Association between Polymorphisms in Bitter Taste Receptor Genes and Clinical Features in Korean Asthmatics

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Key Words

Bitter taste receptor · Asthma · Single nucleotide polymorphism

Abstract

Background: Bitter taste receptors (TAS2R) in human airway smooth muscle have recently been shown to have an important role in bronchodilation, together with β_2 -adrenergic receptors. **Object:** To evaluate the association between genetic variations in *TAS2R* and clinical features, including bronchodilator response and asthma control. **Method:** We analyzed the association between single nucleotide polymorphisms (SNPs) of *TAS2R10* and *TAS2R14* and variables such as demographic data, atopy, duration of disease, and asthma control status, including variables such as asthma control test (ACT) score, percent predicted value of forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), and FEV₁/FVC ratio, as well as bronchodilator response (BDR), in 721 asthma patients in Korea. **Result:** Three novel SNPs of 633G>A, 645C>A, and –79G>A in *TAS2R10* and 3 known SNPs of –815T>C, –1267G>A, and –1897T>C in *TAS2R14* were analyzed. Increased BDR was significantly associated with SNPs of –815T>C [OR (95% CI) = 1.88 (1.01–3.49), $p = 0.04$] [J Gen Physiol 2005;125:535–553; Am J Respir Cell Mol Biol 2010;42:

373–3812], –1267A>G [OR (95% CI) = 2.07 (1.03–4.15), $p = 0.04$] and –1897T>C [OR (95% CI) = 3.05 (1.01–9.23), $p = 0.04$, in a dominant model, and OR = 1.91 (1.08–3.36), $p = 0.02$, in a codominant model] of the *TAS2R14* gene. There was a significant association between –815T>C and a low mean ACT score [OR (95% CI) = 5.84 (1.94–17.61), $p = 0.001$]. In haplotype analysis, TAC, CAT, and TGT, or TG and CA haplotypes on *TAS2R14* were significantly associated with increased BDR; CAT and CA haplotypes were significantly associated with a low ACT score. **Conclusion:** Genetic variations in *TAS2Rs* may be valuable genetic markers to predict therapeutic response and outcomes in asthma. Further research in an independent cohort is needed.

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Introduction

Asthma is a chronic inflammatory disorder characterized by variable airflow obstruction and hyperresponsiveness of bronchi to different stimuli [1–3]. Most asthma types can be well controlled by current asthma medication, including inhaled corticosteroid with or without long-acting β_2 -agonists, leukotriene antagonists, theophylline, tiotropium, and even omalizumab; however, approximate-

ly 5–10% of all patients fail to achieve control despite the availability of optimal asthma treatments [4], leading to a great burden on health costs [5]. Thus, there is an urgent need for new treatment approaches for asthma.

Bronchodilators are a very important asthma treatment modality that directly reverses airway obstruction [6], although inhaled corticosteroids are the mainstay of asthma treatment. Recently, it has become apparent that bronchoconstriction without additional inflammation induces airway remodeling in patients with asthma [7].

G protein-coupled receptors are known to regulate major signaling pathways in the airways that control bronchial relaxation and contraction [8]. Bitter taste receptors (TAS2Rs) are members of the G protein-coupled receptor family and have recently been discovered in airway epithelium and smooth muscle cells [9]. Bitter agonist-induced airway relaxation has been documented in animal studies [10, 11], and researches with human airway tissues also showed concordant results, although there is a difference in the magnitude of airway relaxation [9, 12–14]. Among subtypes of TAS2Rs, TAS2R5, 10, and 14 are considered to have a prime role in bronchodilation [15].

The exact signaling pathways of TAS2R-mediated relaxation are still unclear. Several explanations such as membrane hyperpolarization by the activation of large conductance calcium-activated potassium (K_{Ca}) channels, inhibition of L-type voltage-dependent calcium channels and inhibition of phosphatidylinositol-3-kinases are reported, although none of these pathways could fully explain the mechanism of TAS2R-induced bronchodilation [9, 12, 14]. However, these results imply the possibility that TAS2Rs are involved in airway relaxation via a different path than β_2 -adrenergic receptors (β_2 ARs), which increase intracellular cAMP leading to the activation of protein kinase A [8, 16]. Therefore, TAS2R agonists could be potent novel therapeutic agents for asthma.

Inhaled β_2 -agonists are the mainstay of asthma treatment and showed a heterogeneous response in individuals with asthma [17–19]. Genetic variation in the β_2 AR genes might be partly involved in these phenomena, and the number of studies have shown the relationship between various β_2 AR polymorphisms and the degree of responsiveness or desensitization to the β_2 -agonists and asthma severity [20–23].

Based on these results, we hypothesized that TAS2R polymorphisms may affect various clinical characteristics of asthma, just as genetic variations in β_2 ARs [24, 25]. The aim of this study was to evaluate the association between TAS2R polymorphisms and the response to bronchodilator and asthma control status in asthmatic patients.

