student's *t*-test between cases and controls. [c]Data about VAS score, concomitant asthma, and family history of allergic diseases were not available in some cases.
Abbreviations: *Der p, Dermatophagoides pteronyssinus; Der f, Dermatophagoides farina*; VAS, visual analogue scales.

Table 3 Primary Information of Six SNPs in the *GARP* Gene

SNP No.	NCBI *rs* No.	Chromosome Position[a]	Location	Base Change	MAF			P for HWE[c]	Genotyped (%)
					Database[b]	Case	Control		
1	rs947998	76672762	5' near gene	G>T	0.305	0.310	0.317	0.290	98.1
2	rs79525962	76660374	exon	C>T	0.092	0.129	0.095	0.274	99.2
3	rs1320646	76659003	3'UTR	G>A	0.111	0.093	0.109	0.893	99.3
4	rs3781699	76658741	3'UTR	A>C	0.407	0.326	0.362	0.392	99.8
5	rs1803627	76658300	3'UTR	T>G	0.061	0.087	0.081	0.051	99.2
6	rs7685	76657595	3'UTR	T>G	0.350	0.332	0.355	0.548	99.2

Notes: [a]SNP position in NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp). [b]MAF for CHB from the HapMap databases (http://hapmap.ncbi.nlm.nih.gov) or NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp). [c]HWE *P*-value in the control group using a goodness-of-fit χ^2 test.
Abbreviations: SNP, single nucleotide polymorphism; GARP, glycoprotein A repetitions predominant; MAF, minor allele frequencies; HWE, Hardy-Weinberg equilibrium; UTR, untranslated region; CHB, Han Chinese in Beijing, China.

Stratification Analysis of SNP rs79525962 in PER Subgroups

Considering that rs79525962 showed a significant association with HDM-sensitized PER, we evaluated the distributions of rs79525962 among PER subgroups. PER patients were stratified into six subgroups based on age, gender, concomitant asthma, family history of allergic diseases, rhinitis severity and serum total IgE level. As shown in Table 5, in contrast to wild-type genotype CC, genotype CT+TT of rs79525962 brought with a higher risk of HDM-sensitized PER in the subgroup of age ≥16 years (adjusted OR=1.745, 95% CI=1.103–2.760), and this high risk was also found in the females (adjusted OR=1.708, 95% CI=1.021–2.856). No evident associations were found between rs79525962 with other PER-related variables (all $P > 0.05$).

Association Between *GARP* Polymorphisms and AR-Related Phenotypes

As shown in Table 6, the genotypes of rs947998, rs79525962, rs1320646, rs3781699, rs1803627 and rs7685 in the *GARP* gene were not linked with the susceptibility to PER with or without asthma in the dominant model (all $P > 0.05$). Given the significant difference in serum total IgE levels between cases and controls, the associations of serum total IgE with six SNPs were evaluated separately. As showed in Table 7, both total IgE levels demonstrated no significant difference among individuals classified according to genotypes of six SNPs (all $P > 0.05$). No obvious associations were observed between different SNP genotypes and other AR-related phenotypes, including VAS score and specific IgE levels against *Der p* and *Der f* (all $P > 0.05$).

Association Between *GARP* Haplotypes and PER

As shown in Figure 1, the LD between rs1320646 and rs3781699 was quite strong (D'=1.0, r^2=0.058). We then identified rs1320646-rs3781699 as a potential *GARP* haplotype and evaluated its association with PER risk among cases and controls. As shown in Table 8, compared with the common haplotype G-A of rs1320646-rs3781699, the G-C haplotype exhibited a lower risk of HDM-sensitized PER (adjusted OR=0.819, 95% CI=0.676–0.993).

Functions of SNP rs1320646, rs3781699 and rs79525962

Using HaploReg, we observed that rs1320646, rs3781699 and rs79525962 possessed enhancer histone marks, altered motifs and selected expression quantitative trait locus (eQTL) hits. Besides, GRASP QTL hits were rich in rs1320646 and rs79525962. The RegulomeDB scores of rs1320646, rs3781699 and rs79525962 were 1f, 3a and 4, respectively (Table 9). RNAfold predicted that rs79525962 C to T substitution led to the alteration in GARP secondary structure, with MFE increasing from −21 kcal/mol to −19.1 kcal/mol (Figure 2A), indicating that the C allele had a higher binding affinity to microRNAs than the T allele. We also observed that rs3781699 A to C substitution changed the secondary structure of GARP, with MFE decreasing from −16.4 kcal/mol to −21.6 kcal/mol, suggesting that the C allele had a higher binding affinity to microRNAs than the A allele (Figure 2B). However, no similar results were found in rs1320646 (Figure 2C).

