

# Association between *TAAR6* polymorphisms and airway responsiveness to inhaled corticosteroids in asthmatic patients

Hun Soo Chang<sup>a</sup>, Jeong-Seok Heo<sup>a</sup>, Seung-Woo Shin<sup>b</sup>, Da-Jeong Bae<sup>a</sup>, Hyun Ji Song<sup>a</sup>, Ji Ae Jun<sup>a</sup>, Jeong Dong Kim<sup>a</sup>, Jong-Sook Park<sup>b,c</sup>, Byung Lae Park<sup>d</sup>, Hyung Doo Shin<sup>d,e</sup> and Choon-Sik Park<sup>b,c</sup>

**Background** Genetic polymorphisms may be responsible for the wide variation in response to inhaled corticosteroids in asthmatic patients. We had previously reported that one polymorphism rs7772821, located on the 3'-UTR of trace amine-associated receptor 6 (*TAAR6*), is significantly associated with percentile changes in the forced expiratory volume in 1 s (% $\Delta$ FEV<sub>1</sub>) after inhaled corticosteroid treatment in asthmatics using a genome-wide association study. The aim of the present study was to validate the association between 15 single-nucleotide polymorphisms (SNPs) on the *TAAR6* and airway responsiveness to inhaled corticosteroids in the asthmatics.

**Methods** The % $\Delta$ FEV<sub>1</sub> induced by 4 weeks' treatment with inhaled fluticasone propionate (1000  $\mu$ g daily) was measured in 246 asthmatics. The 15 SNPs of *TAAR6* were genotyped using a TaqMan assay. An association analysis between % $\Delta$ FEV<sub>1</sub> and *TAAR6* polymorphisms was carried out using a linear regression model controlling for age, sex, smoking status, presence of atopy, and baseline FEV<sub>1</sub> as covariates.

**Results** Among the 15 SNPs and seven haplotypes of *TAAR6*, rs7772821 (T > G) on the 3'-UTR showed the strongest correlation with inhaled corticosteroid-induced % $\Delta$ FEV<sub>1</sub> ( $P_{\text{corr}} = 0.002$  in the codominant model,  $P_{\text{corr}} = 0.03$  in the dominant model,  $P_{\text{corr}} = 0.01$  in the recessive model).

## Introduction

Asthma is a heterogeneous disease with subgroups defined by etiology, pathology, severity, physiologic parameters, and response to treatment [1]. Corticosteroids suppress almost every step of the inflammatory cascade. Widespread use of inhaled corticosteroids (ICSs) in particular has revolutionized the treatment of asthmatic symptoms, and these agents are a mainstay of therapy for patients with chronic asthma [2]. Recommendations for patients with moderate persistent asthma include either medium-dose or high-dose ICS therapy, or combination therapy with a low-dose ICS and a long-acting  $\beta_2$  agonist (LABA) [3]. Patients with severe persistent asthma are generally treated with a

The % $\Delta$ FEV<sub>1</sub> of the rs7772821T > G minor homozygotes (60.77%) was higher than that of patients harboring either the rs7772821 T/G or T/T genotypes (21.32 and 31.60%, respectively).

**Conclusion** The *TAAR6* rs7772821 polymorphism may be one of the important genetic factors for predicting the response to treatment with inhaled corticosteroids in asthmatics. *Pharmacogenetics and Genomics* 25:334–342 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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<sup>a</sup>Department of Medical Bioscience, Graduate School, Soonchunhyang University, Chungcheongnam-do, <sup>b</sup>Genome Research Center, Division for Allergy and Respiratory Diseases, <sup>c</sup>Department of Internal Medicine, Division of Allergy and Respiratory Medicine, Soonchunhyang University Bucheon Hospital, Gyeonggi Do, <sup>d</sup>Department of Genetic Epidemiology, SNP Genetics Inc. and <sup>e</sup>Department of Life Science, Sogang University, Seoul, Republic of Korea

Correspondence to Choon-Sik Park, MD, Department of Internal Medicine, Division of Allergy and Respiratory Medicine, Soonchunhyang University Bucheon Hospital, 1174, Jung Dong, Wonmi Ku, Bucheon, Gyeonggi Do 420-021, Republic of Korea  
Tel: + 82 32 621 5105; fax: + 82 32 621 5023; e-mail: mdcspark@hanmail.net

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medium-dose or high-dose ICS together with a LABA. Although these guidelines work well for most patients, the response to ICS therapy varies widely among individuals [4–6].

The wide variation is believed to result, in part, from differences in the underlying genetic backgrounds. However, direct evidence of genetic variations associated with ICS responsiveness remains limited. Glucocorticoid resistance is associated with single-nucleotide polymorphisms (SNPs) on human glucocorticoid receptor (GR) gene *NR3C1* [7] and corticotropin-releasing hormone receptor 1 [8]. The glucocorticoid resistance has also been found to be associated with SNPs on the genes outside of the glucocorticoid pathway: dual-specificity phosphatase 1 [9], *TBX21* [10], *WDR21A* [11], *eNOS G894T* [12], *ORMDL3* [13], *HDAC1* [14], and *ADRB2* [15]. Taken together, these

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data show a clear association between genetic variability and an individual's response to ICS; however, these mutations alone do not fully explain the diversity observed in individual patients.

To identify other biologically relevant polymorphisms associated with variation in ICS responsiveness, several genome-wide association studies (GWAS) have been carried out. In one study carried out in Caucasians, polymorphisms rs1558726, rs2388639, and rs10044254 were shown to be related to the improvement of asthma symptoms in response to ICS in childhood asthmatics, but not in adult patients [16]. Recently, we carried out a GWAS for percentile changes of the forced expiratory volume in 1 s (% $\Delta$ FEV<sub>1</sub>) following ICS treatment in adult asthmatics [17]. This analysis identified 50 SNPs in 38 genes to be associated significantly with % $\Delta$ FEV<sub>1</sub> ( $P < 0.0001$ ), including rs7772821, located in the 3'-UTR of trace amine-associated receptor 6 (TAAR6) ( $P = 6.08 \times 10^{-5}$ ), which is known as one of the trace amine-associated receptors.

