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Effect of β2-Adrenergic Receptor Polymorphism in Asthma Control of Patients Receiving Combination Treatment

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Purpose: Combination treatment of inhaled corticosteroid (ICS) plus long-acting β2-agonist (LABA) is widely used as a maintenance regimen for the management of asthma. This study evaluated the effect of the β2-adrenergic receptor (ADRB2) polymorphism on lung function and asthma control with regular use of combination treatment of an inhaled ICS plus LABA. **Materials and Methods:** 43 Korean asthmatics who were symptomatic despite regular ICS use for at least 3 months were enrolled. For a 2-week run-in period, they received ICS (budesonide 800 μg/day) plus terbutaline (5 μg prn). as needed. During the 24-week active treatment period, they received budesonide 160 μg and formoterol 4.5 μg b.i.d. as maintenance and rescue medication. Pulmonary function and quality of life scores were monitored every 8 weeks; morning/evening peak expiratory flow meter (PEFR) was recorded daily. Patients were genotyped for ADRB2 Arg16Gly using single base extension methodology. **Results:** During the run-in period, there were no significant between-group differences in lung function; after 8 weeks of active treatment, Arg/Arg patients had significantly higher forced expiratory volume in 1 secord (FEV_1) and maximal mid-expiratory flow (MMEF) ($p = 0.023$ and $p = 0.021$, respectively), and better asthma control and quality of life after 24 weeks ($p = 0.016$ and $p = 0.028$, respectively). During treatment, there was a greater improvement in morning/evening PEFR in Arg/Arg patients. **Conclusion:** Asthmatic patients with the Arg/Arg genotype at codon 16 of ADRB2 achieve better asthma control with long-term regular use of combined budesonide and formoterol treatment, suggesting that the ADRB2 genotype may dictate choice of treatment strategy.

Key Words : β2-adrenergic receptor polymorphism, long-acting β2-agonist, bronchodilating effect

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INTRODUCTION

The combination of low or moderate doses of inhaled corticosteroids (ICS) with long-acting β2-agonists (LABA) improves asthma control in adults and reduces exacerbations.[1,2] The combination of ICS plus LABA for maintenance therapy has been endorsed in guidelines for the treatment of moderate to severe asthma.[3] Recent pharmacogenetic studies demonstrated that the β2-adrenergic receptor (ADRB2) polymorphism modifies bronchodilating response to regular use of short-acting[4,5] and long-acting[6-8] β2-agonists. Individuals homozygous for glycine (Gly) at codon 16 of the ADRB2 gene have greater bronchodilation, measured as the forced expiratory volume in 1 second (FEV_1) and maximal mid-expiratory flow (MMEF) to regular treatment of albuterol[4,5] and salmeterol.[6,8] By contrast, a recent finding in a Korean population[9] showed that asthmatic patients with the homozygous Arg/Arg genotype had a significantly greater response of acute brochodilation to a short-acting β2-agonist. Some studies reported that ADRB2 genetic polymorphisms did not result in significant change of bronchodilating response to short

acting β2-agonist[10,11] and LABA,[12-14] and that other ethnic-specific pharmacogenetic differences have been demonstrated in individuals with different ADRB2 genotypes.[15] Therefore, to elucidate whether there is a genotype-dependent influence or any pharmaco-ethnic differences on the effects of regular use of LABA combined with ICS in Korean asthmatics, we conducted a 24-week trial of combined budesonide 160 μg and formoterol 4.5 μg in moderate asthmatics and compared clinical outcomes according to the ADRB2 genotype.

MATERIALS AND METHODS

Subjects and study design

This study was a long-term trial consisting of a 2-week run-in and a 24-week active treatment period. Patients with moderately persistent asthma were recruited, based on the Global Initiative for Asthma (GINA) guidelines. All subjects participated in this study were ethnically Korean. Men and women, aged 18-55 years with a documented history of asthma for at least 6 months, no history of smoking or reported history of viral infection in the proceeding 6 weeks, and an FEV_1 of at least 60% of the predicted value, were recruited at Ajou University Hospital, Suwon, and Dankook University Hospital, Cheonan, Korea. All study patients had not been exposed to long acting β2 agonist for 3 months prior to this study, and they provided written informed consent as approved by the local committee on human research.

