Possible protective effects of the *Glu27* allele of ß$_2$-Adrenergic receptor polymorphism in Thai asthmatic patients

Ticha Limsuwan[1], Ammarin Thakkinstian[2], Oravan Verasertniyom[3], Monchand Vanichapuntu[3], John Attia[4], Suchela Janwityanujit[1], and Kanokrat Nantiruj[1]

Summary

The genetic polymorphisms at the 16th (*Arg→Gly*) and 27th (*Gln→Glu*) amino acid positions of the ß$_2$-adrenergic receptor (ADRB2) may be linked to various asthma-related phenotypes. These include the adverse effects on lung function known to occur following the regular use of albuterol. The study aimed to determine the association between these two ADRB2 SNPs, their haplotypes and the phenotypes in Thai asthmatic patients. One-hundred and thirty asthmatic patients were genotyped at the *Arg16Gly* and *Gln27Glu* polymorphisms. Demographic data, disease severities, pulmonary function tests and medication usages were recorded for each patient. The frequencies of the *Arg16* and *Gln27* alleles were found to be 56.9% and 91.2%, respectively, while the linkage disequilibrium coefficient between the two SNPs was 0.36. Three haplotypes were estimated, i.e. *Arg-Gln, Gly-Gln* and *Gly-Glu* with frequencies of 148 (56.9%), 89 (34.2%) and 23 (8.9%), respectively. The mean percentages for predicted FEV$_1$ (%FEV$_1$) for these corresponding haplotypes were 73.5 (SD = 16.3), 72.4 (SD = 17.4) and 80.7 (SD = 13.1), respectively (p = 0.258). Additionally, the number of hospitalizations, emergency visits and inhaled corticosteroid/long-acting ß$_2$-agonist (ICS/ LABA) usages were lower in *Gln/Glu* subjects than for *Gln/Gln* genotyped patients, with values of 0% versus 11.9% (p = 0.122) for hospitalizations;4.5% versus 18.8% (p = 0.121) for emergency visits; and 50% versus 76.6%, (p = 0.042) for ICS/LABA usages. The presence of the *Glu27* allele in Thai asthmatic patients is associated with a decreased asthma severity, higher %FEV$_1$ values, less frequent hospitalizations and emergency visits, and decreased ICS/LABA usage. *(Asian Pac J Allergy Immunol 2010;28:107-14)*

Key words: *adrenergic receptor-ß$_2$, asthma, genetic polymorphism, haplotype, lung function*

From the [1]Allergy Immunology and Rheumatology Division,
[2] Section for Clinical Epidemiology and Biostatistics,
[3]Research Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
[4]Centre for Clinical Epidemiology and Biostatistics, The University of Newcastle, Newcastle, NSW, AU
Corresponding author: Ticha Limsuwan
Allergy Immunology and Rheumatology Division, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama 6th Road, Phyathai, Bangkok, 10400, Thailand
Email: ratls@mahidol.ac.th, tichalim@hotmail.com

Introduction

Over a decade of research has been conducted on the genetic polymorphisms at the 16th (*Arg→Gly*) and 27th (*Gln→Glu*) amino acid positions of the ß$_2$-adrenergic receptor (ADRB2) and their relationship with asthma phenotypes. The findings have led to a number of different, and sometimes conflicting, conclusions. The ADRB2 gene is a 1242 base-pair intronless gene, located on the long arm of chromosome 5 (5q32-q34, MIM # +109690). A minimum of 9 single nucleotide polymorphisms (SNPs) have been identified within the coding region, but only those at amino acid positions 16 and 27 are present at sufficiently high frequencies across the general population. Recombinant cell studies and primary cultures of human airway smooth muscle cells studies show that the *Gly16* allele enhances agonist-promoted ADRB2 down-regulation while the *Glu27* variant appears to play a protective role against this effect[1,2]. Several studies indicate a correlation between the *Gly16* genotype and nocturnal asthma, moderate (to severe) asthma, increased bronchial hyper-responsiveness (BHR) and steroid-dependent asthma[3-7], while a link between

lower BHR and the *Glu27* genotype has been observed[8]. Other studies, however, fail to show concordance between these alleles and the various phenotypes[9,10].