Endogenous amine compounds, including p-tyramine, m-tyramine,  $\beta$ -phenylethylamine, tryptamine, octopamine, and *N,N*-dimethyltryptamine, are present in the mammalian nervous system in trace amounts [18]. The presence of these compounds has been linked to highly prevalent psychiatric disorders such as depression and schizophrenia [19]. Elsewhere in the body, they have been shown to affect airway constriction.  $\beta$ -Phenylethylamine induces contractions in guinea-pig lung parenchymal strips and bronchoconstriction of perfused lungs [20]. Tyramine elicits a dose-dependent contraction of the airway *in vivo* through the release of noradrenaline and its metabolites [21,22]. Tyramine is subsequently metabolized into octopamine, an adrenergic agent used as a sympathomimetic drug, which can then be packaged with norepinephrine in synaptic vesicles. Other degradation pathways for tyramine include conversion to the methylated alkaloid derivative *N*, *N*-dimethyltyramine [23]. Tryptamine is a monoamine alkaloid similar to the amino acid tryptophan, and can be found in trace amounts in the brains of mammals; it is believed to act both as a neuromodulator and as a neurotransmitter [24]. *N,N*-dimethyltryptamine is a naturally occurring psychedelic compound of the tryptamine family. Along with  $\beta$ -phenylethylamine, these compounds are known to increase serum concentrations of corticotropin and cortisol [25].

On the basis of the effects of trace amines on airway contraction, and their association with adrenocorticotrophic hormones and cortisol, genetic variation in *TAAR6* may be related to individual differences in ICS responsiveness. In the present study, we examined the association of 15 polymorphisms in *TAAR6* with airway responsiveness to ICS in asthmatic patients as a validation of our previous GWAS results [17].

## Methods

### Participants

This prospective controlled trial included 246 ethnic Korean patients with mild-to-severe persistent asthma recruited from the Asthma Genome Research Center, composed of three Korean University hospitals. Of the 246 asthmatics, 67 were newly enrolled patients and 179 were the participants of our previous GWAS [17]. All participants were examined by physicians before enrollment, and diagnosed as asthmatics according to the guidelines set forth by the Global Initiative for Asthma [3]. They had a history of dyspnea and wheezing during the 12 months before diagnosis and showed one or more of the following: (a) at least 15% or at least 12% and 200 ml increase in FEV<sub>1</sub> after inhalation of a short-acting bronchodilator or (b) a provocative concentration of methacholine causing a 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub>) less than 10 mg/ml. Exclusion criteria included a history of asthma duration less than 1 year, the occurrence of acute exacerbation within the previous 4 weeks, a positive skin reaction to pollens, the presence of parenchymal lung disease on chest radiography, use of inhaled or systemic corticosteroids within the previous 4 weeks, and maintenance with theophylline, leukotriene antagonists, or other add-on asthma therapies. An informed written consent to participate in the study was obtained from all study participants. The study protocol was approved by the Ethics Committees of the hospitals (IRB No. SCHBC\_IRB\_05\_02).

Upon the diagnosis of asthma, total immunoglobulin E levels were measured using the CAP fluorescent enzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). All participants were subjected to a skin prick test using 24 aeroallergens including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat and dog epithelium and hairs, cockroach, tree, grass, and weed pollens, *Aspergillus* spp., and *Alternaria* spp. (Bencard, Brentford, UK). Atopy was defined as a positive reaction to one or more of the allergens with weal size of at least 3 mm or a histamine control in diameter.

The participants started to maintain ICS therapy for 4 weeks, as described previously [4]. Participants were instructed to administer 1000- $\mu$ g fluticasone propionate daily through a multidose dry-powder inhaler (Diskhaler; GlaxoSmithKline; Research Triangle Park, North Carolina, USA), to record their symptom scores, and frequency of short-acting bronchodilators as needed, and to switch to a LABA combination ICS or add-on therapy in the event of an exacerbation (defined as a  $\geq 12\%$  reduction in FEV<sub>1</sub> with intolerable aggravation of asthma symptoms). The primary endpoint was the  $\Delta$ FEV<sub>1</sub> after the course of ICS treatment. FEV<sub>1</sub> values were obtained between 8:00 and 10:00 a.m. with no use of short-acting bronchodilators within the previous 8 h. The % $\Delta$ FEV<sub>1</sub> was calculated as [(final FEV<sub>1</sub> – baseline FEV<sub>1</sub>)/baseline FEV<sub>1</sub>]  $\times 100\%$ .

## Genotyping

Using data obtained from the International HapMap database and the National Center for Biotechnology Information (NCBI; build 36), 15 candidate SNPs were selected with a more than 5% minor allele frequency, high linkage disequilibrium (LD), and haplotype tagging status. To calculate LDs, the HapMap DB data for Han Chinese and Japanese populations were used; SNPs with almost absolute LDs ( $r^2 > 0.98$ ) were selected.

The Primer Express software (Applied Biosystems, Foster City, California, USA) was used to design amplifying primers and minor groove-binding TaqMan probes for each of the 15 polymorphic sites of *TAAR6*. For PCR, TaqMan universal master mix (Applied Biosystems) was used without uracyl DNA glycosylase, with a primer concentration of 900 nmol/l. The TaqMan minor groove-binding probe concentration was 200 nmol/l. Reactions were performed in a 384-well format in a total reaction volume of 5  $\mu$ l using 20 ng of genomic DNA. Plates were run on a PE 9700 thermal cycler (Applied Biosystems) under the following conditions: initial incubation at 50°C for 2 min, then 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Assay plates were then transferred to a Prism 7900HT instrument (Applied Biosystems) to measure the fluorescence intensity in each well. Fluorescence data files from each plate were analyzed using automated software (SDS 2.1, Applied Biosystems). For quality control purposes, PCR was run in duplicate for 10% of samples to ensure accuracy; the rate of concordance between duplicates was more than 99%.