Outpatients included were required to have been on maintenance therapy with less than 800 μg of inhaled budesonide for at least 3 months and to have a history of at least 1 asthma exacerbation in the previous 12 months. For at least 30 days before enrollment and during the 2-week run-in period, patients took a constant daily dose of 800 μg of budesonide. To be eligible, patients had to have an FEV_1 of 60-100% of the predicted value with 12% or more reversibility after 100 μg of salbutamol inhalation compared to baseline, and should have used a short-acting β2-agonist for asthma symptoms on at least 5 of the last 7 days of the run-in. During the 24-week active treatment period, all patients were asked to use combination therapy, while consisted of budesonide 160 μg and formoterol 4.5 μg combined in a device, twice a day as maintenance treatment and for relief as needed. The pretreatment peak expiratory flow meter (PEFR) was assessed twice daily using a Mini-Wright PEFR meter (Clement Clark, Harlow, UK). Daily symptoms, nocturnal awakenings, and rescue medication use were recorded on diary cards. FEV_1 was assessed 5 times (at -2, 0, 8, 16, and 24 weeks) by calibrated spirometry. Both PEFR and FEV_1 were presented as percent predicted values. An Asthma Control Questionnaire (ACQ; range: 0, absent, to 6, severe) was completed 4 times (at 0, 8, 16, and 24 weeks), and the Asthma Quality of Life Questionnaire (AQLQ; range: 1, severe impairment, to 7, not impaired) validated by the Korean Society of Allergology[16] was recorded 3 times (at 0, 8, and 24 weeks).

Genotyping

Genotyping of the ADRB2 Arg16Gly polymorphism was performed all study using a single base extension method. The sequences of the amplifying and extension primers for the ADRB2 Arg16Gly polymorphism were forward 5'AAAATTA TGCTCCAGGAGTCTCA-3', reverse 5'-ATAAGTTTCTTGG CTGATTAAGATCA-3', and extension 5'-tttacttgtgatgaatagaa aaatt3'. Primer extension reactions were performed with the SNaPshot ddNTP primer extension kit (Applied Biosystems, Foster City, CA, USA). The results were analysed after completion of this study, using the ABI Prism GeneScan and Genotyper software (Applied Biosystems). Among the study subjects, 13 individuals were Arg/Arg homozygous, 25 individuals were Arg/Gly heterozygous and 5 individuals were Gly/Gly homozygous genotypes. Study subjects were categorized into 2 groups: Arg/Arg and Arg/Gly plus Gly/Gly groups, because of limited number for analysis.

Statistical analysis

Lung function [percent predicted value of FEV_1, forced vital capacity (FVC), and MMEF] and the change in ACQ and AQLQ scores between groups were compared using the Mann-Whitney U test. Student's t-test was used to compare demographic data among the study groups. Values of $p \leq 0.05$ were regarded as significant. The primary outcome of the study was asthma control characterized by FEV_1 and MMEF (% predicted value), measured at every visit. Secondary outcomes were morning/evening PEFR (% predicted value), morning/evening PEFR variability (evening PEFR minus morning PEFR as a percentage of evening PEFR), asthma symptoms (range: 0 absent to 3 severe), frequency of rescue medication use, and ACQ and AQLQ scores. Patients were stratified into two groups depending on the ADRB2 codon 16 genotype, Arg/Arg and Arg/Gly or Gly/Gly. Owing to the repeated measurements of primary and secondary response variables within each phase of experiment, longitudinal data analysis was applied within the context of the intention-to-treat principle. Mean PEFR from each individual was parallel transformed into origin of y axis at starting point of the study. The transformed data were presented as reversible PEFR. The data were fitted and modeled using a non-linear regression (SIGMAPLOT 9.0) for the 2 genotype groups. The regression equations were satisfied by independency, normality and constant variance of residual error.