Table 4 Genotypes and Allele Frequencies in *GARP* Polymorphisms Among Cases and Controls

SNP No.	NCBI rs No.	Genotypes	Case		Control		Crude OR (95% CI)	Adjusted OR (95% CI)[a]	P[b]
			N	%	N	%			
1	rs947998		n=531		n=435				
		GG	249	46.9	198	45.5	1.000 (reference)	1.000 (reference)	0.911
		GT	235	44.3	198	45.5	0.944 (0.724–1.231)	0.937 (0.718–1.225)	
		TT	47	8.9	39	9.0	0.958 (0.603–1.524)	0.988 (0.620–1.574)	
		GT+TT	282	53.1	237	54.5	0.946 (0.734–1.220)	0.946 (0.733–1.221)	0.670
		T allele	0.310		0.317				0.725
2	rs79525962		n=533		n=444				
		CC	406	76.2	362	81.5	1.000 (reference)	1.000 (reference)	**0.034**
		CT	117	22.0	80	18.0	1.304 (0.949–1.792)	1.319 (0.959–1.814)	
		TT	10	1.9	2	0.5	4.458 (0.970–20.481)	4.225 (0.918–19.452)	
		CT+TT	127	23.8	82	18.5	**1.381 (1.011–1.886)**	**1.393 (1.019–1.904)**	**0.042**
		T allele	0.129		0.095				**0.018**
3	rs1320646		n=532		n=446				
		GG	442	83.1	354	79.4	1.000 (reference)	1.000 (reference)	0.169
		GA	81	15.2	87	19.5	0.746 (0.534–1.041)	0.742 (0.531–1.037)	
		AA	9	1.7	5	1.1	1.442 (0.479–4.340)	1.460 (0.484–4.405)	
		GA+AA	90	16.9	92	20.6	0.783 (0.568–1.082)	0.781 (0.565–1.079)	0.138
		A allele	0.093		0.109				0.250
4	rs3781699		n=534		n=449				
		AA	240	44.9	187	41.6	1.000 (reference)	1.000 (reference)	0.151
		AC	240	44.9	199	44.3	0.940 (0.719–1.229)	0.932 (0.713–1.220)	
		CC	54	10.1	63	14.0	0.668 (0.443–1.007)	**0.646 (0.427–0.976)**	
		AC+CC	294	55.1	262	58.4	0.874 (0.679–1.127)	0.863 (0.670–1.114)	0.299
		C allele	0.326		0.362				0.093
5	rs1803627		n=534		n=443				
		TT	446	83.5	377	85.1	1.000 (reference)	1.000 (reference)	0.574
		TG	83	15.5	60	13.5	1.169 (0.816–1.675)	1.171 (0.817–1.678)	
		GG	5	0.9	6	1.4	0.704 (0.213–2.326)	0.701 (0.212–2.320)	
		TG+GG	88	16.5	66	14.9	1.127 (0.796–1.595)	1.128 (0.797–1.597)	0.500
		G allele	0.087		0.081				0.645
6	rs7685		n=532		n=445				
		TT	237	44.5	188	42.2	1.000 (reference)	1.000 (reference)	0.492
		TG	237	44.5	198	44.5	0.949 (0.726–1.242)	0.942 (0.719–1.233)	
		GG	58	10.9	59	13.3	0.780 (0.518–1.175)	0.757 (0.501–1.143)	
		TG+GG	295	55.5	257	57.8	0.911 (0.706–1.174)	0.899 (0.697–1.161)	0.470
		G allele	0.332		0.355				0.281

Notes: [a]Adjusted for age and gender in multivariate logistic regression model. Bold values denote statistical significance at the $P < 0.05$ level. [b]Two-sided χ^2 test for the distributions of genotype and allele frequencies.
Abbreviations: GARP, glycoprotein A repetitions predominant; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 5 Stratification Analysis of rs79525962 in the Dominant Model in PER Subgroups

Variables	Subcategory	Case	Control	Adjusted OR (95% CI)[a]
Age (years)		n=533	n=444	
	< 16	257	225	1.076 (0.693–1.671)
	≥ 16	276	219	**1.745 (1.103–2.760)**
Gender		n=533	n=444	
	Male	356	273	1.241 (0.836–1.840)
	Female	177	171	**1.708 (1.021–2.856)**
Concomitant asthma		n=450		
	No	326		1.000 (reference)
	Yes	124		0.936 (0.565–1.552)
Family history of allergic diseases		n=447		
	No	293		1.000 (reference)
	Yes	154		0.987 (0.620–1.571)
Rhinitis severity (VAS score)		n=455		
	VAS score ≤ 5	207		1.000 (reference)
	VAS score > 5	248		0.909 (0.585–1.411)
Serum total IgE[b]		n=529	n=442	
	Lower level	431	442	1.000 (reference)
	Higher level	98		1.144 (0.696–1.880)

Notes: [a]Adjusted for age and gender in multivariate logistic regression model. Bold values denote statistical significance at the $P < 0.05$ level. [b]Lower level: below the 90th percentile of logarithmic total IgE; higher level: above the 90th percentile of logarithmic total IgE.
Abbreviations: Dominant model: MW+MM/WW; MW: heterozygotes; MM: mutation homozygotes; WW: wild homozygotes. PER, persistent allergic rhinitis; VAS, visual analogue scale; OR, odds ratio; CI, confidence interval.