## Statistical analysis

The LD between loci was measured as Lewontin's  $D'$  ( $ID'$ ) and  $r^2$  [26]. Haplotypes of each individual were inferred using an expectation-maximization algorithm (PHASE v. 2.0) developed by Stephens *et al.* [27]. *TAAR6* was parsed into two haplotype blocks using  $ID'$ . SNPs with more than 0.9  $ID'$  between adjacent SNPs were constructed in the same haplotype blocks. Haplotypic codes in the study participants were as follows: -- as C/C, -/ht as C/R, and ht/ht as R/R. Each haplotype was analyzed using the same methods described for SNP analysis. Analysis of  $\% \Delta FEV_1$  was carried out using a linear regression model with corresponding  $P$ -values after controlling for age, sex, smoking status (nonsmoker, ex-smoker, or smoker), presence of atopy, and baseline  $FEV_1$  as covariates. Statistical analyses were carried out using SAS version 9.1 (SAS Institute, Cary, North Carolina, USA). To correct for multiple testing, the effective number of independent markers was calculated using SNPSpD software (<http://genepi.qimr.edu.au/general/daleN/SNPSpD/>), which is based on the spectral decomposition of matrices of pair-wise LD values between SNPs [28]. The resulting number of independent marker loci in *TAAR6* with three alternative models

( $10.7411 \times 3 = 32.2233$ ) was applied to correct for multiple testing. In addition, a permutation test was used to evaluate the association results using PLINK (1 000 000 permutations).

## Results

### Characteristics of the study participants

This prospective controlled trial initially included 246 patients with moderate-to-severe persistent asthma (Table 1). All participants performed lung function tests at the beginning of the study to determine their baseline  $FEV_1$  and were instructed to administer an ICS (1000  $\mu$ g fluticasone propionate) daily for 4 weeks. Of the 246 participants enrolled in the trial, 230 completed the study. Among these, 211 patients (91.7%) did not experience symptom aggravation, whereas 19 participants (8.2%) added either LABA or other therapeutics at 1–2 weeks after the study because of symptom aggravation and decreases of 12% or more of  $FEV_1$ . In these cases,  $FEV_1$  at the time of exacerbation was considered the final  $FEV_1$ . The mean ratio of ICS compliance was 93.5%. The clinical characteristics of the study participants are summarized in Table 1. ICS treatment was associated with significant increases in the mean FVC and  $FEV_1$  values, although the final  $\% \Delta FEV_1$  varied considerably from –33 to 192%.

### Frequencies and heterozygosity of *TAAR6* SNPs in the study population

The minor allele frequencies of 15 *TAAR6* SNPs are summarized in Table 2. The LD coefficients ( $ID'$  and  $r^2$ ) among *TAAR6* SNPs were calculated for all study participants (Fig. 1a and c). Haplotypes were parsed into two blocks (Fig. 1b); seven haplotypes showing frequencies more than 0.05 (*block1-ht1*, *ht2*, *ht3*, *ht4*, and *block2-ht1*, *ht2*, *ht3*) were used for further analysis.

**Table 1 Clinical profiles of steroid response study participants**

| Description                                          | All participants     |
|------------------------------------------------------|----------------------|
| <i>N</i>                                             | 246                  |
| Age [median (range)] (years)                         | 50.75 (15–75)        |
| Onset of asthma, age [median (range)] (years)        | 40.94 (1–75)         |
| Sex (male/female)                                    | 110/136              |
| Current smoker/ex-smoker (%)                         | 17.89/24.39          |
| Positive rate of skin test (%)                       | 50.41                |
| Baseline FVC (% pred) <sup>a</sup>                   | 79.03 $\pm$ 14.12    |
| Baseline $FEV_1$ (% pred) <sup>a</sup>               | 76.04 $\pm$ 16.98    |
| FVC% pred at 4 weeks' treatment <sup>a</sup>         | 81.98 $\pm$ 15.15    |
| $FEV_1$ % pred at 4 weeks' treatment <sup>a</sup>    | 81.09 $\pm$ 18.72    |
| Change (%) of $FEV_1$ with treatment <sup>b</sup>    | 26.80 (–34 to 182.4) |
| Blood eosinophils (%) <sup>a</sup>                   | 6.28 $\pm$ 5.23      |
| PC <sub>20</sub> , methacholine (mg/ml) <sup>a</sup> | 5.13 $\pm$ 8.17      |
| Total IgE (IU/ml) <sup>a</sup>                       | 374.3 $\pm$ 583.1    |

$FEV_1$ %, percentile changes in the forced expiratory volume.

<sup>a</sup>Values are presented as mean  $\pm$  SE.