RESULTS

The clinical and physical characteristics of 43 enrolled patients are shown in Table 1. There were no significant differences in baseline spirometry results between the 2 genotype groups,

Table 1. Comparison of Clinical Characteristics of Study Subjects Stratified by ADRB2 Genotype Group

	Genotype		*p* value
	Arg/Arg (n = 13)	Arg/Gly or Gly/Gly (n = 30)	
Age (yrs)*	30.8 ± 10.6	41.5 ± 10.9	0.005
Gender (M / F)	5 / 8	21 / 9	0.089
Atopy (+ / -)	5 / 8	11 / 19	1.000
Log total IgE (IU / mL)*	1.89 ± 0.57	2.34 ± 0.5	0.021
Baseline FEV_1 (%)*	77.8 ± 13.6	73.5 ± 12.1	0.316
Asthma duration (yr)*	9.0 ± 6.5	6.2 ± 3.5	0.234
Family history of asthma	4 / 9	7 / 23	0.709
PC_{20} methacholine (mg / mL)*	6.75 ± 2.5	5.10 ± 2.4	0.646
Number of exacerbation (time / yr)*	0.50 ± 0.17	0.62 ± 0.1	0.602
FVC (% predicted)	80.5 ± 2.9	82.3 ± 2.6	0.698
MMEF (% predicted)	67.2 ± 7.9	52.4 ± 4.7	0.104
Morning PEFR (% predicted)	88.8 ± 6.3	83.8 ± 5.0	0.550
Evening PEFR (% predicted)	90.7 ± 6.4	89.3 ± 7.7	0.900
FEV_1 increase after BDT (%)*	19.7 ± 2.3	19.9 ± 1.0	0.930

ADRB2, β2-adrenergic receptor; M, male; F, female; FVC, forced vital capacity; MMEF, maximal mid-expiratory flow; PEFR, peak expiratory flow rate; FEV_1, forced expiratory volume in 1sec; BDT, bronchodilating test.
*mean ± SD.