Several *in vivo* studies examining the effects of the *Gly16* genotype on lung function following $ß_2$-agonist therapy reveals conflicting results. An early study involving formoterol, a long-acting $ß_2$-agonist (LABA), showed an increased desensitization to bronchodilators in *Gly16* subjects as compared to their *Arg16* counterparts[11], while large-scale clinical studies revealed decreases in morning peak expiratory flow rates (PEFRs) in homozygous *Arg16* genotyped patients[12]. Interestingly, homozygous *Arg16* subjects were exclusively linked to the *Gln27* SNP, while *Gly16* was linked with *Glu27*[13,14]. The contrasting data may therefore be explained in part by linkage disequilibrium (LD) between *Gly16* and *Glu27*. In this way, different study populations may contain different LD to produce varying results if individual SNPs are considered. A Human Genome Epidemiology (HuGE) network meta-analysis[15] revealed protective effects of the *Glu27* allele on asthma, and this risk was modified by the amino acid at position 16. This was similar to an *in vivo* study indicating that the *Gly-Glu* haplotype leads to an improved response to bronchodilators as compared to the *Arg-Gln* haplotype[16]. However, only a few studies have been performed in Asian populations, where the LD values between the two SNP may differ with other populations. Therefore, we conducted a study examining the effect of individual ADRB2 polymorphisms and haplotypes on asthma severity phenotypes within a Thai population.

Methods

Patients

A cross-sectional study was completed during December 2005 to June 2006 at the Allergy Clinic, Ramathibodi Hospital, Bangkok, Thailand. Ramathibodi Hospital is a 1200-bed tertiary care hospital. All patients with physician diagnoses of asthma were invited to participate in the study. The study was approved by the Ethical Committee of the Faculty of Medicine, Ramathibodi Hospital. All patients gave informed consent before enrollment. A data record form, including demographic characteristics, and asthma phenotypes (e.g. nocturnal symptoms, hospitalization records, and emergency department (ED) visits in the past year) was completed for each patient. Subjects were classified as physically active if they had reported at least one of the following activities: walking continuously for at least 30 minutes per day, lifting or carrying heavy objects at work daily, or participating in sports or physical exercise for more than 2 hours per week. A history of nocturnal asthma was defined as awakening caused by asthma at least once a week for 12 consecutive months[17].

Pulmonary function tests were performed by one of two trained technicians. Percentage of predicted FEV_1 (% FEV_1) and percent reversibility data were recorded. Reversibility was defined as at least 12% improvement in FEV_1 after administration of 400 µg salbutamol[18]. Atopy was defined as positive skin prick test to at least one common aeroallergen (house dust mite, American cockroach, indoor molds, cat, dog, and grass pollen). Patients were requested to record their daily medication use for one month, noting in particular any rescue $ß_2$-agonists.

Genotyping of ADRB2-16 and ADRB2-27 polymorphisms

Genomic DNA was extracted from whole blood using standard techniques. Identification of ADRB2-16 A46G and ADRB2-27 C79G mutations were performed using a 5'-nuclease assay (TaqMan™). Primer and probe sets were designed and manufactured using the Applied Biosystems 'Assay-by-Design' approach (Applied Biosystems, Foster City, CA, U.S.A.). The assays were performed according to manufacturer's instructions on an ABI 7500 Real-Time PCR System. Primer sets for the A/G nucleotide bases at position 16 and the C/G bases at position 27 were as reported by Martinez *et al*[19]. DNA sequencing was performed in 5 randomly selected DNA samples, using a dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) on an ABI 3100 Sequencer to confirm the specific genotypes at both positions.

