Experimental Allergy – Research Article

Polymorphisms in MicroRNA Target Sites of TGF-β Signaling Pathway Genes and Susceptibility to Allergic Rhinitis

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Abstract

Background: The polymorphisms inside microRNA target sites locating in the 3'-UTR region may introduce the microRNA-binding changes, which may regulate the gene expression and correlate with the potential diseases. ***Objectives:*** We aimed to investigate whether the polymorphisms in microRNA target sites of transforming growth factor beta (TGF-β) signaling pathway genes are associated with the susceptibility of mite-sensitized allergic rhinitis (AR) in a Han Chinese population. ***Methods:*** In this case-control study, 454 AR patients and 448 healthy controls were recruited. Three HapMap single-nucleotide polymorphisms (SNPs) were mapped to putative microRNA recognition sites and genotyped by TaqMan allelic discrimination assay. ***Results:*** The genotype and allele frequencies of 3 SNPs (rs1590 in *TGFBR1*; rs1434536 and rs17023107 in *BMPR1B*) showed lack of significant association with AR. However, in the subgroup analysis, the TG, GG, and TG/GG genotypes of rs1590 exhibited significantly increased risk of AR in the male subgroup (TG: adjusted OR = 1.57, 95% CI = 1.08–2.31; GG: adjusted OR = 1.76, 95% CI = 1.09–2.86; TG/GG: adjusted OR = 1.62, 95% CI = 1.13–2.33). The CT genotypes of rs17023107 might have potential to protect against AR in the patients age of <15 years (adjusted OR = 0.37, 95% CI = 0.14–0.95) and the males (adjusted OR = 0.48, 95% CI = 0.25–0.95). No significant association was found between SNPs and the total serum IgE level. ***Conclusions:*** In a Han Chinese population, stratified by age and gender, susceptibility to mite-sensitized AR may be associated with 2 SNPs (rs1590 and rs17023107) in microRNA target sites of TGF-β signaling pathway genes.

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Introduction

Allergic rhinitis (AR) affects 10–42% of the population worldwide [1]. The prevalence of adult AR in China increased from 11.1% in 2005 to 17.6% in 2011 in major cities [2–4]. As a heterogeneous disorder of the nasal mu-

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Edited by: D.Y. Wang, Singapore.

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cosa, AR is induced by T helper 2 cells and IgE responses that are specific to aeroallergens in allergen-sensitized individuals. Since the 1990s, genetic studies using the candidate gene approaches have found linkage between cytokine-encoding genes and other immunomodulatory mediators [5, 6].

Transforming growth factor beta (TGF-β) is a pleiotropic cytokine that directs cellular responses including proliferation, differentiation, apoptosis, migration, tissue repair, and immunoglobulin isotype switching [7]. Dysfunction of TGF-β signaling pathway shows intimacy with multiple human disorders, such as cancer, asthma, autoimmune, and cardiovascular diseases [8, 9]. TGF-β superfamily is a composite of fibrogenic and immuno-modulatory factors propping up the structure of the upper and lower airways [10, 11]. The elevated levels of TGF-β1 could act as a healing molecule in the airways of asthmatics, promoting the process of tissue repair [12] and diminishing airway hyperresponsiveness [13]. A cytosine to thymine transition within the *TGFB1* promoter (rs1800469) has been reported significantly associated with a higher plasma concentration of TGF-β1 [14] in the development of asthma [15, 16] Moreover, our previous study has confirmed that rs1800469 in *TGFB1* is associated with increased risk and severity of persistent AR in a Chinese population [17]. Allelic variations in the constituents of TGF-β pathway may regulate transcription and expression of target genes and proteins, which in turn changes the function of TGF-β pathway and the process of allergic diseases.

MicroRNAs are a class of endogenous short noncoding RNAs (18–25 nt long) that act posttranscriptionally as negative regulators in gene expression principally via interaction with target sites in the 3′-UTRs of regulated genes in metazoans [18]. The introduction of a single-nucleotide polymorphism (SNP) into a 3′-UTR can have numerous functional consequences potential associated with human disorders, by either introducing or removing microRNA target sequences or changing the binding efficiency [19]. Studies have shown that C minor allele (SNP rs4245739A<C) in the 3′-UTR of *MDM4* has been shown to decrease the risk of cancer, as the C minor SNP creates a new binding site for miR-191 and/or miR-887-3p [20, 21]. On the other hand, it was known that miR-498, miR-155, miR-205, and let-7e may play a role in development of AR [22, 23]. Experimentally validated results suggested a bidirectional cross-talk between microRNAs and TGF-β pathway [18], as evidenced by the role of miR-146a polymorphism in asthma [24, 25]. This study was undertaken, for the first time, to evaluate the associations between SNPs in the putative microRNA target sites of 3′-UTR of TGF-β signaling pathway genes and the susceptibility to mite-sensitized AR in a Han Chinese population.