Methods

Subjects and Study Design

The study subjects were recruited from the COREA (Cohort for Reality and Evolution of Adult Asthma in Korea) cohort. As described previously [26], the study participants were aged ≥ 14 years, with at least one typical asthma symptom, such as chronic cough, dyspnea, sputum or wheezing, for more than 3 months. Asthma was diagnosed based on airway hyperresponsiveness, defined by the provocation concentration of methacholine (≤ 16 mg/ml) that caused a decrease in forced expiratory volume in 1 s (FEV₁) of 20%, or airway reversibility, defined as improvement in FEV₁ of more than 200 ml and 12% of the percentage predicted after the administration of 180 μ g of albuterol by a metered-dose inhaler. This latter measure was used when the patients had a basal predicted FEV₁ of less than 60%. Subjects with destroyed lungs, bronchiectasis, or lung resection were excluded [26, 27].

A total of 721 subjects were eligible for genotyping. All study subjects were optimally managed according to the Global Initiative for Asthma (GINA) guidelines [3]. Demographic data, total immunoglobulin (Ig) E, results of the skin prick test, peripheral blood and sputum eosinophil counts at the time of enrolment into the COREA were obtained. Spirometric measurement, including pre- and post-bronchodilator FEV₁, forced vital capacity (FVC) values, FEV₁/FVC ratio and positivity of the bronchodilator response (BDR) test [28] at enrolment were recorded. If data from follow-up visits were available, we also recorded pulmonary function (prebronchodilator FEV₁, FVC values and FEV₁/FVC ratio) and Asthma Control Test (ACT) [29] score at 3 and 6 months after enrolment.

To measure the improvement of asthma control and pulmonary function after enrolment, we defined new variables: mean ACT score at 6 months; ACT variability during the 6-month period after enrolment; difficulty in treating asthma at 6 months, and FEV₁ improvement at 6 months. The mean ACT score during the 6-month period was the average of ACT scores at 3 and 6 months. Based on previous research which reported that ACT scores ≤ 19 indicate uncontrolled asthma, we divided the study subjects into two groups: the low mean ACT group (mean ACT score ≤ 19) and the high ACT group (mean ACT score > 19) [30–32]. Variability in the ACT score was defined as the difference between ACT scores at 3 and 6 months. Study subjects were divided into two groups based on this value: ACT variability ≥ 4 versus < 4 .

Patients who did not achieve an acceptable level of control at step 4 (using a reliever plus ≥ 2 controllers) treatment 6 months after enrolment were regarded as having difficult-to-treat asthma, according to the GINA guidelines [3]. When the best pre-bronchodilator FEV₁ at 6 months after enrolment was compared with baseline FEV₁, patients who showed no increase in pre-bronchodilator FEV₁ of > 200 ml and 12% were classified into the poor FEV₁ improvement group. To compare genetic variation status, we also included 337 nonasthmatic subjects, who agreed to participate in this study, from another cohort which is consistent with chronic idiopathic urticaria (CIU) patients in our center. All of these control subjects were excluded from the asthma patient group through an interview regarding their clinical symptoms.

All study subjects were fully informed of the aim of this study before enrolment, and informed consent was obtained from each

participant. If study subjects were children, written informed consent was obtained from their parents. The study protocols were approved by the Internal Review Board and Ethics Committee of Asan Medical Center. This study was performed in 2012.

Genotyping and Selection of Single Nucleotide Polymorphisms

DNA was extracted from blood samples collected in ethylenediaminetetraacetic acid using the Wizard Genomic DNA Purification Kit (Promega Co., Madison, Wis., USA) according to the manufacturer's recommendation. After the entire sequencing of the *TAS2R10* and *TAS2R14* genes in 24 healthy controls, candidate single nucleotide polymorphisms (SNPs) were selected by the levels of the linkage disequilibrium (LD) statistic. Due to the low levels of LD ($r^2 < 0.001$) among SNPs, 3 SNPs (−79G>A, 633G>A, and 645C>A) with a minor allele frequency (MAF) $\geq 1\%$ were selected in the *TAS2R10* gene. In case of *TAS2R14*, all polymorphisms with an MAF $\geq 5\%$ were included, and we selected 3 SNPs (−815T>C, −1897T>C, and −1267A>G) using the Tagger program within Haploview ($r^2 > 0.8$).

The TaqMan assay was performed according to the manufacturer's instructions (ABI, Foster City, Calif., USA). The SNaPshot assay was performed according to the manufacturer's instructions (ABI PRISM SNaPshot Multiplex kit, Foster City, Calif., USA). Analysis was carried out using the Genemapper software (version 4.0; Applied Biosystems, San Francisco, Calif., USA).

Statistical Analysis

SNPs and haplotypes were analyzed as 3 component variables (i.e., AA, AB, and BB; A is the major frequency allele or haplotype and B is the minor frequency allele or haplotype). Haplotype analysis was conducted using the Haploview program (version 4.2). To determine whether the effect of any single homozygous genotype differed from the common effects of the heterozygous genotype or another homozygote (i.e., the dominant model, AA vs. AB + BB; the recessive model, BB vs. AB + AA, or the codominant model, AA vs. AB vs. BB), a 2×2 contingency table was created. The χ^2 test for categorical variables and Student's *t* test or ANOVA with Scheffé's posttest for continuous variables were conducted. Mann-Whitney and Kruskal-Wallis tests were used as nonparametric methods. To determine the odds ratios (ORs) and 95% confidence intervals (CIs), multiple logistic regression modelling was performed. All analyses were adjusted for age, gender and body mass index (BMI). Hardy-Weinberg equilibrium was analyzed using the permutation test. All statistical analyses were performed using SAS software (version 9.1.3, Cary, N.C., USA). *p* values < 0.05 were regarded as significant.