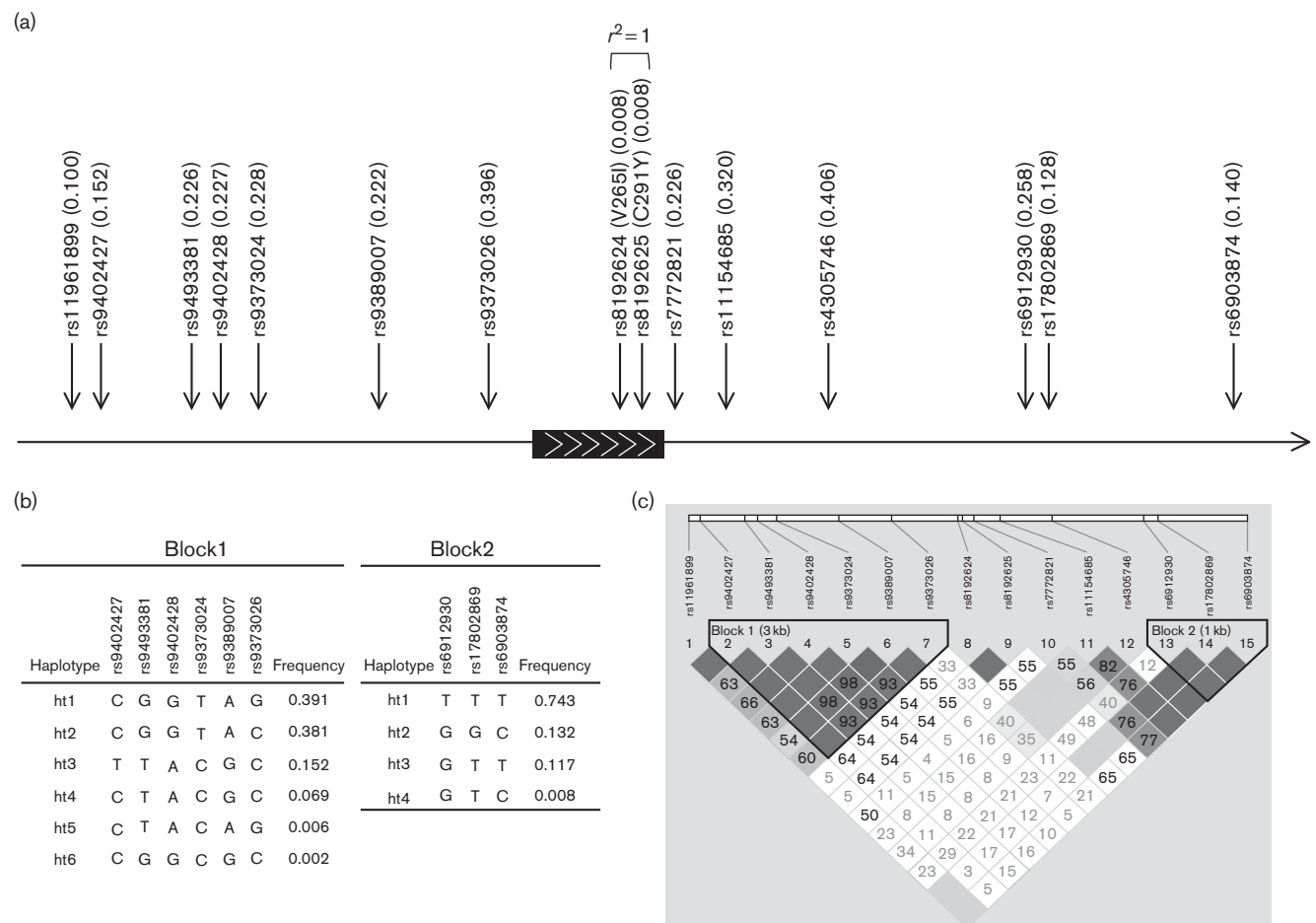
<sup>b</sup>Change (%) of  $FEV_1$  with treatment of inhaled corticosteroid for 4 weeks was calculated using the following formula:  $\% \Delta FEV_1 = [(FEV_1 \text{ at 4 weeks' treatment} - \text{baseline } FEV_1) / \text{baseline } FEV_1] \times 100\%$ .

**Table 2** Allele frequencies and Hardy–Weinberg equilibrium analysis of *TAAR6* polymorphisms in Korean ( $n = 246$ )

| SNP ID                  | Loci       | Alleles | Position | Genotype distribution |     |     | Total | MAF   | Heterozygosity | HWE   |
|-------------------------|------------|---------|----------|-----------------------|-----|-----|-------|-------|----------------|-------|
|                         |            |         |          | C/C                   | C/R | R/R |       |       |                |       |
| rs11961899 <sup>a</sup> | rs11961899 | A > G   | 5'       | 200                   | 39  | 5   | 244   | 0.100 | 0.181          | 0.072 |
| rs9402427               | rs9402427  | C > T   | 5'       | 178                   | 61  | 7   | 246   | 0.152 | 0.258          | 0.526 |
| rs9493381 <sup>a</sup>  | rs9493381  | G > T   | 5'       | 149                   | 83  | 14  | 246   | 0.226 | 0.349          | 0.589 |
| rs9402428               | rs9402428  | G > A   | 5'       | 148                   | 83  | 14  | 245   | 0.227 | 0.350          | 0.603 |
| rs9373024               | rs9373024  | T > C   | 5'       | 148                   | 84  | 14  | 246   | 0.228 | 0.352          | 0.650 |
| rs9389007               | rs9389007  | A > G   | 5'       | 150                   | 83  | 13  | 246   | 0.222 | 0.345          | 0.732 |
| rs9373026               | rs9373026  | C > G   | 5'       | 99                    | 99  | 48  | 246   | 0.396 | 0.479          | 0.013 |
| rs8192624               | rs8192624  | G > A   | Exon     | 242                   | 4   | 0   | 246   | 0.008 | 0.016          | 0.898 |
| rs8192625               | rs8192625  | G > A   | Exon     | 241                   | 4   | 0   | 245   | 0.008 | 0.016          | 0.897 |
| rs7772821 <sup>a</sup>  | rs7772821  | T > G   | 3'       | 146                   | 89  | 11  | 246   | 0.226 | 0.349          | 0.579 |
| rs11154685 <sup>a</sup> | rs11154685 | A > G   | 3'       | 111                   | 111 | 23  | 245   | 0.320 | 0.435          | 0.528 |
| rs4305746 <sup>a</sup>  | rs4305746  | G > A   | 3'       | 91                    | 109 | 45  | 245   | 0.406 | 0.482          | 0.224 |
| rs6912930               | rs6912930  | T > G   | 3'       | 138                   | 89  | 19  | 246   | 0.258 | 0.383          | 0.385 |
| rs17802869              | rs17802869 | T > G   | 3'       | 188                   | 48  | 7   | 243   | 0.128 | 0.223          | 0.079 |
| rs6903874               | rs6903874  | T > C   | 3'       | 185                   | 53  | 8   | 246   | 0.140 | 0.241          | 0.095 |

C/C, major homozygote; C/R, heterozygote; HWE,  $P$ -value of Hardy–Weinberg equilibrium analysis; MAF, minor allele frequency; R/R, minor homozygote; *TAAR6*, trace amine-associated receptor 6.

<sup>a</sup>SNPs assessed in both our previous GWAS [18] and this study.