Materials and Methods

Subjects

A total of 454 AR patients (304 males and 150 females) were recruited from the First Affiliated Hospital of Nanjing Medical University since May 2008. AR was diagnosed according to the ARIA 2008 update [26]. The presence of other allergic diseases, like asthma, eczema, atopic dermatitis, urticaria, and food and drug allergies, as well as a family history of allergy was determined according to a questionnaire. Patients enrolled were sensitized mainly by dust mites, including *Dermatophagoides pteronyssinus (Der p)* or *Dermatophagoides farinae (Der f)*. According to the questionnaire results, 105 AR (23.1%) patients were accompanied with asthma, and 272 (59.9%) patients without asthma; 77 (17.0%) patients' asthma information was missing because the related items in their questionnaires were not answered. The 448 healthy controls (277 males and 171 females) were recruited from the annual physical exams, which confirmed that they had normal nasal examination, no clinical features of nasal diseases, and no clinical features or family history of allergy. The Phadiatop assay exhibited negative allergen-specific IgE in their serum. Age and gender of the patients and the controls were matched. The response rate in the study was >85%. After the interview, 5 mL peripheral blood was taken by venipuncture from each subject. However, in all cases before taking peripheral venous blood, glucocorticoids were not used within 4 weeks; H1 antihistamines, leukotriene receptor antagonists, and other antiallergic drugs were not used within 2 weeks. The study was approved by the Ethics Committee of Nanjing Medical University (20080305). Written informed consent was obtained from all participants who were genetically unrelated Han Chinese from Jiangsu and Anhui provinces in eastern China.

Quantitative IgE Measurements

Serum total IgE and specific IgE were measured by the ImmunoCAP system (Phadia, Uppsala, Sweden). Total IgE was determined in all the subjects. Phadiatop tests were performed in the healthy controls. Specific IgE antibodies to common aeroallergens were determined in the patients, including *Der p* (d1), *Der f* (d2), cat epithelium and dander (e1), dog dander (e5), *Blatella germanica* (i6), *Alternaria alternate* (m6), *Ambrosia elatior* (w1), and *Artemisia vulgaris* (w6). When the serum allergen-specific IgE was higher than 0.35 kUA/L, the result was considered positive. We chose patients who are allergic to dust mites (d1 and/or d2), a type of allergen that is the most common in East China. The positive rates of other aeroallergens (e1, e5, i6, m6, w1, and w6) were low and not the main allergens causing symptoms.

Selection of Polymorphisms within the TGF-β Signaling Pathway

Forty-six potential SNPs derived from HapMap consortium were mapped to prospective microRNA target sites in 15 genes (*TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, TGFBR3, BMP2, BMP4, BMPR1A, BMPR1B, SMAD1, SMAD2, SMAD3, SMAD4,* and *SMAD7*). The combined predictions by TargetScan [27], Pa-

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Table 1. Primers and probes for genotypes screening by TaqMan allelic discrimination

Target gene	SNPs	Primers	Probes
TGFBR1	rs1590	F: 5′-ACAAATGTGCTGACCCAAAGG-3′ R: 5′-GGCTTTTCTCCACATGCTTAGG-3′	C Allele: 5′-FAM-CATCATGCCCCA**C**TG-MGB-3′ A allele: 5′-HEX-CATCATGCCCCA**A**TG-MGB-3′
BMPR1B	rs1434536	F: 5′-TCCTTCTGGGAGCTTCTCTGTCT-3′ R: 5′-TGCTTCCCAGTGGGTTCAG-3′	G allele: 5′-FAM-CTCCCTGA**G**AGGTGA-MGB-3′ A allele: 5′-HEX-CTCCCTGA**A**AGGTGA-MGB-3′
BMPR1B	rs17023107	F: 5′-CCGCCTGTCTAGGACCACAT-3′ R: 5′-CATATTGCCTGAAGCTGCAAAA-3′	T Allele: 5′-FAM-TGGAATTCCTG**T**AGGTGG-MGB-3′ C Allele: 5′-HEX-TGGAATTCCTG**C**AGGTG-MGB-3′

Bold represents mutation sites. SNPs, single-nucleotide polymorphisms.