Results

Clinical Characteristics of Subjects

Of the 721 patients, 304 (42.2%) were male and the mean age was 54.5 years. Due to the availability of the blood sample volume, patients under the age of 19 years could not be included in the current study. More than half of the patients (371, 51.5%) were never-smokers. The average baseline prebronchodilator FEV₁ was 2.18 liters

Table 1. Clinical characteristics of 721 asthma subjects

Characteristic	Asthma subjects in the current study (n = 721)
Male sex	304 (42.2) ⁱ
Age, years	54.47 ± 42.46 ^a
BMI	24.01 ± 3.4 ^a
Smoking status	102/231/371
(current/ex-/never-smoker)	(14.1/32/51.5) ⁱ
Atopy ^c	214 (29.7) ⁱ
Rhinosinusitis	510 (70.7) ⁱ
Duration of disease, years	13.1 ± 12.3 ^a
Duration of treatment, years	6.5 ± 6.2 ^a
Eosinophils, blood, % (n = 612)	3.8 (0–28.4) ^b
Eosinophils, sputum, % (n = 75)	3.0 (0–96) ^b
Total IgE, serum, IU/ml (n = 516)	178 (2–4,156) ^b
Baseline lung function (n = 721)	
FVC, liters	3.1 ± 1.2 ^a
FVC ^d , %	88.9 ± 18.4 ^a
FEV ₁ , liters	2.18 ± 0.82 ^a
FEV ₁ ^d , %	75.6 ± 19.6 ^a
Bronchodilator response (n = 311)	41 (13.2) ⁱ
3-month follow-up after enrolment (n = 494)	
FVC, liters	3.2 ± 0.9 ^a
FVC ^d , %	90.03 ± 17.3 ^a
FEV ₁ , liters	2.36 ± 0.32 ^a
FEV ₁ ^d , %	81.02 ± 18.2 ^a
6-month follow-up after enrolment (n = 432)	
FVC, liters	3.24 ± 0.96 ^a
FVC ^d , %	92.4 ± 17.8 ^a
FEV ₁ , liters	2.87 ± 9.6 ^a
FEV ₁ ^d , %	82.4 ± 18.9 ^a
Mean ACT ^e score at 6 months	22.1 ± 3.3 ^a
ACT variability at 6 months ^f (n = 296)	62 (20.9) ⁱ
Difficult-to-treat asthma at 6 months ^g (n = 338)	147 (43.5) ⁱ
Poor FEV ₁ improvement at 6 months ^h (n = 434)	298 (68.9) ⁱ

^a Mean ± SD. ^b Median (range). ^c Positive skin test response to one or more common allergens. ^d Percentage predicted. ^e Total number and percentage of patients who showed positive bronchodilator response. ^f The difference between ACT scores at 3 and 6 months (shown as the number of subjects who showed ≥ 4 ACT variability only). ^g Patients who did not achieve control status at step 4 (reliever + ≥ 2 controllers) treatment at 6 months after enrolment (shown as the number of subjects who did not achieve control status). ^h When the best prebronchodilator FEV₁ during 6 months after enrolment was compared with baseline FEV₁, patients whose prebronchodilator FEV₁ increased by < 200 ml and 12% were classified as poor FEV₁ improvement group. ⁱ Number of patients (%).

Table 2. Haplotype frequency in *TAS2R10* and *TAS2R14* genes

<i>TAS2R10</i>	633G>A	645C>A	-79G>A	Frequency, %
Ht1	G	C	A	96.8

<i>TAS2R14</i>	-815T>C	-1267A>G	-1897T>C	Frequency, %
Ht2	T	A	C	45.8
Ht3	C	A	T	23.9
Ht4	T	G	T	20.2
Ht5	T	G	C	6.4
Ht6	T	A		49.7
Ht7	T	G		26.5
Ht8	C	A		23.8

(75.6% predicted), and the follow-up FEV₁ values at 3 and 6 months were 2.36 liters (81.02%) and 2.87 liters (82.4%), respectively. The mean ACT score at 6 months was 22.1, and the number of patients showing poor FEV₁ improvement at 6 months was 298 (68.9%; table 1). Controls showed a similar gender distribution (131, 37.6%), but the mean age was lower than that of asthmatic patients (54.5 vs. 47.8 years, data not shown).

In the subgroup analysis, we compared the baseline characteristics depending on the ACT score (low ACT vs. high ACT group). Except for the lung function 3 and 6 months after enrolment, most of the baseline characteristics, including age, gender and laboratory findings, did not show any significant difference (online suppl. table 1; see www.karger.com/doi/10.1159/000443796 for all online suppl. material).