Table 6 Genotype Frequencies of *GARP* Polymorphisms in the Dominant Model in PER Patients with and without Asthma

SNP No.	NCBI rs No.	Case with Asthma vs Control	Case without Asthma vs Control
		Adjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]
1	rs947998	0.670 (0.445–1.008)	1.140 (0.853–1.525)
2	rs79525962	1.249 (0.765–2.041)	1.410 (0.993–2.003)
3	rs1320646	0.948 (0.570–1.576)	0.714 (0.490–1.041)
4	rs3781699	0.919 (0.612–1.380)	0.951 (0.712–1.271)
5	rs1803627	0.950 (0.536–1.684)	1.199 (0.813–1.768)
6	rs7685	1.060 (0.703–1.598)	0.945 (0.707–1.263)

**Note**: [a]Adjusted for age and gender in multivariate logistic regression model.
Abbreviations: Dominant model, MW+MM/WW; MW, heterozygotes; MM, mutation homozygotes; WW, wild homozygotes; GARP, glycoprotein A repetitions predominant; PER, persistent allergic rhinitis; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 7 Associations Between *GARP* Polymorphisms and AR-Related Phenotypes

SNP No.	NCBI rs No.	*P* for Serum Total IgE[a]		*P* for Specific IgE[b]		*P* for VAS Score[c]
		Case	Control	*Der p*	*Der f*	
1	rs947998	0.782	0.780	0.179	0.213	0.139
2	rs79525962	0.608	0.270	0.820	0.800	0.524
3	rs1320646	0.713	0.442	0.543	0.244	0.518
4	rs3781699	0.173	0.938	0.803	0.434	0.736
5	rs1803627	0.944	0.232	0.874	0.678	0.717
6	rs7685	0.361	0.956	0.977	0.628	0.529

Notes: [a]Selective variables were transformed into logarithmic model to normalize the distribution before analysis of covariance adjusted for age and gender. [b]Nonparametric test for selective variables with non-normal distribution. [c]Analysis of covariance adjusted for age and gender for selective variables with normal distribution.
Abbreviations: GARP, glycoprotein A repetitions predominant; AR, allergic rhinitis; SNP, single nucleotide polymorphism; *Der p, Dermatophagoides pteronyssinus; Der f, Dermatophagoides farinae*.
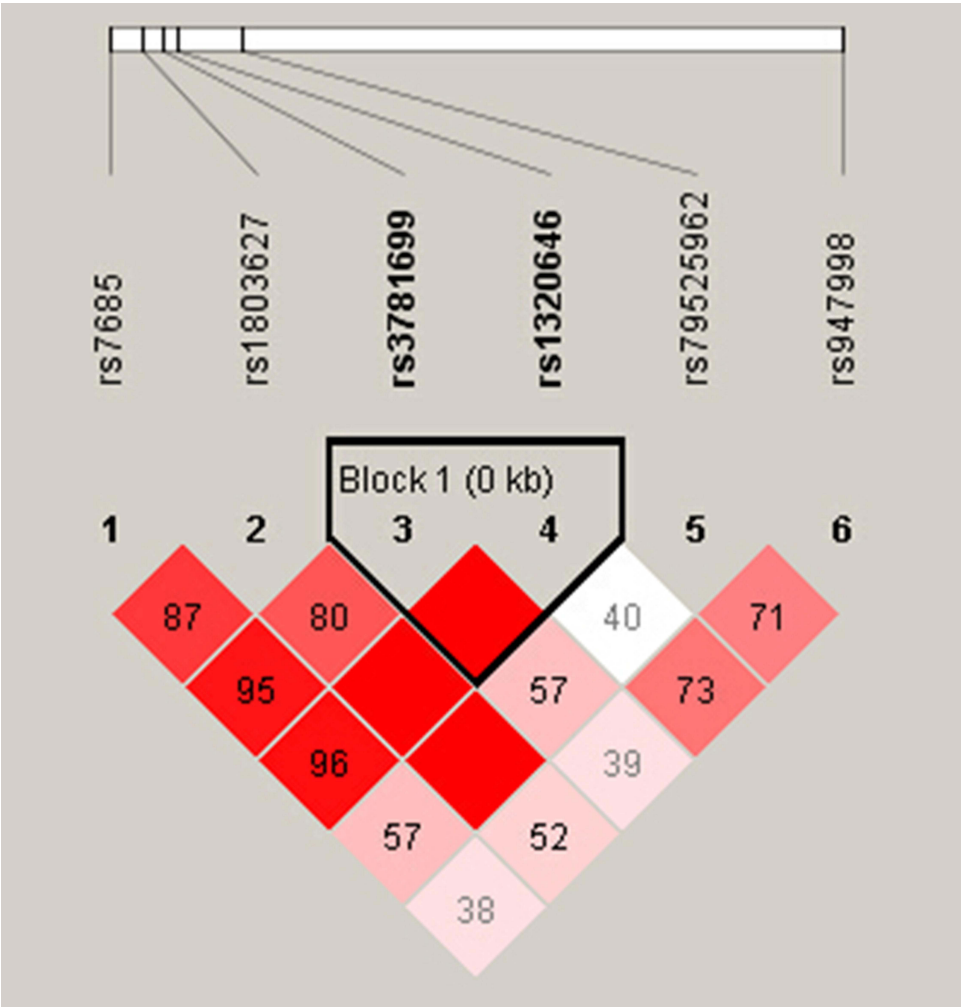
Figure 1 Linkage disequilibrium (LD) of six SNPs in the *GARP* gene. LD of six SNPs was determined using the solid spine of LD option of Haploview 4.2. *D'* values are displayed in the squares. Empty red squares have a pairwise *D'* of 1.0. Red squares indicate strong pairwise LD, gradually coloring down to white squares of weak pairwise LD.