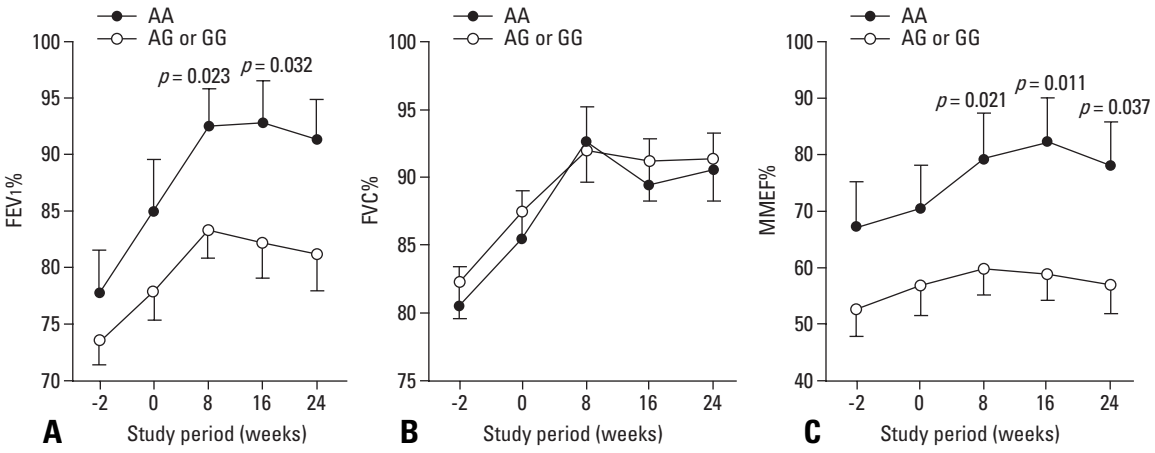

Fig. 1. Mean (SD) % predicted value of FEV_1 (A), FVC (B) and MMEF (C) during the study period (-2-0 weeks: run-in period; 0-24 weeks: active treatment) for both ADRB2 codon 16 genotype groups (Arg/Arg; Arg/Gly or Gly/Gly). *p values for Arg/Arg compared with Arg/Gly or Gly/Gly at each time point. FEV_1, forced expiratory volume in 1sec; FVC, forced vital capacity; MMEF, maximal mid-expiratory flow; ADRB2, β2-adrenergic receptor.

with the Arg/Arg genotype group having a mean FEV_1 of 77.8% and the Arg/Gly or Gly/Gly genotype group having a mean FEV_1 of 73.5%. Mean age and serum total immuno globulin (Ig) E level were significantly higher in the Arg/Gly or Gly/Gly genotype group than in the Arg/Arg genotype group (41.5 ± 10.9 vs. 30.8 ± 10.6 years, 2.34 ± 0.5 vs. 1.89 ± 0.57 IU/mL, $p < 0.05$, respectively). However, there were no significant differences in gender, atopy rate, PC_{20} methacholine, asthma duration, family history of asthma and frequency of exacerbation according to the genotype (all $p > 0.05$).

During the 2-week run-in period, both groups exhibited increase of lung function, but no significant differences were noted between the 2 groups. There was a significant increase of FEV_1 during the regularly scheduled combined treatment ($p < 0.05$) (Fig. 1). After 8 weeks of active treatment, treatment effects differed significantly between the genotype groups: patients with an Arg/Arg genotype had significantly higher FEV_1% and MMEF% than those with an Arg/Gly or Gly/Gly genotype ($p < 0.05$), whereas no significant differences between the groups were noted for FVC%. This improvement appeared to persist through subsequent 16 weeks, and the mean FEV_1 and PEFR levels were then decreased, with statistical significance lost by

24 weeks.

There was a significant increase in the quality of life score during the regularly scheduled combined treatment (Fig. 2B). After 24 weeks of active treatment, the treatment effects showed significant differences between the genotype groups; the Arg/Arg group had a significantly higher quality of life than the Arg/Gly or Gly/Gly group ($p < 0.05$), whereas there was a significant decrease in the symptom score during treatment (Fig. 2A). After 24 weeks of active treatment, the treatment effects showed significant differences between the 2 genotypes ($p < 0.05$). However, there was no significant difference in the requirement for rescue medicine according to genotype ($p > 0.05$).

The morning PEFR, measured daily in all subjects, increased after active treatment (Fig. 3A); the regression equations were y = 89.59 + 5.99×(1 - 0.97^ time) for the Arg/Arg genotype and y = 74.91 + 3.13×(1 - 0.95^ time) for the Arg/Gly or Gly/Gly genotypes. The evening PEFR level was also increased after active treatment, and the equations were y = 90.78 + 6.49×(1 - 0.97^ time) for the Arg/Arg genotype and y = 75.30 + 3.52×(1 - 0.97^ time) for the Arg/Gly or Gly/Gly genotype (Fig. 3B). The slopes were calculated using the differential equation of the best-fit line (y = -b×c^ time×In<c>), the plotted line of each genotype showed different inclination; the slope for the Arg/Arg genotype was steeper than that for the Arg/Gly or Gly/Gly genotype during the active treatment period, however it wasn't calculated by a statistical tool. The time to reach the plateau morning and evening PEFR levels was longer for the Arg/Arg genotype (87 days for the morning PEFR, 98 days for the evening PEFR) than for the Arg/Gly or Gly/Gly genotype (52 and 81 days, respectively).