SNP Identification in the *TAS2R* Genes

Because the *TAS2R10* and *TAS2R14* subtypes are the most highly expressed in human airway smooth muscle [9], we sought to sequence these genes. After sequencing the *TAS2R10* gene, 15 SNPs were identified. Of these, 3 SNPs (-79G>A, 633G>A, and 645C>A) were newly discovered SNPs with a >0.1% MAF. We selected all 3 SNPs as tagging SNPs by considering the level of LD (online suppl. table 2). Twenty-three SNPs were identified through sequencing of the *TAS2R14* gene. Three of these SNPs (-815T>C, -1267A>G, and -1897T>C) had a >10% MAF and were selected as tagging SNPs (online suppl. table 3). Eight haplotypes were reconstructed on both genes [*TAS2R10*: Ht1 (GCG); *TAS2R14*: Ht2 (TAC), Ht3 (CAT), Ht4 (TGT), Ht5 (TGC), Ht6 (TA), Ht7 (TG), Ht8 (CA); table 2].

Table 3. Frequency of *TAS2R* genotypes in asthmatic patients and healthy controls

Genotype	Controls (n = 337)	Asthmatics (n = 721)	p value
-79G>A			
GG	335 (99.7%)	705 (99.3%)	n.s. ^a
GA	2 (0.3%)	5 (0.7%)	
AA	0	0	
645C>A			
CC	331 (98.2%)	710 (99%)	n.s. ^a
CA	6 (1.8%)	7 (1%)	
AA	0	0	
633G>A			
GG	322 (95.8%)	681 (95.1%)	n.s.
GA	13 (3.9%)	35 (4.9%)	
AA	1 (0.3%)	0	
-815T>C			
TT	201 (60.7%)	414 (57.7%)	n.s.
TC	112 (33.8%)	265 (37%)	
CC	18 (5.4%)	38 (5.3%)	
-1267A>G			
AA	188 (56%)	395 (55.2%)	n.s.
AG	124 (36.9%)	261 (36.5%)	
GG	24 (7.1%)	59 (8.3%)	
-1897T>C			
CC	98 (29.2%)	207 (29%)	n.s.
CT	173 (51.5%)	330 (46.2%)	
TT	65 (19.3%)	177 (24.8%)	
Haplotypes			
Ht1 (GCG)	335 (99.4%)	707 (98.1%)	n.s.
Ht2 (TAC)	247 (73.2%)	489 (67.8%)	0.04 ^b
Ht3 (CAT)	130 (38.5%)	301 (41.7%)	n.s.
Ht4 (TGT)	119 (35.3%)	253 (35.1%)	n.s.
Ht5 (TGC)	35 (10.4%)	86 (11.9%)	n.s.
Ht6 (TA)	254 (75.3%)	521 (72.2%)	n.s.
Ht7 (TG)	147 (43.6%)	320 (44.3%)	n.s.
Ht8 (CA)	130 (38.5%)	302 (41.8%)	n.s.

n.s. = Not significant. -79G>A, 645C>A, and 633G>A were from *TAS2R10*; -815C>T, -1267A>G, and -1897T>C were from *TAS2R14*. ^a χ^2 test, $p > 0.05$. ^b Determined using a dominant model (TAC/TAC + TAC/others vs. others/others) and the χ^2 test.

Comparison of Genotype Frequency of *TAS2R* SNPs between Asthma Patients and Healthy Controls

When we compared the genotype frequency of *TAS2R* SNPs between 721 asthmatics and 337 nonasthmatic adult controls, there were no significant differences in the frequency of the 6 tagging SNPs (-79G>A, 633G>A, 645C>A, -815T>C, -1267A>G, and -1897T>C) between

Table 4. Genetic effect of SNPs in *TAS2R10* on airway reversibility and asthma control status

		BDR positive ^c	Mean ACT score during 6 months ^d	ACT variability during 6 months ^e	Difficult-to-treat asthma at 6 months ^f	Poor FEV ₁ improvement at 6 months ^g
-79G>A						
Dominant ^a	p value ^b	0.99	0.18	0.99	0.98	0.96
	OR (95% CI)	0	6.58 (0.4, 116.69)	0	0	1.07 (0.06, 17.9)
Recessive ^a	p value	n.a.	n.a.	n.a.	n.a.	n.a.
	OR (95% CI)					
Codominant ^a	p value	0.99	0.18	0.99	0.98	0.96
	OR (95% CI)	0	6.58 (0.4, 116.69)	0	0	1.07 (0.06, 17.9)
645C>A						
Dominant	p value	0.45	0.98	0.72	0.21	0.91
	OR (95% CI)	2.17 (0.21, 32.42)	0	1.5 (0.15, 15.16)	3.89 (0.4, 37.78)	1.14 (0.09, 13.35)
Recessive	p value	n.a.	n.a.	n.a.	n.a.	n.a.
	OR (95% CI)					
Codominant	p value	0.45	0.98	0.83	0.16	0.92
	OR (95% CI)	2.62 (0.21, 32.45)	0	1.283 (0.13, 12.56)	5.19 (0.52, 52.1)	1.09 (0.23, 5.13)
633G>A						
Dominant	p value	0.97	0.97	0.79	0.78	0.68
	OR (95% CI)	0	1.02 (0.21, 4.97)	1.2 (0.31, 4.71)	1.15 (0.43, 3.06)	1.27 (0.4, 3.98)
Recessive	p value	n.a.	n.a.	n.a.	n.a.	n.a.
	OR (95% CI)					
Codominant	p value	0.98	0.92	0.69	0.74	0.7
	OR (95% CI)	0	1.086 (0.23, 5.13)	1.316 (0.35, 5.02)	1.18 (0.44, 3.17)	1.25 (0.4, 3.94)