Table 2. Distribution of selected variables between cases and controls

Variables	Cases ($n = 454$)		Controls ($n = 448$)		p value
	n	%	n	%	
Age, median (IQR), years	15.0 (11.0–26.0)		15.5 (9.9–29.0)		0.890
Gender					
Male	304	67.0	277	61.8	0.108
Female	150	33.0	171	38.2	
Concomitant asthma[a]					
Yes	105	23.1			
No	272	59.9			
Serum total IgE, median (IQR), kU/L	284.0 (128.5–594.2)		25.0 (10.4–47.4)		**<0.001**
Allergen-specific IgE, median (IQR), kU_A/L					
Dermatophagoides pteronyssinus	29.7 (6.6–72.3)				
Dermatophagoides farinae	24.6 (6.2–66.9)				

Bold represents statistical significance. IQR, interquartile range. [a] Information of concomitant asthma was unavailable in some cases.

trocles [28], PolymiRTS [29], and microRNA SNP [30], based on different biological parameters, empowered the identification of SNPs inside 3′-UTR that could potentially alter microRNA binding (referred to as target SNPs). Related documents published in MEDLINE/PubMed databases from January 2008 were reviewed, and 30 target SNPs were found. Consequently, 3 target SNPs in *TGFBR1* (rs1590) and *BMPR1B* (rs1434536 and rs17023107) that may affect the binding affinity of microRNA::mRNA constituted the selection set to be genotyped. The primers and TaqMan probes are shown in Table 1.

DNA Extraction and Genotyping

Genomic DNA was purified from peripheral blood leukocytes using a commercial kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions and stored at −70°C until usage. Genotyping was performed with the TaqMan SNP Genotyping Assay using the 384-well ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the following protocol: 50°C for 2 min, 95°C for 10 min followed by 40–45 cycles of 95°C for 15 s, and 60°C for 1 min. For the TaqMan assay, both PCR primers and MGB TaqMan probes are shown in Table 1. The genotype analysis was performed by 2 persons independently blinded to the study. More than 15% of the samples were randomly selected for confirmation, and the discordance rate between genotypes was below 0.3%. All SNPs were in Hardy-Weinberg equilibrium (HWE).

Statistical Analysis

Demographic characteristics were compared by Student's t test (for continuous variables) and χ^2 test (for categorical variables). Departure from HWE proportions of each SNP was tested by a goodness-of-fit χ^2 test among controls. The abnormally distributed values were described by quartiles. Unconditional logistic regression analysis was performed to evaluate the effect of SNP genotypes on AR status, with age and sex as covariates; linear regression analysis was used to evaluate the effect of specific IgE level on AR outcome. Stratification analyses were performed by age, gender, asthma, and total IgE. $p < 0.05$ was considered statistically significant. The Bonferroni method was used for multiple comparisons. All tests were 2-sided and performed with Statistical Analysis System software (version 9.1.3; SAS Institute, Cary, NC, USA) and Stata 8.2 statistical package (StataCorp LP, College Station, TX, USA).

Table 3. Primary information of genotyped SNPs in microRNA target sites of TGF-β signaling pathway genes

Target gene	SNPs	Location	Base change	MAF			*p* value for HWE test
				HapMap	Case	Control	
TGFBR1	rs1590	3′UTR	T>G	0.439	0.459	0.444	0.611
BMPR1B	rs1434536	3′UTR	C>T	0.326	0.381	0.391	0.797
BMPR1B	rs17023107	3′UTR	C>T	0.067	0.037	0.041	0.838

SNPs, single-nucleotide polymorphisms; TGF-β, transforming growth factor beta; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

Table 4. Genotype and allele frequencies of *TGFBR1* and *BMPR1B* among cases and controls