**Fig. 1**

Map and haplotypes of the *TAAR6* gene. (a) Gene map and SNPs locations in *TAAR6* on chromosome 6q23.2. Coding exons are indicated by black boxes. (b) Haplotypes of *TAAR6*. (c) Linkage disequilibrium coefficients ( $|D'|$ ) among *TAAR6* SNPs. SNP, single-nucleotide polymorphism; *TAAR6*, trace amine-associated receptor 6.

Table 3 Association analyses of TAAR6 polymorphisms with the %ΔFEV1 following inhaled corticosteroid treatment

| SNP ID     | Alleles | Position            | C/C                 | C/R                 | R/R                | P <sub>a</sub> | P <sub>a</sub> * | P <sub>b</sub> | P <sub>b</sub> * | P <sub>c</sub> | P <sub>c</sub> * | P <sup>†</sup> | Statistical power |
|------------|---------|---------------------|---------------------|---------------------|--------------------|----------------|------------------|----------------|------------------|----------------|------------------|----------------|-------------------|
| rs11961899 | A > G   | 5'                  | 200 (28.11 ± 32.12) | 39 (17.35 ± 31.31)  | 5 (23.04 ± 27.90)  | 0.11           | 1                | 0.08           | 1                | 0.79           | 1                | 0.55           | 0.662             |
| rs9402427  | C > T   | 5'                  | 178 (24.98 ± 29.87) | 61 (31.18 ± 37.33)  | 7 (34.95 ± 52.05)  | 0.29           | 1                | 0.3            | 1                | 0.63           | 1                | 0.74           | 0.473             |
| rs9493381  | G > T   | 5'                  | 149 (25.64 ± 28.69) | 83 (29.15 ± 37.95)  | 14 (25.26 ± 38.66) | 0.86           | 1                | 0.68           | 1                | 0.69           | 1                | 1              | 0.368             |
| rs9402428  | G > A   | 5'                  | 148 (25.77 ± 28.75) | 83 (29.15 ± 37.95)  | 14 (25.26 ± 38.66) | 0.87           | 1                | 0.7            | 1                | 0.68           | 1                | 1              | 0.355             |
| rs9373024  | T > C   | 5'                  | 148 (25.42 ± 28.66) | 84 (29.50 ± 37.85)  | 14 (25.26 ± 38.66) | 0.76           | 1                | 0.58           | 1                | 0.69           | 1                | 1              | 0.373             |
| rs9389007  | A > G   | 5'                  | 150 (25.08 ± 28.62) | 83 (30.27 ± 37.80)  | 13 (24.57 ± 40.15) | 0.66           | 1                | 0.45           | 1                | 0.62           | 1                | 1              | 0.385             |
| rs9373026  | C > G   | 5'                  | 99 (24.11 ± 32.95)  | 99 (32.59 ± 34.22)  | 48 (20.43 ± 26.46) | 0.97           | 1                | 0.24           | 1                | 0.16           | 1                | 1              | 0.366             |
| rs8192624  | G > A   | Exon                | 242 (27.07 ± 32.75) | 4 (10.57 ± 11.66)   | —                  | 0.32           | 1                | 0.32           | 1                | —              | —                | 1              | 0.459             |
| rs8192625  | G > A   | Exon                | 241 (27.09 ± 32.82) | 4 (10.57 ± 11.66)   | —                  | 0.32           | 1                | 0.32           | 1                | —              | —                | 1              | 0.458             |
| rs7772821  | T > G   | 3'                  | 146 (21.32 ± 24.69) | 89 (31.50 ± 36.99)  | 11 (60.77 ± 56.64) | 0.0005         | 0.002            | 0.001          | 0.03             | 0.0004         | 0.01             | 0.003          | 0.989             |
| rs11154685 | A > G   | 3'                  | 111 (27.15 ± 33.32) | 111 (26.49 ± 33.61) | 23 (25.46 ± 24.09) | 0.77           | 1                | 0.84           | 1                | 0.76           | 1                | 1              | 0.393             |
| rs4305746  | G > A   | 3'                  | 91 (26.25 ± 34.14)  | 109 (29.16 ± 34.36) | 45 (21.82 ± 24.09) | 0.55           | 1                | 0.95           | 1                | 0.23           | 1                | 1              | 0.402             |
| rs6912930  | T > G   | 3'                  | 138 (25.36 ± 31.52) | 89 (27.21 ± 33.79)  | 19 (35.36 ± 34.78) | 0.26           | 1                | 0.38           | 1                | 0.29           | 1                | 0.92           | 0.486             |
| rs17802869 | T > G   | 3'                  | 188 (24.71 ± 29.67) | 48 (32.18 ± 40.46)  | 7 (36.22 ± 26.74)  | 0.09           | 1                | 0.09           | 1                | 0.45           | 1                | 0.59           | 0.646             |
| rs6903874  | T > C   | 3'                  | 185 (24.70 ± 29.99) | 53 (31.02 ± 38.74)  | 8 (47.40 ± 40.16)  | 0.03           | 0.97             | 0.07           | 1                | 0.07           | 1                | 0.28           | 0.725             |
| BL1_h1     |         | 101 (23.97 ± 32.72) | 98 (32.57 ± 34.40)  | 47 (20.87 ± 26.57)  | 0.89               | 1              | 0.22             | 1              | 0.2              | 1              | 1                | 1              | 0.368             |
| BL1_h2     |         | 99 (26.43 ± 33.45)  | 106 (29.30 ± 34.48) | 41 (21.26 ± 24.32)  | 0.69               | 1              | 0.76             | 1              | 0.25             | 1              | 1                | 1              | 0.380             |
| BL1_h3     |         | 178 (24.98 ± 29.87) | 61 (31.18 ± 37.33)  | 7 (34.95 ± 52.05)   | 0.29               | 1              | 0.3              | 1              | 0.63             | 1              | 1                | 1              | 0.473             |
| BL1_h4     |         | 215 (27.45 ± 32.33) | 29 (23.47 ± 35.03)  | 2 (5.19 ± 24.41)    | 0.34               | 1              | 0.44             | 1              | 0.3              | 1              | 1                | 1              | 0.452             |
| BL2_h1     |         | 19 (35.36 ± 34.78)  | 89 (27.21 ± 33.79)  | 138 (25.36 ± 31.52) | 0.26               | 1              | 0.29             | 1              | 0.38             | 1              | 1                | 1              | 0.486             |
| BL2_h2     |         | 189 (24.52 ± 29.71) | 49 (32.26 ± 40.04)  | 8 (47.40 ± 40.16)   | 0.02               | 0.64           | 0.04             | 1              | 0.07             | 1              | 0.21             | 1              | 0.783             |
| BL2_h3     |         | 193 (28.03 ± 34.23) | 48 (20.93 ± 24.95)  | 5 (35.78 ± 29.04)   | 0.42               | 1              | 0.28             | 1              | 0.66             | 1              | 1                | 1              | 0.429             |