n.a. = Not assessed. ^a Models of the minor allele (dominant: AA vs. AB + BB, recessive: AA + AB vs. BB, codominant: AA vs. AB vs. BB). ^b p value from multiple logistic regression analysis after adjusting for age, gender and BMI. ^c Control (reference): negative BDR; case: positive BDR. ^d Control (reference): the average of ACT scores at 3 and 6 months >19; case: ≤19. ^e Control (reference): the difference between ACT scores at 3 and 6 months ≥4; case: <4. ^f Control (reference): patients who achieved control status at step 4 (reliever + ≥2 controllers) treatment at 6 months after enrolment; case: patients who did not achieve control status. ^g Control (reference): the increase in the best prebronchodilator FEV₁ value during 6 months after enrolment ≥200 ml and 12% predicted; case: <200 ml and 12% predicted.

the two groups. The only difference was with Ht2 (TAC), which had a significantly lower frequency in asthmatic patients in a dominant model (table 3).

Association between TAS2R SNPs and Haplotypes and Clinical Characteristics of Asthma

We first evaluated the association between genetic polymorphisms in *TAS2R* and baseline demographic and laboratory characteristics including the duration of disease, the duration of treatment, the proportion of positive skin prick tests, the total serum IgE level and sputum eosinophil counts. We found no significant associations between *TAS2R* SNPs and those parameters (online suppl. tables 4 and 5).

When we analyzed the relationship between genetic variations in the *TAS2R* gene and airway reversibility, we found no significant association between a positive BDR and *TAS2R10* SNPs (table 4). However, a positive BDR

was significantly associated with -815T>C [OR (95% CI) = 1.88 (1.01–3.49), *p* = 0.04, in a codominant model], -1267A>G [OR (95% CI) = 2.07 (1.03–4.15), *p* = 0.04, in a dominant model] and -1897T>C [OR (95% CI) = 3.05 (1.01–9.23), *p* = 0.04, in a dominant model and OR = 1.91 (1.08–3.36), *p* = 0.02, in a codominant model] in the *TAS2R14* gene (table 5).

When we analyzed the relationship between genetic variations in the *TAS2R* gene and the mean ACT score during the 6-month period after enrolment, we found a significant association between -815T>C in the *TAS2R14* gene and a low mean ACT score [OR (95% CI) = 5.84 (1.94–17.61), *p* = 0.001, in a recessive model; table 5]. However, there were no statistically significant associations between *TAS2R* SNPs and other variables, including ACT variability, difficult-to-treat asthma and poor FEV₁ improvement during the 6-month follow-up (tables 4, 5).

Table 5. Genetic effect of SNPs in *TAS2R14* on airway reversibility and asthma control status

		BDR positive ^c	Mean ACT score during 6 months ^d	ACT variability during 6 months ^e	Difficult-to-treat asthma at 6 months ^f	Poor FEV ₁ improvement at 6 months ^g
-815T>C						
Dominant ^a	p value ^b	0.06	0.8	0.3	0.6	0.1
	OR (95% CI)	2.16 (0.96, 4.82)	0.93 (0.48, 1.75)	0.74 (0.41, 1.35)	1.09 (0.7, 1.72)	1.49 (0.88, 2.49)
Recessive ^a	p value	0.2	0.001	0.1	0.4	0.5
	OR (95% CI)	2.39 (0.58, 9.8)	5.84 (1.94, 7.61)	2.33 (0.74, 7.30)	1.55 (0.54, 4.46)	1.38 (0.44, 4.34)
Codominant ^a	p value	0.04	0.3	0.7	0.5	0.1
	OR (95% CI)	1.88 (1.01, 3.49)	1.33 (0.79, 2.23)	0.93 (0.56, 1.54)	1.13 (0.77, 1.65)	1.37 (0.89, 2.11)
-1267A>G						
Dominant	p value	0.04	0.5	0.7	0.8	0.2
	OR (95% CI)	2.07 (1.03, 4.15)	0.829 (0.43, 1.58)	1.12 (0.62, 2.02)	1.05 (0.67, 1.63)	0.71 (0.42, 1.2)
Recessive	p value	0.2	0.2	0.8	0.1	0.2
	OR (95% CI)	0.29 (0.04, 2.26)	0.411 (0.09, 1.81)	1.08 (0.38, 3.04)	1.78 (0.83, 3.8)	0.54 (0.20, 1.47)
Codominant	p value	0.2	0.3	0.7	0.4	0.1
	OR (95% CI)	1.34 (0.79, 2.24)	0.782 (0.47, 1.3)	1.08 (0.69, 1.68)	1.14 (0.82, 1.6)	0.73 (0.49, 1.1)
-1897T>C						
Dominant	p value	0.04	0.3	0.9	0.4	0.9
	OR (95% CI)	3.05 (1.01, 9.23)	0.745 (0.38, 1.45)	0.99 (0.53, 1.85)	1.24 (0.76, 2.03)	0.98 (0.56, 1.69)
Recessive	p value	0.09	0.9	0.9	0.5	0.7
	OR (95% CI)	2.08 (0.88, 4.87)	1.011 (0.49, 2.08)	0.96 (0.48, 1.89)	1.17 (0.69, 1.97)	1.12 (0.61, 2.05)
Codominant	p value	0.02	0.6	0.9	0.4	0.8
	OR (95% CI)	1.91 (1.08, 3.36)	0.893 (0.58, 1.37)	0.98 (0.66, 1.46)	1.15 (0.84, 1.56)	1.03 (0.72, 1.45)