Discussion

In this study, we report SNP rs79525962 in *GARP* appears to mark a genuine HDM-sensitized PER risk locus and the *GARP* gene may contribute to susceptibility of HDM-sensitized PER in this Chinese Han population. Moreover, we predict a putative function of the SNPs. Current literature suggests our research unprecedented in exploring the relationship between *GARP* variations and AR risk in Chinese population.

GARP protein, encoded by the *GARP* gene localized in the 11q13.5 chromosomal region, is a 80-kDa transmembrane protein with its extracellular region primarily composed of 20 leucine-rich repeats.[14] GARP protein is highly expressed

Table 8 Associations Between PER Risk and the Frequencies of Haplotypes

Haplotype	Haplotype Frequencies		Adjusted OR (95% CI)[a]
rs1320646-rs3781699	Case	Control	
G-A	0.581	0.529	1.000 (reference)
G-C	0.326	0.362	**0.819 (0.676–0.993)**
A-A	0.093	0.109	0.777 (0.573–1.050)

Notes: [a]Derived from logistic regression model performed by HAPSTAT 3.0 based on observed genotypes. Bold values denote statistical significance at the *P* < 0.05 level.
Abbreviation: PER, persistent allergic rhinitis; OR, odds ratio; CI, confidence interval.

Table 9 Functional Annotation of 3 SNPs in silico Analysis

Chr: Position[a]	Variant	Score[b]	Enhancer Histone Marks	Motifs Changed	GRASP QTL Hits	Selected eQTL Hits	dbSNP Func Annot
11: 76659003	rs1320646	1f	7 tissues	7 altered motifs	2 hits	3 hits	3′UTR
11: 76658741	rs3781699	3a	8 tissues	Hltf	–	3 hits	3′UTR
11: 76660374	rs79525962	4	7 tissues	7 altered motifs	2 hits	3 hits	Missense

Notes: [a]Based on NCBI build 38 of the human genome. [b]Based on RegulomeDB (https://www.regulomedb.org/regulome-search/).
Abbreviation: SNP, single nucleotide polymorphisms; GRASP, Genome-Wide Repository of Associations Between SNPs and Phenotypes; eQTL, expression quantitative trait locus; UTR, untranslated region.

on the surface of activated Tregs and increases the immune-suppressing function of Tregs.[29] In addition, GARP, functioning as a carrier and a cell surface receptor for latent transforming growth factor (TGF)-β, binds directly to the latent TGF-β, then modifies latent TGF-β into active TGF-β.[14] Tregs can regulate immune tolerance and inflammatory responses,[30,31] which in turn makes it a potential target in the treatment of allergic diseases. TGF-β is a pleiotropic cytokine critical to the generation of Treg cells.[32] It plays crucial roles in the remodeling and immunosuppression of allergic inflammatory airway diseases[33] and mediates pro- and anti-inflammatory responses.[34]

Our recent studies have found that a *TGFB1* promoter polymorphism may be associated with the susceptibility and the severity of PER,[12] and SNPs in microRNA target sites of TGF-β signaling pathway genes may be associated with risk of HDM-sensitized PER stratified by age and gender in Chinese Hans.[13] Studies have demonstrated that GARP promotes TGF-β secretion and activation, then increases the suppressive function of Tregs through TGF-β.[35–37] Moreover, GARP/ latent TGF-β1 complexes can induce Th17 differentiation.[14] Studies have also reported that human B lymphocytes, upon stimulation, produce active TGF-β1 from surface GARP/latent TGF-β1 complexes and induce IgA isotype switching.[38,39] As GARP mediates the immune-suppressing activity of Treg cells,[36,37,40,41] and allergen-dependent inflammation is suppressed by Tregs via soluble GARP,[29] we proposed that GARP might be implicated in the pathogenesis of AR.