Genotype distribution of each SNP is presented as the number of participants (%ΔFEV<sub>1</sub> after inhaled corticosteroid treatment, mean and SD). P-values were calculated by linear regression analysis controlling age, sex, smoking status, and atopy as covariates. C/C, major homozygote; C/R, heterozygote; FEV<sub>1</sub>%, percentile changes in the forced expiratory volume; R/R, minor homozygote; TAAR6, trace amine-associated receptor 6. \*The corrected P-values for multiple comparisons (x32.2233). P<sub>a</sub>, P<sub>b</sub> and P<sub>c</sub> mean P-values of codominant, dominant, and recessive model, respectively. †P-values obtained by 1 000 000 permutation test for the codominant model.

Table 4 Association analyses of *TAAR6* polymorphisms with the basal FEV1

| SNP ID     | Alleles | Position | All participants ( <i>n</i> = 246) |                     |                     |                      |                      |                      |
|------------|---------|----------|------------------------------------|---------------------|---------------------|----------------------|----------------------|----------------------|
|            |         |          | C/C                                | C/R                 | R/R                 | <i>P<sub>a</sub></i> | <i>P<sub>b</sub></i> | <i>P<sub>c</sub></i> |
| rs11961899 | A > G   | 5'       | 200 (65.12 ± 13.03)                | 38 (67.95 ± 9.74)   | 5 (61.00 ± 10.98)   | 0.41                 | 0.21                 | 0.46                 |
| rs9402427  | C > T   | 5'       | 178 (65.35 ± 12.12)                | 60 (65.37 ± 12.56)  | 7 (63.71 ± 25.03)   | 0.46                 | 0.55                 | 0.49                 |
| rs9493381  | G > T   | 5'       | 149 (65.06 ± 12.30)                | 82 (65.73 ± 12.31)  | 14 (65.43 ± 18.43)  | 0.96                 | 0.99                 | 0.88                 |
| rs9402428  | G > A   | 5'       | 148 (65.01 ± 12.32)                | 82 (65.73 ± 12.31)  | 14 (65.43 ± 18.43)  | 0.99                 | 0.96                 | 0.90                 |
| rs9373024  | T > C   | 5'       | 148 (65.13 ± 12.31)                | 83 (65.60 ± 12.29)  | 14 (65.43 ± 18.43)  | 0.92                 | 0.95                 | 0.88                 |
| rs9389007  | A > G   | 5'       | 150 (65.10 ± 12.34)                | 82 (65.76 ± 12.19)  | 13 (64.85 ± 19.05)  | 0.86                 | 0.97                 | 0.70                 |
| rs9373026  | C > G   | 5'       | 98 (66.42 ± 13.23)                 | 99 (64.77 ± 12.68)  | 48 (64.15 ± 11.42)  | 0.35                 | 0.31                 | 0.62                 |
| rs8192624  | G > A   | Exon     | 241 (65.16 ± 12.68)                | 4 (74.00 ± 8.29)    | 0                   | 0.16                 | 0.16                 | –                    |
| rs8192625  | G > A   | Exon     | 240 (65.14 ± 12.70)                | 4 (74.00 ± 8.29)    | –                   | 0.16                 | 0.16                 | –                    |
| rs7772821  | T > G   | 3'       | 146 (66.08 ± 11.39)                | 88 (64.92 ± 13.68)  | 11 (58.09 ± 18.18)  | 0.14                 | 0.33                 | 0.06                 |
| rs11154685 | A > G   | 3'       | 110 (64.97 ± 13.78)                | 111 (65.53 ± 11.75) | 23 (66.48 ± 11.57)  | 0.73                 | 0.78                 | 0.77                 |
| rs4305746  | G > A   | 3'       | 91 (65.20 ± 13.94)                 | 108 (65.19 ± 12.06) | 45 (65.44 ± 11.45)  | 0.97                 | 0.93                 | 0.97                 |
| rs6912930  | T > G   | 3'       | 137 (64.48 ± 11.70)                | 89 (66.45 ± 14.29)  | 19 (65.89 ± 11.20)  | 0.38                 | 0.22                 | 0.84                 |
| rs17802869 | T > G   | 3'       | 187 (64.97 ± 11.97)                | 48 (66.44 ± 15.29)  | 7 (70.86 ± 6.72)    | 0.21                 | 0.26                 | 0.37                 |
| rs6903874  | T > C   | 3'       | 184 (64.62 ± 11.92)                | 53 (67.45 ± 14.91)  | 8 (66.88 ± 12.87)   | 0.20                 | 0.14                 | 0.86                 |
|            |         |          | –/–                                | ht/–                | ht/ht               | <i>P<sub>a</sub></i> | <i>P<sub>b</sub></i> | <i>P<sub>c</sub></i> |
| BL1_ht1    |         |          | 100 (66.59 ± 13.16)                | 98 (64.40 ± 12.75)  | 47 (64.47 ± 11.32)  | 0.32                 | 0.22                 | 0.75                 |
| BL1_ht2    |         |          | 99 (64.87 ± 13.15)                 | 105 (64.80 ± 11.99) | 41 (67.66 ± 13.13)  | 0.27                 | 0.57                 | 0.16                 |
| BL1_ht3    |         |          | 178 (65.35 ± 12.12)                | 60 (65.37 ± 12.56)  | 7 (63.71 ± 25.03)   | 0.46                 | 0.55                 | 0.49                 |
| BL1_ht4    |         |          | 214 (64.94 ± 12.93)                | 29 (68.79 ± 9.99)   | 2 (53.50 ± 6.36)    | 0.40                 | 0.19                 | 0.16                 |
| BL2_ht1    |         |          | 19 (65.89 ± 11.20)                 | 89 (66.45 ± 14.29)  | 137 (64.48 ± 11.70) | 0.38                 | 0.84                 | 0.22                 |
| BL2_ht2    |         |          | 188 (64.91 ± 11.97)                | 49 (66.55 ± 15.15)  | 8 (66.88 ± 12.87)   | 0.40                 | 0.35                 | 0.86                 |
| BL2_ht3    |         |          | 192 (65.41 ± 12.71)                | 48 (64.77 ± 13.02)  | 5 (66.40 ± 7.70)    | 0.80                 | 0.79                 | 0.94                 |