^a Models of the minor allele (dominant: AA vs. AB + BB, recessive: AA + AB vs. BB, codominant: AA vs. AB vs. BB). ^b p value from multiple logistic regression analysis after adjusting age, gender and BMI. ^c Control (reference): negative BDR; case: positive BDR. ^d Control (reference): the average of ACT scores at 3 and 6 months >19; case: ≤19. ^e Control (reference): the difference between ACT scores at 3 and 6 months ≥4; case: <4. ^f Control (reference): patients who achieved control status at step 4 (reliever + ≥2 controllers) treatment at 6 months after enrolment; case: patients who did not achieve control status. ^g Control (reference): the increase in the best prebronchodilator FEV₁ value during 6 months after enrolment ≥200 ml and 12% predicted; case: <200 ml and 12% predicted.

Haplotype analysis demonstrated that haplotypes Ht3 (CAT), Ht4 (TGT), Ht7 (TG) and Ht8 (CA) of *TAS2R14* were significantly more common in patients with a positive BDR. By contrast, patients with Ht2 (TAC) and Ht6 (TA) of *TAS2R14* had a reduced frequency of positive BDR. In addition, Ht3 (CAT) and Ht8 (CA) were significantly associated with a low mean ACT score during the 6 months after enrolment. We found no association between haplotypes of *TAS2R* and other variables (table 6).

The relationship between genetic variations of the *TAS2R* gene and serial spirometry findings, including FVC and FEV₁ at the time of enrolment and after 3 and 6 months, was also analyzed. FVC and FEV₁ at 3 months were associated with 633G>A in *TAS2R10* (online suppl. table 6A, B).

Discussion

Bitter taste receptors are extensively expressed from the tongue to the intestinal mucosa and the pancreas [33–35]. They protect against the ingestion of potentially toxic substances by producing nausea and vomiting or by interrupting gastric emptying, leading to a delayed delivery of the toxin to the gastrointestinal system [36, 37]. In recent years, the expression of bitter taste receptors has been found in extraoral tissues, including respiratory epithelia [38] and airway smooth muscle cells [39]. A number of *TAS2R* subtypes were confirmed to be expressed in human bronchi and among them, *TAS2R5*, 10 and 14 are supposed to be mainly involved in the relaxation of these tissues. In the current study, we analyzed the genetic variation in *TAS2R10* and 14 [15].

Although it was not as marked as initially reported by Deshpande et al. [9], several recent studies with human

Table 6. Genetic effect of haplotypes in *TAS2R* on airway reversibility and asthma control status