Genotypes	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)[b, c]
	n	%	*n*	%		
TGFBR1						
rs1590	*n* = 453		*n* = 446			
TT	127	28.0	140	31.4	1.00	1.00
TG	236	52.1	215	48.2	1.21 (0.89–1.64)	1.21 (0.89–1.63)
GG	90	19.9	91	20.4	1.09 (0.75–1.59)	1.09 (0.75–1.60)
TG/GG	326	72.0	306	68.6	1.17 (0.88–1.56)	1.17 (0.88–1.56)
G allele[a]					1.06 (0.88–1.28)	1.07 (0.88–1.28)
BMPR1B						
rs1434536	*n* = 452		*n* = 442			
CC	183	40.5	165	37.3	1.00	1.00
CT	194	42.9	208	47.1	0.84 (0.63–1.21)	0.84 (0.63–1.21)
TT	75	16.6	69	15.6	0.98 (0.66–1.45)	0.99 (0.56–1.16)
CT/TT	269	59.5	277	62.7	0.88 (0.67–1.15)	0.88 (0.67–1.15)
T allele[a]					0.96 (0.79–1.16)	0.96 (0.79–1.16)
rs17023107	*n* = 454		*n* = 446			
CC	420	92.5	409	91.7	1.00	1.00
CT	34	7.5	37	8.3	0.90 (0.55–1.45)	0.92 (0.57–1.50)
TT	0	0.0	0	0.0	–	–
CT/TT	34	7.5	37	8.3	0.90 (0.55–1.45)	0.92 (0.57–1.50)
T allele[a]					0.90 (0.56–1.45)	0.92 (0.57–1.49)

[a] Additive model. [b] Adjusted for age and gender in logistic regression model. [c] Thousand times permutation test.

Results

Characteristics of the Subjects

The characteristics of the studied population are presented in Table 2. A total of 454 mite-sensitized AR patients showed a median age of 15.0 (IQR, 11.0–26.0) years, including 304 (67.0%) males and 150 (33.0%) females. The 448 healthy controls had a median age of 15.5 (IQR, 9.9–29.0) years and 277 males (61.8%) and 171 females (38.2%). There were no statistically differences in age or gender between 2 groups ($p > 0.05$).

Total serum IgE levels (284.0 [128.5–594.2] kU/L) in the patients were significantly higher than those in healthy controls (25.0 [10.4–47.4] kU/L) ($p < 0.001$). In AR patients, the serum levels of allergen-specific IgE against *Der p* and *Der f* were 29.7 (6.6–72.3) and 24.6 (6.2–66.9) kU$_A$/L, respectively.

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Information of the Polymorphisms

The primary information and allele frequencies observed are summarized in Table 3. All genotyped distributions of control subjects were consistent with HWE ($p > 0.05$). In addition, the minor allele frequency of all the 3 SNPs were in accordance with that reported in the HapMap database of CHB (Han Chinese in Beijing, China).

Association between Target SNPs and AR

As shown in Table 4, the genotypic and allele frequency analysis of the selected SNPs (rs1590 in *TGFBR1*; rs1434536, and rs17023107 in *BMPR1B*) were not significantly associated with the susceptibility to mite-sensitized AR.

Stratification Analysis in Subgroups

The results of stratification analysis are shown in Table 5. AR patients were divided into 4 subgroups by age, gender, presence of asthma, and total IgE levels. Compared with the wild-type TT genotype, the TG, GG, and TG/GG genotypes of rs1590 in *TGFBR1* exhibited a significantly increased risk of AR in the subgroup of males (TG: adjusted OR = 1.57, 95% CI = 1.08–2.31; GG: adjusted OR = 1.76, 95% CI = 1.09–2.86; TG/GG: adjusted OR = 1.62, 95% CI = 1.13–2.33). Interestingly, the homozygote GG genotype of rs1590 exhibited a significantly decreased risk of AR in the subgroup of females (GG: adjusted OR = 0.44, 95% CI = 0.23–0.83). As for the rs1434536 in *BMPR1B*, the CT genotype showed a significantly decreased risk of AR in the subgroup of patients without asthma (CT: adjusted OR = 0.70, 95% CI = 0.50–0.98). Compared with the wild-type CC genotype, the CT genotype of rs17023107 in *BMPR1B* exhibited a significantly decreased risk of AR in the subgroup of age <15 years (CT: adjusted OR = 0.37, 95% CI = 0.14–0.95) and also in the subgroup of males (CT: adjusted OR = 0.48, 95% CI = 0.25–0.95). All the significances remained obvious after a thousand times of permutation tests. However, none of these SNPs in the TGF-β pathway was correlated with the serum total IgE levels.