Statistical analysis

Mean (SD) and frequency (percentage) were used to describe continuous and categorical data, respectively. Genotype frequencies were assessed whether they complied with the Hardy-Weinberg equilibrium (HWE) rule using an exact test since deviation from the HWE rule might be due to

genotyping error[20]. Analysis of variance (ANOVA) was applied to compare means of asthmatic phenotypes (e.g., %FEV$_1$, reversibility) between three genotype groups if the data were in normal distribution, otherwise the Kruskal-Wallis test was applied to compare median values by genotype. Linkage disequilibrium between the two SNPs, in term of D', was assessed as previously described[21]. Haplotype frequencies were estimated using the EM algorithm method[22]. Logistic regression was applied to assessed association between haplotypes and disease severity (i.e., presence or absence of nocturnal symptom, ED visits, and hospitalization). The odds ratio (OR) and its 95% confidence interval (CI) were estimated. All analyses were performed using STATA version 10.0, except estimating haplotypes in which the SimHap[23] was used. *P* values less than 0.05 were considered as statistically significant.

Results

Patient Characteristics

There were 130 patients included in the present study. The mean age was 48.74 (SD = 13.1) years, and females comprised 86.9% of the total. The mean BMI was 25.2 (SD = 4.7) kg/m^2 and the majority were non-smokers (91%). Skin prick tests was positive for aeroallergens in 102 of 108 cases (95.4%). Paternal and maternal asthma were reported by 31.5% and 23.4% of patients, respectively. Less than half of the patients reported nocturnal symptoms, hospitalizations, or emergency department (ED) visits within the past year (46.2%, 9.8% and 16.3%, respectively).

The most common medication used was inhaled corticosteroid (ICS) therapy (n = 89); 27 of 89 (30.3%) used ICS alone, while the remaining 62 (69.7%) used ICS with long-acting ß$_2$-agonist (LABA). Others treatments were short-acting ß$_2$-agonists (61.4%) and allergen immunotherapy treatment (43.2%) (Table 1).

Gly16 and *Glu27* allele frequencies were 43.1% (95% CI: 37.0% - 49.3%) and 8.8% (95% CI: 5.7% - 13.0%). The genotype frequencies for *Arg/Arg*, *Arg/Gly* and *Gly/Gly* were 44 (33.8%), 60 (46.2%) and 26 (20%), respectively, whereas *Gln/Gln* and *Gln/Glu* were 107 (82.3%) and 23 (17.7%), respectively. The *Glu/Glu* genotype was not detected in any samples used in this study. Both *Arg16Gly* and *Gln27Glu* polymorphisms were consistent with HWE (p = 0.480 and p = 0.597, respectively) (Table 2).

ADRB2 polymorphisms and pulmonary function tests

Ninety-four patients (72.3%) were able to complete pulmonary function tests. Reasons for the missing data included: few or no symptoms after long term allergen immunotherapy (n = 14), poor technique (n = 3; 2 were elderly, 1 wearing dental prosthesis), unable to contact participants (n = 9), loss to follow up (n = 1), or refusal for personal reasons (n = 9). Patient characteristics and disease severities between patients who either completed or did not complete pulmonary function tests were similar (Table 3).

Table 1. Characteristics of the asthmatic patients used in this study

Characteristics	**N = 130**
	Mean (SD)
Age, year	48.7 (13.1)
BMI, kg/m^2	25.2 (4.7)
ICS dose, µg, of BUD equivalence	588.1 (384.8)
	Number (%)
Gender	
Male	17 (13.1)
Female	113 (86.9)
Smoking	
Current smoker	1 (0.8)
Ex-smoker	10 (8.2)
Non-smoker	111 (91)
Education	
≥ High school	86 (72.9)
< High school	32 (27.1)
Parental asthma	
Maternal asthma	29 (23.4)
Paternal asthma	18 (14.5)
Both	4 (3.2)
Atopy	
Yes	126 (96.9)
No	1 (0.8)
History of hay fever	
Yes	125 (98.4)
No	2 (1.6)
Skin prick test result	
Positive result	102 (95.4)
Negative result	6 (4.6)
Nocturnal symptom	
Yes	54 (46.2)
No	63 (53.8)
Admit in the past year	
Yes	12 (9.8)
No	111 (90.2)
ED visit in the past year	
Yes	20 (16.3)
No	103 (83.7)
Current ICS use	
Yes	89 (80.2)
No	22 (19.8)
Rescue ß$_2$-agonist use	
Yes	70 (61.4)
No	44 (38.6)
Ever desensitization	
Yes	54 (43.2)
No	71 (56.8)