		BDR positive ^c	Mean ACT score during 6 months ^d	ACT variability during 6 months ^e	Difficult-to-treat asthma at 6 months ^f	Poor FEV ₁ improvement at 6 months ^g
Ht1 (GCG)						
Dominant ^a	p value ^b OR (95% CI)	n.a.	n.a.	n.a.	n.a.	n.a.
Recessive ^a	p value OR (95% CI)	0.5 1.98 (0.24, 15.71)	0.9 0.94 (0.25, 3.46)	0.8 0.89 (0.27, 2.89)	0.8 0.93 (0.39, 2.23)	0.6 0.81 (0.3, 2.17)
Codominant ^a	p value OR (95% CI)	0.5 1.98 (0.24, 15.75)	0.9 0.94 (0.25, 3.46)	0.8 0.89 (0.27, 2.89)	0.8 0.93 (0.39, 2.23)	0.6 0.81 (0.3, 2.17)
Ht2 (TAC)						
Dominant	p value OR (95% CI)	0.01 0.37 (0.16, 0.84)	0.9 0.99 (0.5, 1.98)	0.7 0.89 (0.47, 1.68)	0.6 0.9 (0.55, 1.46)	0.7 1.08 (0.61, 1.92)
Recessive	p value OR (95% CI)	0.1 0.37 (0.11, 1.29)	0.5 1.27 (0.62, 2.58)	0.7 0.89 (0.44, 1.78)	0.5 0.85 (0.51, 1.44)	0.6 1.16 (0.64, 2.11)
Codominant	p value OR (95% CI)	0.01 0.47 (0.25, 0.86)	0.6 1.08 (0.71, 1.67)	0.6 0.92 (0.61, 1.37)	0.5 0.91 (0.67, 1.23)	0.6 1.08 (0.76, 1.55)
Ht3 (CAT)						
Dominant	p value OR (95% CI)	0.06 2.13 (0.95, 4.78)	0.8 0.92 (0.48, 1.74)	0.3 0.73 (0.41, 1.34)	0.7 1.07 (0.68, 1.67)	0.1 1.46 (0.87, 2.46)
Recessive	p value OR (95% CI)	0.3 2.39 (0.58, 9.78)	<0.001 5.75 (1.91, 17.36)	0.2 2.31 (0.73, 7.2)	0.4 1.57 (0.54, 4.52)	0.6 1.38 (0.44, 4.32)
Codominant	p value OR (95% CI)	0.04 1.87 (1.01, 3.47)	0.2 1.32 (0.78, 2.21)	0.7 0.93 (0.56, 1.53)	0.5 1.11 (0.76, 1.62)	0.2 1.36 (0.88, 2.09)
Ht4 (TGT)						
Dominant	p value OR (95% CI)	0.03 2.14 (1.06, 4.28)	0.3 0.71 (0.35, 1.43)	0.8 0.94 (0.5, 1.74)	0.4 1.18 (0.74, 1.88)	0.4 0.81 (0.46, 1.41)
Recessive	p value OR (95% CI)	0.4 0.47 (0.06, 3.77)	0.2 0.25 (0.03, 1.95)	0.7 0.78 (0.22, 2.8)	0.07 2.25 (0.93, 5.45)	0.3 0.58 (0.17, 1.95)
Codominant	p value OR (95% CI)	0.1 1.506 (0.87, 2.6)	0.2 0.68 (0.38, 1.21)	0.7 0.92 (0.56, 1.51)	0.2 1.26 (0.88, 1.81)	0.3 0.81 (0.52, 1.26)
Ht5 (TGC)						
Dominant ^a	p value ^b OR (95% CI)	0.8 1.1 (0.34, 3.51)	0.4 1.52 (0.57, 4.04)	0.2 1.7 (0.69, 4.14)	0.3 0.72 (0.35, 1.49)	0.4 0.72 (0.33, 1.57)
Recessive ^a	p value OR (95% CI)	0.9 0	0.9 0	0.9 0	0.8 1.41 (0.08, 23.57)	0.9 0
Codominant ^a	p value OR (95% CI)	0.9 1.03 (0.34, 3.09)	0.4 1.4 (0.55, 3.55)	0.1 1.84 (0.81, 4.21)	0.4 0.77 (0.39, 1.51)	0.3 0.68 (0.33, 1.41)
Ht6 (TA)						
Dominant	p value OR (95% CI)	0.003 0.29 (0.12, 0.67)	0.5 0.78 (0.39, 1.57)	0.6 0.86 (0.45, 1.67)	0.4 0.82 (0.49, 1.36)	0.9 0.98 (0.53, 1.79)
Recessive	p value OR (95% CI)	0.05 0.28 (0.083, 1)	0.5 1.22 (0.61, 2.43)	0.8 1.06 (0.55, 2.04)	0.2 0.73 (0.44, 1.21)	0.7 1.07 (0.61, 1.91)
Codominant	p value OR (95% CI)	0.002 0.39 (0.21, 0.72)	0.9 0.98 (0.64, 1.52)	0.8 0.97 (0.65, 1.45)	0.2 0.82 (0.61, 1.12)	0.8 1.02 (0.71, 1.46)
HT7 (TG)						
Dominant	p value OR (95% CI)	0.01 2.88 (1.24, 6.68)	0.6 0.85 (0.44, 1.62)	0.6 1.14 (0.63, 2.07)	0.8 1.04 (0.66, 1.62)	0.2 0.72 (0.43, 1.22)
Recessive	p value OR (95% CI)	0.03 0.38 (0.05, 2.99)	0.2 0.42 (0.09, 1.86)	0.8 1.11 (0.39, 3.14)	0.1 1.76 (0.82, 3.77)	0.2 0.55 (0.2, 1.48)
Codominant	p value OR (95% CI)	0.1 1.57 (0.88, 2.81)	0.3 0.79 (0.47, 1.32)	0.7 1.1 (0.71, 1.72)	0.4 1.14 (0.81, 1.59)	0.1 0.74 (0.49, 1.11)

Table 6 (continued)

		BDR positive ^c	Mean ACT score during 6 months ^d	ACT variability during 6 months ^e	Difficult-to-treat asthma at 6 months ^f	Poor FEV ₁ improvement at 6 months ^g
Ht8 (CA)						
Dominant	p value	0.06	0.7	0.3	0.7	0.1
	OR (95% CI)	2.13 (0.95, 4.78)	0.91 (0.48, 1.73)	0.73 (0.4, 1.33)	1.08 (0.69, 1.7)	1.46 (0.87, 2.46)
Recessive	p value	0.2	0.002	0.1	0.4	0.5
	OR (95% CI)	2.39 (0.58, 9.78)	5.78 (1.92, 17.45)	2.31 (0.74, 7.23)	1.54 (0.53, 4.44)	1.38 (0.44, 4.32)
Codominant	p value	0.04	0.2	0.7	0.5	0.2
	OR (95% CI)	1.87 (1.01, 3.48)	1.32 (0.78, 2.21)	0.92 (0.56, 1.52)	1.12 (0.76, 1.64)	1.36 (0.88, 2.09)

n.a. = Not assessed. ^a Models of Ht (dominant: Ht/Ht + Ht/others vs. others/others, recessive: Ht/Ht vs. Ht/others + others/others, codominant: Ht/Ht vs. Ht/others vs. others/others). ^b p value from multiple logistic regression analysis after adjusting for age, gender and BMI. ^c Control (reference): negative bronchodilator response (BDR); case: positive BDR. ^d Control (reference): the average of ACT scores at 3 and 6 months >19; case: ≤19. ^e Control (reference): the difference between ACT score at 3 and 6 months ≥4; case: <4. ^f Control (reference): patients who achieved control status at step 4 (reliever + ≥2 controllers) treatment at 6 months after enrolment; case: patients who did not achieve control status. ^g Control (reference): the increase in the best prebronchodilator FEV₁ value during 6 months after enrolment ≥200 ml and 12% predicted; case: <200 ml and 12% predicted.

airway tissues also demonstrated a TAS2R-mediated bronchodilator effect. Based on these data, TAS2R agonists are currently being considered as a new target for treatment of severe asthma.