Interaction Study

The genotypes of rs1590, rs1434536, and rs17023107 were renamed as A1, A2, and A3, respectively. The multifactor dimensionality reduction software was used to assess the joint effects of the 3 SNPs in *TGFBR1* and *BMPR1B*. As presented in Table 6, no significant associations were detected between the 3 selected SNPs.

Table 5. Stratification analyses of *TGFBR1* and *BMPR1B* in subgroups

Variables	Subgroup[b]	rs1590 adjusted OR (95% CI)[c, d]			rs1434536 adjusted OR (95% CI)[c, d]			rs17023107 adjusted OR (95% CI)[c, d]		
		XY[e]	YY[e]	XY/YY[e]	XY[e]	YY[e]	XY/YY[e]	XY[e]	YY[e]	XY/YY[e]
Age	<15 years	1.34 (0.83–2.09)	1.25 (0.71–2.21)	1.31 (0.86–2.01)	0.89 (0.58–1.36)	1.04 (0.59–1.83)	0.93 (0.63–1.38)	**0.37 (0.14–0.95)**	–	**0.37 (0.14–0.95)**
	≥15 years	1.11 (0.73–1.68)	0.96 (0.57–1.60)	1.06 (0.72–1.57)	0.80 (0.54–1.19)	0.96 (0.56–1.65)	0.84 (0.58–1.22)	1.32 (0.72–2.42)	–	1.32 (0.72–2.42)
Gender	Male	**1.57 (1.08–2.31)**	**1.76 (1.09–2.86)**	**1.62 (1.13–2.33)**	0.89 (0.62–1.27)	0.91 (0.55–1.49)	0.89 (0.64–1.25)	**0.48 (0.25–0.95)**	–	**0.48 (0.25–0.95)**
	Female	0.71 (0.43–1.19)	**0.44 (0.23–0.83)**	0.62 (0.38–1.01)	0.74 (0.46–1.22)	1.12 (0.60–2.08)	0.84 (0.53–1.32)	1.96 (0.92–4.18)	–	1.96 (0.92–4.18)
Asthma	Yes	1.48 (0.89–2.46)	0.93 (0.47–1.84)	1.32 (0.81–2.15)	1.13 (0.70–1.83)	1.29 (0.69–2.43)	1.17 (0.74–1.84)	0.59 (0.22–1.55)	–	0.59 (0.22–1.55)
	No	1.10 (0.78–1.57)	1.04 (0.68–1.61)	1.09 (0.78–1.51)	**0.70 (0.50–0.98)**	0.88 (0.56–1.38)	0.75 (0.55–1.02)	1.23 (0.73–2.07)	–	1.23 (0.73–2.07)
IgE level[a]	Lower	1.16 (0.85–1.58)	1.02 (0.69–1.51)	1.12 (0.83–1.50)	0.78 (0.58–1.05)	0.95 (0.64–1.42)	0.82 (0.63–1.09)	1.03 (0.63–1.68)	–	1.03 (0.63–1.68)
	Higher	2.03 (0.89–4.63)	2.32 (0.91–5.91)	2.11 (0.96–4.67)	1.62 (0.79–3.34)	1.61 (0.63–4.14)	1.62 (0.81–3.23)	–	–	–