* BMI, body mass index; BUD; budesonide; ED, emergency department; ICS, inhaled corticosteroid; ICS/LABA, inhaled corticosteroid-long-acting ß$_2$-agonist combination

Table 2. Genotype and allele frequency for *Arg16Gly* and *Gln27Glu* polymorphisms in asthmatic patients

SNP	Number (%)	HWE*
Arg16Gly		0.480
Arg/Arg	44 (33.8)	
Arg/Gly	60 (46.2)	
Gly/Gly	26 (20.0)	
Arg	148 (56.9)	
Gly	112 (43.1)	
Gln27Glu		0.597
Gln/Gln	107 (82.3)	
Gln/Glu	23 (17.7)	
Glu/Glu	0	
Gln	237 (91.2)	
Glu	23 (8.8)	

HWE; Hardy-Weinberg equilibrium

*Exact test

The mean % FEV_1 for *Arg/Arg, Arg/Gly,* and *Gly/Gly* genotypes were 73.1, 74.2, and 73.4, respectively. ANOVA tests revealed that these means were not statistically different (p = 0.953). However, the percent FEV_1 showed greater differences for the *Gln27Glu* genotypes, (comparison of mean values of 72.5 and 80.7 for *Gln/Gln* and *Gln/Glu,* respectively) but again these did not reach statistical significance (p = 0.098) (data not shown).

ADRB2 polymorphisms and disease severity phenotypes

We explored a possible relationship between disease severity (i.e., nocturnal symptom, ED visits, and hospitalization within 1 year) and the ADRB2 polymorphisms. As shown in Table 4, there was not association between either *Gln27Glu* or *Arg16Gly* polymorphism and disease severity. However, dosages of ICS and percentage of ICS/LABA usage in patients with *Gln/Glu* genotypes were significantly lower than in patients with *Gln/Gln* (i.e., 462.5 μg versus 616.9 μg; p = 0.040 for ICS dose, and 50% versus 76.6%, p = 0.042 for ICS/LABA usage). While patients with *Gln/Glu* genotypes generally experienced fewer nocturnal symptoms (31.8% versus 49.5%), and a reduction in emergency visits (4.5% versus 18.8%) and hospitalizations (0% versus 11.9%) than patients possessing *Gln/Gln* genotypes, these values were found to be statistically insignificant.

Arg16Gly and Gln27Glu haplotypes and pulmonary function tests

An estimated LD coefficient (D') of the *Arg16Gly* and *Gln27Glu* polymorphisms was 0.36, which indicated that the *Arg16Gly* and *Gln27Glu* polymorphisms were somewhat linked. Three haplotypes were estimated: *Arg-Gln* (148, 56.9%),

Table 3. Comparison of characteristics between responders versus non responders*

Characteristics	Responders N = 94	Non-responders N = 31	*p* value
Age, year, mean (SD)	48.8 (13.7)	49.6 (11.4)	0.787
Female, no (%)	81 (86.2)	28 (90.3)	0.759
Smoker, no (%)	8 (9.0)	3 (10.3)	0.974
BMI, kg/m^2, mean (SD)	25.6 (4.3)	24.2 (5.4)	0.149
Physical active, no (%)	35 (40.2)	10 (37.0)	0.825
Maternal asthma, no (%)	21/91 (23.1)	6/29 (20.7)	1.000
Paternal asthma, no (%)	15/91 (16.5)	3/29 (10.3)	0.557
Disease Severity			
Presence of nocturnal symptom, no (%)	39/84 (46.4)	15/29 (51.7)	0.670
Ever admit in the past year, no (%)	10/88 (11.4)	2/31 (6.5)	0.729
Ever ED visit in the past year, no (%)	17/88 (19.3)	3/31 (9.7)	0.273
Current ICS use, no (%)	70/86 (81.4)	17/23 (73.9)	0.559
Current ICS dose of BUD equivalence, μg, mean (SD)	591.5 (375.3)	572.5 (451.8)	0.862
Rescue use, no (%)	50/82 (61.0)	19/30 (63.3)	1.000

*Responders are the patients with pulmonary function test done, and non-responders are those without.