Genotype distribution of each SNP is presented as the number of participants (basal FEV1, mean and SD).

*P*-values were calculated by linear regression analysis controlling age, sex, smoking status, and atopy as covariates.

AA, amino acid; C/C, major homozygote; C/R, heterozygote; FEV1%, percentile changes in the forced expiratory volume; R/R, minor homozygote; *TAAR6*, trace amine-associated receptor 6.

*P<sub>a</sub>*, *P<sub>b</sub>* and *P<sub>c</sub>* mean *P*-values of codominant, dominant and recessive model, respectively.

### Association between *TAAR6* polymorphisms and $\Delta$ FEV1 induced by ICS treatment

The 15 SNPs and seven haplotypes were analyzed in terms of their association with ICS responsiveness in the participants using various linear regression models (Table 3). Association analyses showed a significant association between the *TAAR6* rs7772821T>G and  $\Delta$ FEV1 ( $P_{\text{corr}}=0.002$  in the codominant model,  $P_{\text{corr}}=0.03$  in the dominant model, and  $P_{\text{corr}}=0.01$  in the recessive model). The  $\Delta$ FEV1 value of rs7772821T>G minor homozygotes (60.77%) was higher than that of those harboring the rs7772821 T/G or T/T genotype (21.32 and 31.60%, respectively). To exclude the effect of initial lung function on post-therapeutic lung function, SNPs and haplotypes were analyzed in terms of their association with initial FEV1 using regression models. This analysis failed to show an association between any SNP or haplotype with the initial FEV1 (Table 4).

In addition, we analyzed the haplotypes comprising six SNPs on the 3' flanking region of *TAAR6* because rs7772821 was in relatively high LD with these SNPs (Fig. 1c). Among the five common haplotypes (frequency > 0.05), haplotype 1 (*ht1*) and 5 (*ht5*) showed significant associations with  $\Delta$ FEV1 after a correction of multiple comparisons, as presented in Table 5. The *ht1* and *ht5* had T and G alleles on rs7772821, respectively, but had the same alleles on the other five loci (*AGTTT*). Thus, the asthmatics with *ht1* showed lower  $\Delta$ FEV1

( $P_{\text{corr}}=0.032$  in dominant model); in contrast, those with *ht5* showed higher  $\Delta$ FEV1 than the others ( $P_{\text{corr}}=0.004$  in the codominant model). This observation supported our primary finding of a significant association between the *TAAR6* rs7772821T>G SNP and ICS-induced  $\Delta$ FEV1.

### Discussion

In this prospective study, we examined the association between genetic variants of *TAAR6* and airway responsiveness to high-dose ICS therapy in moderate-to-severe asthma. Of the 15 polymorphisms tested, one SNP, rs7772821, located on the 3'-UTR of *TAAR6*, was significantly associated with  $\Delta$ FEV1 after a 4-week course of fluticasone propionate. The  $\Delta$ FEV1 of patients homozygous for the minor allele (rs7772821G/G) was three-fold greater than that of those homozygous for the common allele (the rs7772821T>G).

Although asthma is identified by the presence of reversible airflow obstruction, over time, the obstruction can become permanent in some patients [29]. Therefore, ICS intervention therapy should be initiated early in disease, and be maintained long term, even in patients with relatively mild asthma [30]. The airway structural changes associated with asthma have both steroid-sensitive and steroid-insensitive components. Steroid-insensitive components may be responsible for the incomplete restoration of FEV1, even in the presence of high-dose

Table 5 Association result of additional haplotypes comprised of six SNPs on the 3' flanking region of the *TAAR6* gene with %ΔFEV1 induced by ICS treatment in asthmatics

|     | %ΔFEV1    |            |           |           |            |           |           |                  |                  |                 | P <sub>a</sub> | P <sub>a</sub> * | P <sub>b</sub> | P <sub>b</sub> * | P <sub>c</sub> | P <sub>c</sub> * |
|-----|-----------|------------|-----------|-----------|------------|-----------|-----------|------------------|------------------|-----------------|----------------|------------------|----------------|------------------|----------------|------------------|
|     | rs7772821 | rs11154685 | rs4305746 | rs6912930 | rs17802869 | rs6903874 | Frequency | -/-              | ht/-             | ht/ht           |                |                  |                |                  |                |                  |
| HT  | T         | A          | G         | T         | T          | T         | 0.343     | 111 (34.37±3.72) | 101 (18.58±2.07) | 34 (26.54±5.58) | 0.002          | 0.064            | 0.001          | 0.032            | 0.966          | 1.000            |
| ht1 | T         | G          | A         | T         | T          | T         | 0.254     | 135 (25.95±2.69) | 97 (27.91±3.57)  | 14 (27.38±7.66) | 0.937          | 1.000            | 0.740          | 1.000            | 0.959          | 1.000            |
| ht2 | G         | A          | G         | G         | G          | C         | 0.112     | 196 (24.38±2.10) | 45 (33.18±6.11)  | 5 (64.33±19.27) | 0.008          | 0.258            | 0.018          | 0.580            | 0.010          | 0.322            |
| ht3 | T         | A          | A         | G         | T          | T         | 0.098     | 202 (27.70±2.37) | 40 (21.45±4.09)  | 4 (34.89±16.73) | 0.577          | 1.000            | 0.434          | 1.000            | 0.647          | 1.000            |
| ht4 | G         | A          | G         | T         | T          | T         | 0.081     | 207 (24.55±1.99) | 38 (35.94±7.20)  | 1 (146.51)      | 0.00011        | 0.004            | 0.009          | 0.290            | 0.00023        | 0.007            |