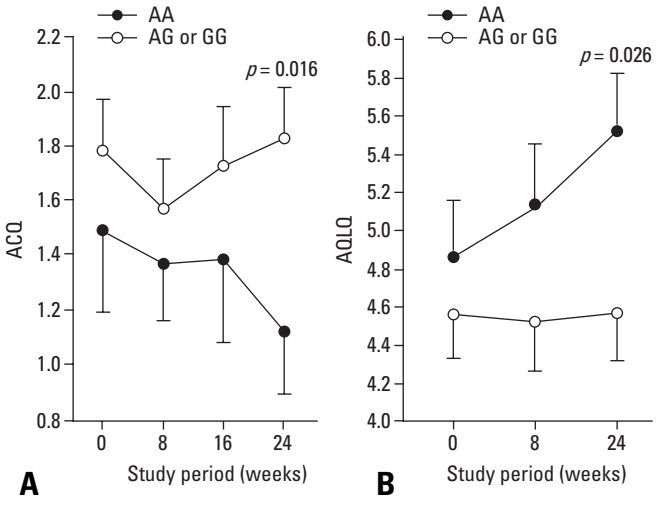

Fig. 2. Mean (SD) symptom scores (A) and quality of life scores (B) during the active treatment period for both ADRB2 codon 16 genotype groups (Arg/Arg; Arg/Gly or Gly/Gly). ADRB2, β2-adrenergic receptor; ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire. *p values for Arg/Arg compared with Arg/Gly or Gly/Gly at each time point.