The present study is the first to evaluate the genetic association of *GARP* polymorphisms with AR in Chinese population. The results suggested that SNP rs79525962 was significantly correlated with HDM-sensitized PER risk, and genotype CT+TT with a high risk of HDM-sensitized PER, compared with the wild-type genotype CC. The C/T polymorphism of rs79525962 located within the exon in the *GARP* gene yields missense coding sequence. This polymorphism drives the transition of alanine (C allele) into threonine (T allele), consequently compromising the conformation and function of GARP protein and might affect the suppressive role of Tregs. Moreover, we observed that rs79525962 C to T substitution altered the secondary structure of *GARP*. Previous study reported that structural changes affect the stability and translation of mRNA.[42] Hence, rs79525962 might regulate the translation of GARP and subsequent Tregs' function by this way. Manz et al[17] have shown a significant excess of *LRRC32* variants, such as rs79525962, in patients with atopic dermatitis. Structural protein modeling and bioinformatic analysis have revealed that protein transport is disrupted upon these variants. Deficient Tregs responses are associated with various allergic and autoimmune diseases,[43,44] suggesting that rs79525962 polymorphism of the *GARP* gene may have a role in these diseases.

In the stratification analysis of rs79525962 in PER subgroups, we found rs79525962 exhibited a significant association with HDM-sensitized PER risk in the subgroups of age ≥16 years and females. Therefore, age of ≥16 years and female may be factors for susceptibility to PER caused by *GARP* variants. This susceptibility may arise from the immature immunity in adolescents and the cycle-mediated endocrine function in women. On the other hand, lack of association was found between rs79525962 and AR concomitant asthma. We speculate that *GARP* polymorphisms increase the risk of allergic sensitization which, in turn, increases the risk of subsequent development of AR and asthma. Ferreira et al[45] found a genetic variant (rs7130588) in *GARP* was significantly associated with atopic status among asthmatics, suggesting that it is a risk factor for allergic but not non-allergic asthma, which was consistent with our results.

Furthermore, as various SNPs may co-work to raise the risk of AR, we identified rs1320646-rs3781699 as a potential haplotype. Compared with G-A of rs1320646-rs3781699, the G-C haplotype decreased the susceptibility to HDM-sensitized
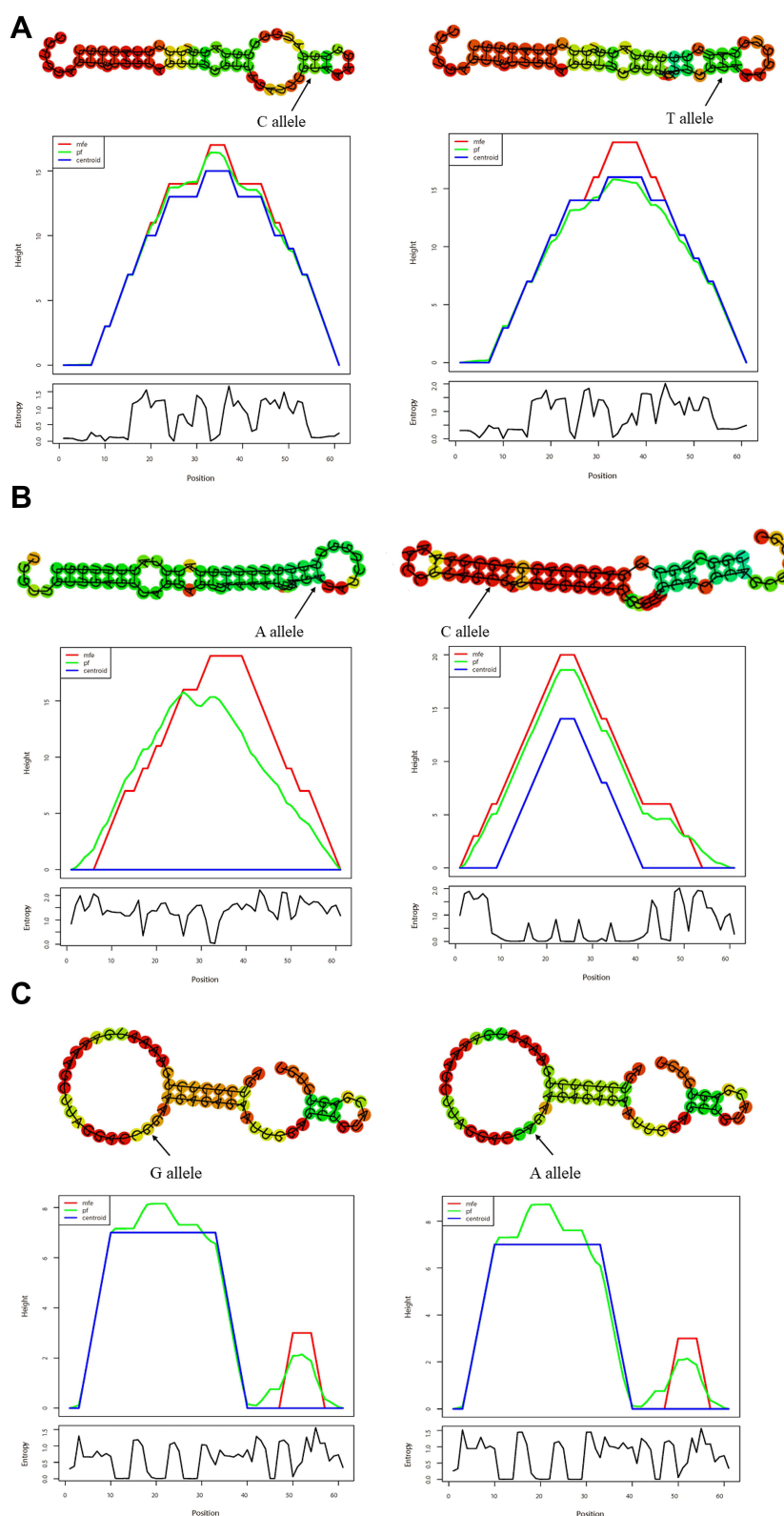
Figure 2 In silico prediction of secondary structures and minimum free energy (MFE) changes corresponding to *GARP* SNPs. (**A**) The MFE changed from −21 kcal/mol to −19.1 kcal/mol caused by rs79525962 (C > T), indicating a stronger binding affinity of microRNAs to the C allele. (**B**) The MFE changed from −16.4 kcal/mol to −21.6 kcal/mol caused by rs3781699 (A > C), indicating a stronger binding affinity of microRNAs to the C allele. (**C**) No change was found in MFE caused by rs1320646.
Abbreviation: GARP, glycoprotein A repetitions predominant.