To date, several studies reported the association between genetic variation in the *TAS2R* gene and specific diseases. A coding SNP, *rs846664* in the *TAS2R16* gene, is believed to exert an influence on the risk of alcohol dependence, especially in African Americans, and another research also noted a significant association between variation in the *TAS2R13* gene and alcohol consumption in patients with head and neck cancer [40–42]. In contrast, Campa et al. [43] reported that the common polymorphism of the *TAS2R14* gene was not a major risk factor in the development of colorectal cancer. However, to the best of our knowledge, this is the first study examining the relationship between genetic variation in bitter taste receptors and respiratory disease.

In the current study, the genetic variations –815T>C, –1267A>G and –1897T>C in *TAS2R14* were associated with a positive BDR, i.e., with airway reversibility. Moreover, there was a significant association between some haplotypes with minor alleles at –815T>C and –1267A>G [Ht3 (CAT), Ht4 (TGT), Ht7 (TG) and Ht8 (CA)] and a positive BDR. By >T, SNPs [Ht2 (TAC), Ht6 (TA)] demonstrated an inverse correlation with airway reversibility. Indeed, we consider that asthma medication or control state at enrolment might not affect the response to the BDR test because bronchodilators were stopped at least 3 days before we per-

formed the test. A possible explanation of these results is that TAS2R- and β_2 AR-induced bronchodilation might be sharing the mechanism of action, at least in part. Bronchodilation by TAS2R agonists has initially been supposed to be mediated by K_{Ca} channels, and later, Zhang et al. [14] suggested that TAS2R agonists induced relaxation in contracted airway smooth muscle cells by inhibiting L-type voltage-dependent calcium channels via G $\beta\gamma$ signaling pathways [9, 14]. However, several discrepancies were reported in these results [12, 13, 44]. Recently, Tan and Sanderson [46] have demonstrated that TAS2R agonists reversed bronchoconstriction by reducing the frequency of calcium oscillations and calcium sensitivity using mouse lung tissues. A similar mechanism has been reported in β_2 AR-induced bronchodilation as shown in previous research [2, 45, 46]. Taken together, the results in the current study implied that genetic variation in the *TAS2R* gene affects the airway reversibility by varying the degree of calcium oscillation and sensitivity of lung tissues. However, the crosstalk between β_2 AR and TAS2R remains poorly known, thus further study in this area is needed.

Interestingly, the C allele in –815T>C as an individual SNP, and haplotypes including the same allele [Ht2 (TAC) and Ht6 (TA)], were associated with a low mean ACT score during the 6-month period following enrolment, indicating that this genetic variation was associated with poorly controlled asthma as well as with a positive BDR response to a short-acting β_2 -agonist. Indeed, variability of airflow limitation is a principal characteristic of

asthma [47], and several studies have demonstrated that BDR correlates with airway inflammation leading to poor control of asthma [47–50]. Therefore, we can speculate that polymorphisms in *TAS2R* might affect therapeutic outcomes of asthma treatment by affecting airway reversibility, because the subjects involved in this study received optimal asthma treatment, including inhaled corticosteroids and bronchodilators, during the follow-up period.

There are some limitations in this study. First, we used a conventional gene-based analysis and a tagging approach, which is currently accepted as an ideal model for gene association studies [51]. However, the current study had a relatively small sample size, and we acknowledge that this point may act as a limitation. Nevertheless, previous studies have indicated that a sample size of 500 individuals is sufficient to discover causative polymorphisms [52]. Second, to assess the asthma control status of study subjects, we used an average ACT score at 3 and 6 months. Therefore, the current study did not fully reflect the status of the patients who changed from one group to the other (e.g. from the low ACT group at 3 months to the high ACT group at 6 months) during 3 months. To compensate for this, we have included another variable, i.e. ACT variability. However, this variable

also has a limit for tracing the changes in each individual. Further research with an increased sample size, including the trace of some variables which have a high variability, such as the ACT score, is needed to achieve greater statistical power. In addition, replication of our results in another dataset is also necessary.

In summary, the minor alleles in the –815T>C, –1267A>G and –1897T>C SNPs in *TAS2R14*, and haplotypes including these SNPs, were associated with airway reversibility. In particular, the minor allele in –815T>C has a strong association with poor asthma control. Our findings suggest that genetic variations in bitter taste receptors may be valuable genetic markers to predict therapeutic responses and outcome in asthma patients. A large population-based prospective study and a functional study are needed to fully understand the importance of bitter taste receptors in asthma.

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