Other haplotypes (frequency <0.05): TGGTTT, GGATT, TAGGTC, TGAGTT, TAATT, GAGTC, GAAGTT, GGGTTT, TAGGTC.  
FEV1%, percentile changes in the forced expiratory volume; ICS, inhaled corticosteroid; *TAAR6*, trace amine-associated receptor 6.  
*P<sub>a</sub>*, *P<sub>b</sub>*, and *P<sub>c</sub>* mean *P*-values of codominant, dominant, and recessive models, respectively.  
\*The corrected *P*-values for multiple comparisons (x32.2233).

corticosteroids. Mechanisms of glucocorticoid insensitivity include changes in GRs, abnormalities in GR cofactors, and transcription factor-induced alterations in the translocation of glucocorticoid-bound GRs to the promoter regions of glucocorticoid-responsive genes [31–33].

The present study suggests an additional molecular function of *TAAR6* gene underlying the responsiveness of asthmatic patients to ICS treatment. In our previous GWAS study, 63 SNPs were genotyped on six members of the *TAAR* gene family, including nine in the *TAAR1* gene, 21 in *TAAR2*, 13 in *TAAR5*, 12 in *TAAR6*, five in *TAAR8*, and four in *TAAR9* [17]. Among these, only rs7772821 was associated with %ΔFEV1 after ICS treatment (Supplement Table 1, Supplemental digital content 1, <http://links.lww.com/FPC/A843>). Trace amine-associated receptors, including *TAAR6*, are a class of guanine nucleotide-binding-protein-coupled receptors (GPCR) identified in 2001 associated strongly with both schizophrenia and bipolar illness [34,35]. A set of *TAAR6* genetic variations (rs7452939, rs4305745, rs6903874, rs6937506, and rs8192625) are associated with clinical features of depression, including antidepressant treatment response, in Koreans [36]. However, this study did not include the rs7772821 genotype described here. In 265 pedigrees of the Irish Study of High-Density Schizophrenia Families, haplotypes harboring the rs7772821 minor allele were associated strongly with delusions and hallucinations, consistent with a family-based association for schizophrenia [37]. Interestingly, atopic disorders in the general population and asthma, in particular, are associated with an increased risk of developing schizophrenia according to two Caucasian population-based registers [38] and in an Asian population [39].

The heterotrimeric G-proteins transduce signals from GPCRs, a receptor family containing ~1000 members. G-proteins have three subunits, designated Gα, Gβ, and Gγ; both the dissociated Gα protein and the Gβ/Gγ complex interact with downstream effector molecules to exert their biological effects. Gβ specifically interacts with GRs, whereas Gβ<sub>1</sub> and Gβ<sub>2</sub> comigrate into the nucleus with GRs to suppress GR-induced transactivation of glucocorticoid-responsive genes [40]. Alteration of *TAAR6* expression in target organs may therefore play a role in the development of resistance or hypersensitivity to glucocorticoids, although the signal transduction pathways downstream of *TAAR* remain poorly characterized. The implications of this study are limited by the absence of an independent replicate study and the absence of functional characterization. The rs7772821T>G SNP is located in the 3'-UTR of *TAAR6*. As SNPs often affect transcript processing through changes in stability [41], we performed a microRNA search using MIRDDB (MicroRNA Target Prediction and Functional Study Database-<http://mirdb.org/mirDB>) and found no candidate microRNAs targeting the 3'-UTR of

TAAR6. To evaluate a possible role of rs7772821 in the expression of TAAR6, we additionally carried out eQTL analysis using GeneVar (<http://www.sanger.ac.uk/resources/software/genevar>) and found that rs7772821 was associated with TAAR6 mRNA expression in T cells, which was assessed using Illumina HumanWG-6 v3 in the Geneva GeneCord study ( $P=0.01$  by 10 000 permutation test) [42]. These data suggest that rs7772821 may be involved in the regulation of TAAR6 expression.

To confirm our observations, these results should be replicated in other populations. Identification of the same findings in replication studies would provide strong evidence for the association of genetic variants in asthma and pharmacogenetics. Here, we did not carry out a replication study for the TAAR6 SNP because of the rarity of this patient cohort. Another limitation of our study was the absence of functional validation of the TAAR6 SNP. By searching TAAR6 expression profiles in Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>), we found that TAAR6 may be highly expressed in lung tissue, trachea, and airway epithelial cells in normal patients and asthmatics. Thus, further studies should be carried out to validate the functional association between rs7772821 and % $\Delta$ FEV<sub>1</sub>. In addition, compliance with medication could affect the results of this study by causing a null bias. According to a systemic review [43], overall patient compliance with ICS ranged from 63 to 92%. The highest compliance (92%) occurred after an intensive education program [44]. Thus, the 93.5% compliance in our study was high for general ICS medication. Furthermore, no short-term complication was reported by the patients or physicians. Taken together, our observations were unlikely to be affected by a null bias.

In summary, our fine-mapping and validation study of 15 human TAAR6 gene polymorphisms showed a significant association between the TAAR6 rs7772821T>G SNP and ICS treatment-induced % $\Delta$ FEV<sub>1</sub>, consistent with the results of our previous GWAS analysis. The response of rs7772821T>G minor allele homozygous patients to high-dose ICS treatment was twofold higher than that of rs7772821T/T-homozygous or T/C heterozygous patients. This difference in responsiveness may result from variation in RNA stabilities. Together, these data suggest an additional role for TAAR6 in the responsiveness of asthmatics to ICS treatment.

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## Conflicts of interest

There are no conflicts of interest.

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