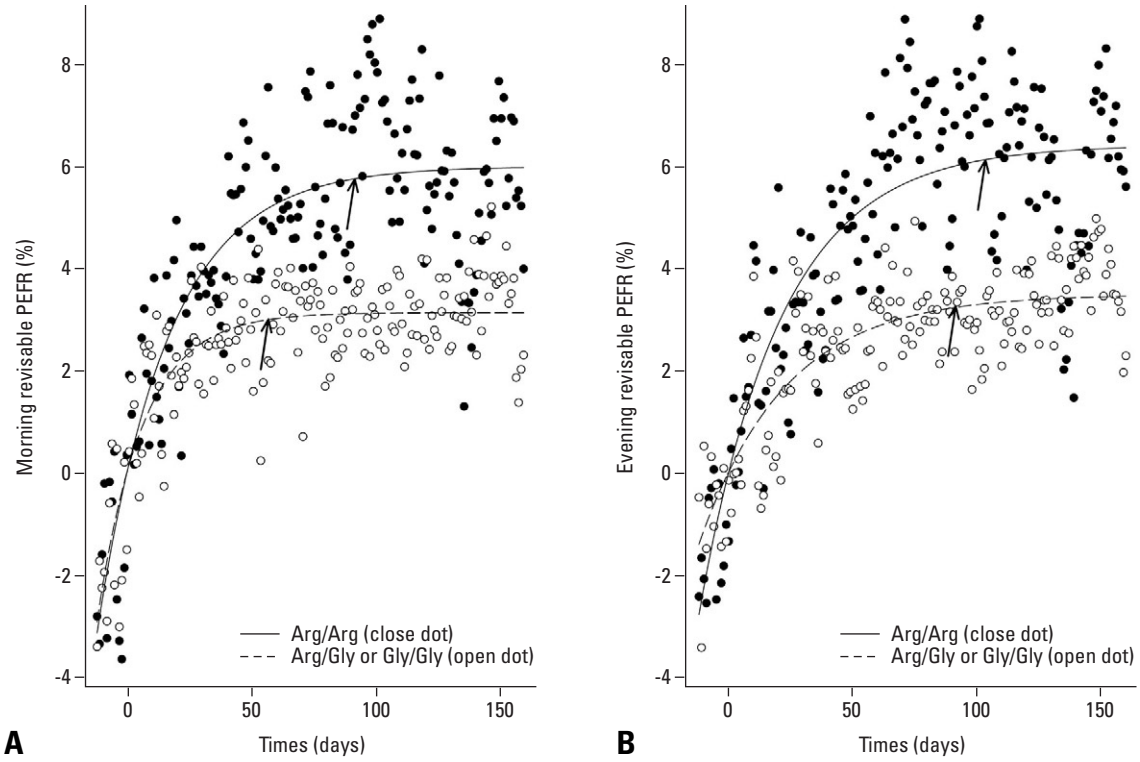

Fig. 3. Changes of morning PEF [(A): Arg/Arg genotype group (●), y = 89.59 + 5.99 × <1 - 0.97^TIME>; Arg/Gly or Gly/Gly genotype group (○), y = 74.81 + 3.13 × < 1 - 0.95^TIME>], and evening PEF [B: Arg/Arg genotype group (●), y = 90.78 + 6.49 × <1 - 0.97^TIME>; Arg/Gly to Gly/Gly genotype group (○), y= 75.30 + 3.52 × <1 - 0.97^TIME>] during the study period, analyzed by non-linear regression and plotted as individual revisable PEFR. ↑ indicates days to reach the plateau PEFR value in each genotype. PEFR, peak expiratory flow rate.

DISCUSSION

The ADRB2 is a G protein-coupled receptor that mediates the actions of catecholamines in multiple tissues. Nine single-nucleotide polymorphism (SNPs) have been identified in the ADRB2 gene,[17] 2 of which are more frequent and give rise to amino acid substitutions in the putative extracellular amino-terminus region of the gene; Arg for Gly at codon 16 and Gln for Glu at codon 27. The Gly16 variant has positively been associated with severe asthma[18,19] and nocturnal asthma.[20] The bronchodilating response to inhaled short- and long-acting β2-agonists shows significant inter-individual variation, depending on the ADRB2 codon 16 genotype,[4-9] although some studies suggested that the bronchodilating effect caused by rescue treatment with β2-agonists is influenced by haplotypes of the ADRB2 gene.[21,22] Furthermore, ethnic differences in the pharmacogenetic effects of ADRB2 Arg16Gly polymorphism has been suggested.[15] Choudhry et al.[15] conducted comprehensive family-based pharmacogenetic study of ADRB2 Arg16Gly polymorphism and demonstrated that Arg16 allele was significantly associated with bronchial responsiveness in Puerto Rican, but not in Mexican, subjects with asthma.

Among two common polymorphisms, Arg16Gly and Gly27Gln, of ADRB2 gene, only the Arg16Gly polymorphism was investigated in this study because of very low frequency of minor allele of the Gly27Gln polymorphism in Korean[9] and Japanese population.[23] Study subjects having the Arg16Gly polymorphism were categorized into two groups, such as Arg/Arg and Arg/Gly plus Gly/Gly groups, because of limited number for analysis.

Our results suggest that the ADRB2 Arg16Gly genotype significantly modifies the level of asthma control, as indicated by improvements in lung function, symptom score, and quality of life, in patients who underwent initially regular treatment with a combined ICS (budesonide) and LABA (formoterol). Our findings suggest that patients with the Arg/Arg genotype would receive greater benefit from the regular use of the combined treatment of ICS and LABA as a maintenance treatment than patients with the Arg/Gly or Gly/Gly genotype. Our primary outcome variables were FEV_1% and MMEF% predicted values, which are recognized as useful indicators that correlate well with clinical outcomes in asthma. Along with these improvements in lung function, patients in the Arg/Arg group exhibited parallel changes in other outcome variables, such as the symptom and quality of life scores and morning/evening PEFR, all suggesting a clinically relevant genotype-specific effect. Although the Arg/Arg group was younger in age, asthma duration was not different between the 2 groups and lung function parameters were presented as % predicted value, and therefore, the effect of age is likely minimal. Moreover, these findings on the difference in the rates of increase of morning and evening PEFR were replicated, as seen in 2 different genotype-specific equations. Therefore this model could be applied to predict morning/evening PEFR responses with long-term regular use of combination treatment.