Table 4. Comparisons of asthma symptoms, use of treatment and polymorphic genotypes

Severity	Polymorphisms						
	Arg16Gly				*Gln27Glu*		
	Arg/Arg N = 44	*Arg/Gly* N = 60	*Gly/Gly* N = 26	P value	*Gln/Gln* N = 107	*Gln/Glu* N = 23	*P* value
Symptom - based, no. (%)							
Presence of nocturnal symptom							
Yes	22(52.4)	25(44.6)	7 (36.8)	0.504	47 (49.5)	7 (31.8)	0.159*
No	20(47.6)	31(55.4)	12(63.2)		48 (50.0)	15 (68.2)	
History of hospitalization in the past year							
Yes	8 (18.6)	3 (5.4)	1 (4.2)	0.065	12 (11.9)	0 (0)	0.122*
No	35 (81.4)	53(94.6)	23(95.8)		89 (88.1)	22 (100)	
History of ED visit in the past year							
Yes	10 (23.3)	6 (10.7)	4 (16.7)	0.245	19 (18.8)	1 (4.5)	0.121*
No	33 (76.7)	50(89.3)	20(83.3)		82 (81.2)	21 (95.5)	
Treatment-based							
ICS dose of BUD equivalence, µg, mean (SD)	588.6 (370.6)	563.4 (375.2)	647.1 (444.6)	0.757	616.7 (409.6)	462.5 (215.6)	0.040
Rescue ß$_2$-agonist use, puff per month, median (range)	8(1-60)	14(1-99)	10(1-47)	0.191†	12.5 (1-99)	9 (2-47)	0.835††
ICS/LABA usage, no. (%)							
Yes	20 (71.4)	30(68.2)	12(70.6)		53 (76.6)	9 (50.0)	0.042
No	8 (28.6)	14(31.8)	5 (29.4)	0.954	18 (25.4)	9 (50.0)	

*P values were calculated by Exact test (*), Kruskal-Wallis test (†), or Mann-Whitney test (††)

Gly-Gln (89, 34%), and *Gly-Glu* (23, 8.9%), whereas *Arg-Glu* was not detected. Pulmonary function according to haplotype is described in Table 5; the *Gly-Glu* haplotype showed a higher mean % FEV_1 than the other two haplotypes, but this was not statistically significant (p = 0.258). We further tested for a dose-response relationship in the *Gly-Glu* haplotypes. Ideally, subjects should be categorized as 0, 1, 2 if they had 0, 1, or 2 copies of the *Gly-Glu* substitution. However, no subjects containing 2 *Gly-Glu* haplotypes (diplotype) were identified. The mean % FEV_1 in those subjects possessing a single haplotype copy was approximately 8.2% higher than subjects who lacked this haplotype. However, statistical analysis did not reveal significant differences (p = 0.098), i.e., there was no correlation between happlotypes and reversibility in FEV_1 .

Arg16Gly and *Gln27Glu* haplotypes and disease severity phenotypes

Associations between haplotypes and disease severity phenotypes were also examined (Table 6). Subjects containing haplotype *Gly-Gln/Gly-Glu* showed a 68% (95% CI: 10% - 82%) decrease in hospitalization than the *Arg-Gln* haplotype. Although there were less chances of having nocturnal symptoms, ED visits, and using ICS/LABA in patients with *Gly-Glu* than *Arg-Gln* haplotypes (52%, 79%, and 58%, respectively), these were not statistically significant.