Bold represents statistical significance. [a] Lower: below the 90th percentile of logarithmic total IgE level; higher: above the 90th percentile of logarithmic total IgE level. [b] Controls were stratified accordingly when dividing cases into age and gender subgroups, but they were kept as a whole in the situation of the other 2 subgroups since the controls in the study had neither asthma nor higher total IgE level in the first place (see Table 1). [c] Adjusted for age and gender in the logistic regression model. [d] Thousand times permutation test. [e] XY, heterozygote genotype; YY, homozygote mutant genotype; XY/YY, heterozygote genotype/homozygote mutant genotype.
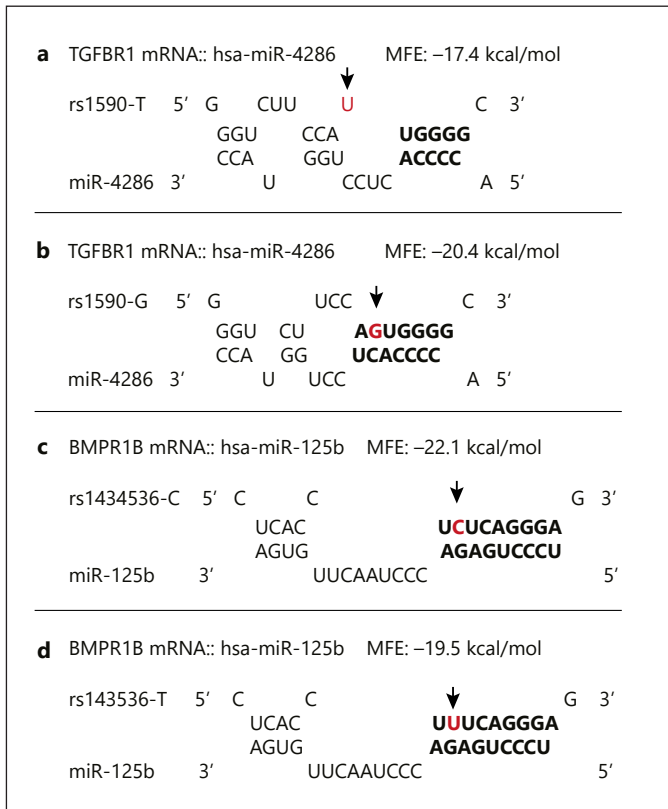
Fig. 1. Predicted binding of miR-4286 to *TGFBR1* (**a**, **b**) and miR-125b to *BMPR1B* (**c**, **d**). The seed region of the target site is shown in bold letter and every SNP is indicated by an arrow. The MFE of the RNA duplex was analyzed by RNA hybrid. MFE, minimum free energy.

Table 6. MDR models for locus-locus interactions

Model[a]	Training balance accuracy	Test balance accuracy	Cross-validation consistency	*p* value
A2	0.5253	0.4815	6/10	0.8281
A1 A2	0.5322	0.5004	10/10	0.6230
A1 A2 A3	0.5351	0.4832	10/10	0.9990

MDR, multifactor dimensionality reduction. [a] The genotypes of rs1590, rs1434536, and rs17023107 were renamed as A1, A2, and A3, respectively.

Discussion

The airway inflammation is regulated by a network of mutually interacting cytokines and effector cells in asthma or rhinitis that could be triggered by genetic and environmental factors [31]. TGF-β represents a master switch in inflammation and remodeling processes in both the upper and lower airways [32]. Experimental studies show that the effect of TGF-β is decided by its level, but also some regulatory proteins [33]. Ierodiakonou et al. [34] indicated that *TGFB1* SNPs could influence serum TGF-β1 serum level and asthma severity.

Genetic variants of TGF-β signaling pathway genes are implicated in the development of allergic disease, yet only a few studies have further investigated how these variants relate to specific dysfunction [35, 36]. MicroRNAs are dysregulated in various human diseases, such as cancer, rheumatoid arthritis, asthma, rhinosinusitis, and AR [37]. So far, none has delved into the roles of microRNA-related SNPs in AR. The present study evaluated the contribution of the polymorphisms inside microRNA target sites in 3′-UTR of TGF-β pathway to mite-sensitized AR susceptibility in a Han Chinese population. Using bioinformatic tools, we obtained *TGFBR1* rs1590T/G, *BMPR1B* rs1434536C/T, and rs17023107C/T as target SNPs that may modify (increase/decrease) the binding affinity of putative microRNA-mRNA duplexes.

TGFBR1 (56 kb in length and consisting of 9 exons) maps to 9q22.33. Functioning as the central propagator of TGF-β signaling, *TGFBR1* encodes a serine/threonine protein kinase receptor. We employed RNA hybrid [38] to model the targeting between specific microRNA and *TGFBR1* mRNA. According to the minimum free energy, miR-4286 was more stably bound to rs1590-G allele than to 1590-T allele (Fig. 1). These results are consistent with those in DNA microarray in the nasal mucosa, showing that attenuated TGF-β signaling may contribute to AR [39]. Polymorphisms in 3′-UTRs may decrease mRNA stability and interfere with its translation through disrupting microRNA-mRNA interaction and polyadenylation in protein-mRNA [40]. Moreover, allele-specific expression could reduce the expression of genes encoding *TGFBR1* in TG mutants [41]. Although subtle, the reduction in constitutive *TGFBR1* expression may alter Smad-mediated TGF-β signaling and AR pathophysiology. Collectively, these data point out that repressed TGF-β signaling contributes to AR risk. However, we cannot rule out the chance that TGF-β signaling is also enhanced in a critical developmental stage and/or cell type-specific manner.