PER. Genetic variant rs3781699 was located in the 3′UTR region of *GARP*. At the post-transcriptional level, microRNAs bind to the 3′UTR of their target messenger RNA to regulate *GARP* expression.[46] Notably, the mutation (A to C allele) in rs3781699 decreased MFE from −16.4 kcal/mol to −21.6 kcal/mol, indicating that the C allele has a higher binding affinity to microRNAs than the A allele.[26] Thus, we speculated that the G-C haplotype of rs1320646-rs3781699 might regulate the expression of *GARP* through this pathway, therefore decreasing the susceptibility to HDM-sensitized PER.

Several limitations of this study should be addressed. First, the homozygous genotype CC of rs3781699 was associated with a low risk of HDM-sensitized PER, compared with the wild-type genotype AA; however, no evident associations were established between the genotype and allele frequencies of rs3781699 and HDM-sensitized PER, which might be explained by that our sample size was not large enough and the effect of rs3781699 on AR pathogenesis was mild. Therefore, further larger-scale studies should be required in Chinese population. Second, the cases were collected from one single tertiary hospital setting in East China, and they may not be representative enough for the whole Han Chinese. Last but not least, *GARP*-related genes were not analyzed in the present study.

Conclusions

SNP rs79525962 in the *GARP* gene marks a risk locus of HDM-sensitized PER, suggesting that *GARP* polymorphisms might drive the development of HDM-sensitized PER in this population of Han Chinese. Further functional studies are required to demonstrate their molecular mechanisms in PER, and more detailed environmental exposure data are required to verify the effect of gene–environment interaction on HDM-sensitized PER.

Abbreviations

AR, allergic rhinitis; ARIA, Allergic Rhinitis and its Impact on Asthma; CHB, Han Chinese in Beijing; CIs, confidence intervals; *Der f, Dermatophagoides farinae; Der p, Dermatophagoides pteronyssinus*; eQTL, expression quantitative trait locus; GARP, glycoprotein A repetitions predominant; GRASP, Genome-Wide Repository of Associations Between SNPs and Phenotypes; HWE, Hardy-Weinberg equilibrium; IgE, Immunoglobulin E; LD, linkage disequilibrium; LRRC32, leucine-rich repeat containing 32; MAF, minor allele frequency; MFE, minimum free energy; ORs, odds ratios; PER, persistent allergic rhinitis; SNPs, single nucleotide polymorphisms; TGF-β, transforming growth factor-β; Treg, regulatory T lymphocytes; UTR, untranslated region; VAS, visual analogue scale.

Ethics Approval and Informed Consent

The research protocol was approved by the Ethics Committee of Nanjing Medical University (20080305), 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.

Acknowledgments

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.

Funding

Disclosure

The authors report no conflicts of interest in this work.