The pharmacogenetic response to β2-agonist in this study was not consistent with previous studies in Caucasian subjects that subjects carrying the Gly/Gly genotype at codon 16 of ADRB2 achieved a greater bronchodilation response to a short-acting β2-agonist and a long-acting β2-agonist without ICS,[4-8] however similar to a previous study in a Korean population[9] that individuals carrying the Arg/Arg genotype achieved a better response to short-acting β2-agonist. This inconsistency result might be due to pharmaco-ethnic difference: ethenic-specific pharmacogenetic difference of ADRB2 Arg16Gly was previously reported between Puerto Rican and Mexican subjects with asthma.[15] There are several polymorphisms in ADRB2 gene, and these functional SNPs can change in receptor expression and signal transduction.[24-27] This means drug response to inhaled β2-agonists can be altered according to specific haplotype which is constructed by polymorphisms. In the present study, however, we focused on the Arg16Gly because of its important clinical relevance and high minor allele frequency compared to other polymorphisms. Therefore, we couldn't exclude possibility of any pharmacogenetic effect of other polymorphisms and the pharmaco-ethnic difference observed might be related to other polymorphisms in linkage with the ADRB2 Arg16Gly which we studied.

Different pharmacogenetic effect of ADRB2 polymorphism on the combination therapy (ICS plus LABA) may be explainable by different clinical trial. A recent 6-month double blind randomized study[14] revealed no genotype effect of ADRB2 on the combination therapy in 2,250 asthmatics. These investigators enrolled larger numbers of Arg/Arg homozygous in a longer treatment period than that of Wechsler et al.[6] The size of the study population and design of clinical trial are important for observing pharmacogenetic effect of ADRB2.

In the present study, LABA treatment was combined with ICS, therefore it is quite possible that the differences in response to ICS between genotype groups could account for the observed differences. However, this possibility is quite unlikely, because that all the study subjects used the same regimen of ICS during the 2-week run-in, and showed improvement in both FEV_1 and MMEF, with no significant differences observed between genotype groups. Our preliminary study demonstrated that genetic variance of ADRB2 at Arg16Gly could not influence the effect of ICS in a Korean population (unpublished data). Therefore, we propose that the genotype-specific effects found in this study may have been derived from the effects of the long-acting β2-agonist and the ADRB2 Arg16Gly polymorphism, although the effects of ICS on the differential response to LABA according to ADRB2 genotype could not completely be excluded.

Another explanation for the difference between this study and that of Wechsler et al.[6] involves the choice of LABA;

formoterol vs. salmeterol. Cho et al.[9] studied the response to a short-acting β2-agonist in a Korean population using albuterol, and found that those carrying the Arg/Arg genotype had better responses to albuterol. This result is similar to our present result, however, dissimilar the finding of long-term albuterol treatment reported by Israel et al.[4,5] Further investigation is needed to replicate these observations in other Asian populations and confirm these apparent pharmaco-ethnic differences.

In this study, FEV_1 and MMEF levels in patients with the Arg/Arg genotype decreased with disappearance of a significant difference in the FEV_1 level between the 2 genotype groups at 24 weeks. However, these findings were observed in subjects with the Arg/Gly or Gly/Gly genotype from 16 weeks. Moreover, the morning/evening daily PEFR results demonstrated that the time to reach the plateau of PEFR levels was significantly shorter in the Arg/Gly or Gly/Gly genotype, suggesting that adverse effects such as downregulation of β2-adrenergic receptor occur earlier in the Arg/Gly or Gly/Gly type, although the mechanism of this effect remains to be elucidated.

In conclusion, Korean asthmatic patients carrying the Arg/Arg genotype at codon 16 of ADRB2 achieve better asthma control with long-term use of combined treatment with a long-acting β2-agonist and ICS, as evidenced by airway function, asthma symptoms, and quality of life. Because of these effects and potential pharmaco-ethic differences in response, ADRB2 genotyping is likely to play an increasingly important role in the choice of treatment for asthmatic patients.

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