In this study, we found lack of association between the *TGFBR1* and *BMPR1B* polymorphisms and the susceptibility of mite-sensitized AR in a Han Chinese population. However, it has been proposed that the effects of polymorphisms may rely on factors such as age, sex, and eth-

nicity. Compared with the wild-type TT genotype, the TG, GG, and TG/GG genotypes of *TGFBR1* rs1590 exhibited a significantly increased risk of AR in the subgroup of males. On the contrary, the GG genotype of rs1590 exhibited a significantly decreased risk of AR in the subgroup of females. Moreover, compared with the wild-type CC genotype, the CT genotype of *BMPR1B* rs17023107 exhibited a significantly decreased risk of AR in the subgroup of males. The significant association between the 2 SNPs and AR in subgroups of gender may result from the interaction between the genotypes and sexual hormones. It has been reported that estrogen can suppress the function of bone morphogenetic proteins (BMPs) by interfering with Smad transcriptional activity and by inhibiting *BMP* gene expression [42].

BMPs are a body of conserved signaling molecules from TGF-β superfamily [43]. BMPs can lead to tumorigenesis and regulate cancer progression in different stages [44]. In a mouse model of asthma, BMP receptors were activated upon allergen provocation in the airway epithelium [45], suggesting BMP signaling may be involved in the tissue repair and inflammatory processes. As shown in Figure 1, it suggested that replacing C allele by T allele reduced binding ability of miR-125b to *BMPR1B* mRNA. In the present study, rs1434536C/T in the *BMPR1B* gene showed no association with AR risk. However, in the subgroup of AR without asthma, we found a significant decreased risk of AR in the CT genotype of rs1434536. Compared with the wild-type genotype CC, the CT genotype of rs17023107 in *BMPR1B* exhibited a significantly decreased risk of AR in the subgroup of age <15 years old. The reason may be that the immune system of the adolescent has not fully developed.

IgE is an atopy-related quantitative trait; however, the underlying mechanisms remain incompletely defined [46]. TGF-β can downregulate the T helper 2 response to reduce IgE release. Serum total IgE and eosinophil cationic protein were found significantly associated with *TGFB1* rs1800469 [47]. In the present study, the *TGFBR1* genotypes did not show a significantly multiplicative interaction effect with *BMPR1B* genotypes for AR risk. That might be explained by the complexity of immune mechanism. In the bronchial inflammation of asthmatics, IgE synthesis is realized by several biological networks that could modulate the effects of TGF-β1 on IgE production [14].

Major strengths of this study include a stratified study design, a large sample size and a comprehensive analysis of TGF-β pathway genes. However, limitations should also be considered. First, only the Smad-dependent TGF-β pathway was evaluated. Second, the effect of environmental and genetic polymorphisms was ignored. Environmental influence is a crucial factor in the progression of allergic diseases. More research on gene-environment interaction is needed in the future.

In summary, our data suggested that in this Han Chinese population stratified by age and gender, the susceptibility of mite-sensitized AR may be associated with 2 SNPs (rs1590 in *TGFBR1* and rs17023107 in *BMPR1B*) inside microRNA target sites of TGF-β signaling pathway genes. Larger, better-designed studies are to be carried out using appropriate molecular and statistical methods to further analyze this functional association.

Statement of Ethics

This study has been reviewed and approved by the Ethics Committee of Nanjing Medical University (20080305).

Conflict of Interest Statement

None of the authors have a conflict of interest in relation to this work. The authors have no ethical conflicts to disclose.

Author Contributions

L.C., R.-X.C., and W.-M.L. mainly designed the study. R.-X.C., W.-M.L., M.-P.L., X.-J.Z., Z.-F.W., H.-Q.T., and L.-P.Z. collected and compiled the data. R.-X.C. and W.-M.L. performed the statistical analysis and drafted the manuscript. M.-L.W. and Z.-D.Z. provided the technical support for the experiment. All authors jointly discussed, reviewed, and amended the manuscript. All authors reviewed the final manuscript version and consented to its submission.

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