References

1. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(Suppl 86):8–160. doi:10.1111/j.1398-9995.2007.01620.x
2. Brożek JL, Bousquet J, Agache I, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol*. 2017;140(4):950–958. doi:10.1016/j.jaci.2017.03.050
3. Cheng L, Chen J, Fu Q, et al. Chinese Society of Allergy guidelines for diagnosis and treatment of allergic rhinitis. *Allergy Asthma Immunol Res*. 2018;10(4):300–353. doi:10.4168/aair.2018.10.4.300
4. Samitas K, Carter A, Kariyawasam HH, et al. Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: the one airway concept revisited. *Allergy*. 2018;73(5):993–1002. doi:10.1111/all.13373
5. Wang J, Zhang Y, Li B, et al. Asthma and allergic rhinitis among young parents in China in relation to outdoor air pollution, climate and home environment. *Sci Total Environ*. 2021;751:141734. doi:10.1016/j.scitotenv.2020.141734
6. Nordeide Kuiper I, Svanes C, Markevych I, et al. Lifelong exposure to air pollution and greenness in relation to asthma, rhinitis and lung function in adulthood. *Environ Int*. 2021;146:106219. doi:10.1016/j.envint.2020.106219
7. Bousquet J, Schünemann HJ, Samolinski B, et al. Allergic Rhinitis and its Impact on Asthma (ARIA): achievements in 10 years and future needs. *J Allergy Clin Immunol*. 2012;130(5):1049–1062. doi:10.1016/j.jaci.2012.07.053
8. Marseglia GL, Caimmi S, Marseglia A, et al. Rhinosinusitis and asthma. *Int J Immunopathol Pharmacol*. 2010;23(1 Suppl):29–31.
9. Laulajainen-Hongisto A, Lyly A, Hanif T. Genomics of asthma, allergy and chronic rhinosinusitis: novel concepts and relevance in airway mucosa. *Clin Transl Allergy*. 2020;10(1):45. doi:10.1186/s13601-020-00347-6
10. Tang L, Chen Y, Xiang Q, et al. The association between IL18, FOXP3 and IL13 genes polymorphisms and risk of allergic rhinitis: a meta-analysis. *Inflamm Res*. 2020;69(9):911–923. doi:10.1007/s00011-020-01368-4
11. Gao Y, Li J, Zhang Y, et al. Replication study of susceptibility variants associated with allergic rhinitis and allergy in Han Chinese. *Allergy Asthma Clin Immunol*. 2020;16:13. doi:10.1186/s13223-020-0411-9
12. Zhu XJ, Lu MP, Chen RX, et al. Polymorphism -509C/T in *TGFB1* promoter is associated with increased risk and severity of persistent allergic rhinitis in a Chinese population. *Am J Rhinol Allergy*. 2020;34(5):597–603. doi:10.1177/1945892420913441
13. Chen RX, Lu WM, Lu MP, et al. Polymorphisms in microRNA target sites of TGF-β signaling pathway genes and susceptibility to allergic rhinitis. *Int Arch Allergy Immunol*. 2021;182(5):399–407. doi:10.1159/000511975
14. Shevach EM. Garp as a therapeutic target for modulation of T regulatory cell function. *Expert Opin Ther Targets*. 2017;21(2):191–200. doi:10.1080/14728222.2017.1275568
15. Chen J, Chen Q, Wu C, et al. Genetic variants of the C11orf30-LRRC32 region are associated with childhood asthma in the Chinese population. *Allergol Immunopathol (Madr)*. 2020;48(4):390–394. doi:10.1016/j.aller.2019.09.002
16. Li J, Zhang Y, Zhang L. Discovering susceptibility genes for allergic rhinitis and allergy using a genome-wide association study strategy. *Curr Opin Allergy Clin Immunol*. 2015;15(1):33–40. doi:10.1097/ACI.0000000000000124
17. Manz J, Rodríguez E, ElSharawy A, et al. Targeted resequencing and functional testing identifies low-frequency missense variants in the gene encoding GARP as significant contributors to atopic dermatitis risk. *J Invest Dermatol*. 2016;136(12):2380–2386. doi:10.1016/j.jid.2016.07.009
18. Nasrallah R, Imianowski CJ, Bossini-Castillo L, et al. A distal enhancer at risk locus 11q13.5 promotes suppression of colitis by Treg cells. *Nature*. 2020;583(7816):447–452. doi:10.1038/s41586-020-2296-7
19. Lehmkuhl P, Gentz M, Garcia de Otezya AC, et al. Dysregulated immunity in PID patients with low GARP expression on Tregs due to mutations in LRRC32. *Cell Mol Immunol*. 2021;18(7):1677–1691. doi:10.1038/s41423-021-00701-z
20. Marenholz I, Esparza-Gordillo J, Rüschendorf F, et al. Meta-analysis identifies seven susceptibility loci involved in the atopic march. *Nat Commun*. 2015;6:8804. doi:10.1038/ncomms9804
21. Chen RX, Dai MD, Zhang QZ, et al. TLR signaling pathway gene polymorphisms, gene-gene and gene-environment interactions in allergic rhinitis. *J Inflamm Res*. 2022;15:3613–3630. doi:10.2147/JIR.S364877
22. Bousquet PJ, Combescure C, Neukirch F, et al. Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines. *Allergy*. 2007;62(4):367–372. doi:10.1111/j.1398-9995.2006.01276.x
23. Chen M, Zheng R, Li F, et al. Genetic variants in Hippo pathway genes are associated with house dust mite-induced allergic rhinitis in a Chinese population. *Clin Transl Allergy*. 2021;11(10):e12077. doi:10.1002/clt2.12077
24. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*. 2016;44(D1):D877–81. doi:10.1093/nar/gkv1340
25. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res*. 2012;22(9):1790–1797. doi:10.1101/gr.137323.112
26. Wang W, Yang C, Nie H, et al. LIMK2 acts as an oncogene in bladder cancer and its functional SNP in the microRNA-135a binding site affects bladder cancer risk. *Int J Cancer*. 2019;144(6):1345–1355. doi:10.1002/ijc.31757
27. Tian HQ, Chen XY, Lu Y, et al. Association of *VDR* and *CYP2R1* polymorphisms with mite-sensitized persistent allergic rhinitis in a Chinese population. *PLoS One*. 2015;10(7):e0133162. doi:10.1371/journal.pone.0133162

28. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21(2):263–265. doi:10.1093/bioinformatics/bth457

29. Miyazono K, Katsuno Y, Koinuma D, et al. Intracellular and extracellular TGF-β signaling in cancer: some recent topics. *Front Med*. 2018;12(4):387–411. doi:10.1007/s11684-018-0646-8

30. Meyer-Martin H, Hahn SA, Beckert H, et al. GARP inhibits allergic airway inflammation in a humanized mouse model. *Allergy*. 2016;71(9):1274–1283. doi:10.1111/all.12883

31. Grant CR, Liberal R, Mieli-Vergani G, et al. Regulatory T-cells in autoimmune diseases: challenges, controversies and–yet–unanswered questions. *Autoimmun Rev*. 2015;14(2):105–116. doi:10.1016/j.autrev.2014.10.012

32. Iizuka-Koga M, Nakatsukasa H, Ito M, et al. Induction and maintenance of regulatory T cells by transcription factors and epigenetic modifications. *J Autoimmun*. 2017;83:113–121. doi:10.1016/j.jaut.2017.07.002

33. DeVries A, Vercelli D. Of pleiotropy and trajectories: Does the TGF-β pathway link childhood asthma and chronic obstructive pulmonary disease? *J Allergy Clin Immunol*. 2018;141(6):1992–1996. doi:10.1016/j.jaci.2018.04.012

34. Ojiaku CA, Yoo EJ, Panettieri RA Jr. Transforming growth factor β1 function in airway remodeling and hyperresponsiveness. The missing link? *Am J Respir Cell Mol Biol*. 2017;56(4):432–442. doi:10.1165/rcmb.2016-0307TR

35. Metelli A, Salem M, Wallace CH, et al. Immunoregulatory functions and the therapeutic implications of GARP-TGF-β in inflammation and cancer. *J Hematol Oncol*. 2018;11(1):24. doi:10.1186/s13045-018-0570-z

36. Salem M, Wallace C, Velegraki M, et al. GARP dampens cancer immunity by sustaining function and accumulation of regulatory T cells in the colon. *Cancer Res*. 2019;79(6):1178–1190. doi:10.1158/0008-5472.CAN-18-2623

37. Vermeersch E, Liénart S, Collignon A, et al. Deletion of GARP on mouse regulatory T cells is not sufficient to inhibit the growth of transplanted tumors. *Cell Immunol*. 2018;332:129–133. doi:10.1016/j.cellimm.2018.07.011

38. Dedobbeleer O, Stockis J, van der Woning B, et al. Cutting Edge: Active TGF-β1 released from GARP/TGF-β1 complexes on the surface of stimulated human B lymphocytes increases class-switch recombination and production of IgA. *J Immunol*. 2017;199(2):391–396. doi:10.4049/jimmunol.1601882

39. Wallace CH, Wu BX, Salem M, et al. B lymphocytes confer immune tolerance via cell surface GARP-TGF-β complex. *JCI Insight*. 2018;3(7):e99863. doi:10.1172/jci.insight.99863

40. Lodyga M, Hinz B. TGF-β1 - A truly transforming growth factor in fibrosis and immunity. *Semin Cell Dev Biol*. 2020;101:123–139. doi:10.1016/j.semcdb.2019.12.010

41. de Streel G, Bertrand C, Chalon N, et al. Selective inhibition of TGF-β1 produced by GARP-expressing Tregs overcomes resistance to PD-1/PD-L1 blockade in cancer. *Nat Commun*. 2020;11(1):4545. doi:10.1038/s41467-020-17811-3

42. Sharma Y, Miladi M, Dukare S, et al. A pan-cancer analysis of synonymous mutations. *Nat Commun*. 2019;10(1):2569. doi:10.1038/s41467-019-10489-2

43. Noval Rivas M, Chatila TA. Regulatory T cells in allergic diseases. *J Allergy Clin Immunol*. 2016;138(3):639–652. doi:10.1016/j.jaci.2016.06.003

44. Eggenhuizen PJ, Ng BH, Ooi JD. Treg enhancing therapies to treat autoimmune diseases. *Int J Mol Sci*. 2020;21(19):7015. doi:10.3390/ijms21197015

45. Ferreira MA, Matheson MC, Duffy DL, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet*. 2011;378(9795):1006–1014. doi:10.1016/S0140-6736(11)60874-X

46. Ali Syeda Z, Langden SSS, Munkhzul C, et al. Regulatory mechanism of microRNA expression in cancer. *Int J Mol Sci*. 2020;21(5):1723. doi:10.3390/ijms21051723