Discussion

Our results did not show a statistical significant association between the ADRB2 *Gln27Glu* polymorphism and better asthma parameters. However, results have suggested that the *Glu27* allele had higher % FEV_1, less nocturnal symptoms, less frequent hospitalization and ED visits, and less corticosteroid dose and ICS/LABA usage, in analyses using both the single SNP data and inferred haplotype data.

These results were consistent with previous reports using Chinese hamster fibroblast cells and human bronchial smooth muscle cell cultures[1,2] which showed that the *Glu27* allele was resistant to down-regulation after prolonged stimulation with ß$_2$-agonists than that observed for the *Gln27* allele. The *Gly-Glu* haplotype has also been reported as having a higher bronchodilator response[16] and a protective effect against diurnal PEFR variations in asthmatic patients with regular use of inhaled ß$_2$-agonists, as compared to *Arg-Gln* patients[24]. Together, these results indicate a

Table 5. Comparisons of Lung Function according to haplotypes

Haplotypes	Frequency (number, %)	Lung Function			
		% FEV_1 (mean, SD)	*P* value	Percent reversibility (mean, SD)*	*P* value
Arg-Gln	148 (56.9)	73.5 (16.3)	0.258	16.2 (11.5)	0.897
Gly-Gln	89 (34)	72.4 (17.5)		16.1 (11.3)	
Gly-Glu	23 (8.9)	80.7 (13.1)		14.6 (10.6)	

*Bartless' test for equal variances = 0.931

protective role of *Glu27* in asthma, as also suggested in HuGENet meta-analysis reports and other studies[15,16,24]. The effects of *Arg16* on *Glu27* observed previously[15] could not be replicated here since the *Arg-Glu* haplotype was not identified in this Thai population. The absence of *Arg-Glu* was consistent across several haplotype studies conducted in different ethnic populations, including African-Americans, Asians and Hispanic-Latinos [16, 25].

Our study could not detect association between haplotype and % FEV_1, which might be due to a lack of power for detection of association. The observed means of % FEV1 were 73.5 and 80.7 in *Arg-Gln* and *Gly-Glu* haplotypes. In order to detect a difference between the two haplotypes of 7% of % FEV1, with 80% power of detection and type one error of 5%, we need to recruit at least 188 subjects.

Table 6. Assessing association between asthma symptoms, use of treatments and haplotypes

Severity	Haplotype			P value	OR_1*(95% CI)	OR_2**(95% CI)
	Arg-Gln	*Gly-Gln*	*Gly-Glu*			
Symptom - based, no. (%)						
Presence of nocturnal symptom						
Yes	69 (49.3)	32 (44.4)	7 (31.8)	0.293	0.8 (0.5 - 1.5)	0.5 (0.2 - 1.3)
No	71 (50.7)	40 (55.6)	15 (68.2)			
History of hospitalization in the past year						
Yes	19 (13.4)	5 (6.1)	0	0.053	0.3 (0.1 - 0.9)***	
No	123 (86.6)	77 (93.9)	22 (100.0)			
History of ED visit in the past year						
Yes	26 (18.3)	13 (15.9)	1 (4.6)	0.264	0.8 (.4 -1.7)	0.2 (0.03 -1.7)
No	116 (81.7)	69 (84.1)	21 (95.4)			
Treatment-based						
ICS/LABA usage, no. (%)						
Yes	70 (70.0)	45 (75.0)	9 (50.0)	0.128	1.3 (0.6 – 2.7)	0.4(0.2-1.2)
No	30 (30.0)	15 (25.0)	9 (50.0)			
ICS dose of BUD equivalence, µg, median (range)	400 (100, 1600)	640(100, 1600)	400(200,800)	0.571		
Rescue ß$_2$-agonist use, puff per month, mean (SD)	17.4 (18.9)	20.2 (20.2)	16.1 (16.6)	0.701		

* OR= odds ratio, OR_1= *Gly-Gln* vs *Arg-Gln*

** OR_2= *Gly-Glu* vs *Arg-Gln*

***OR_1= *Gly-Gln* +*Gly-Glu* vs *Arg-Gln*

The association between each haplotypes and asthma severity phenotypes, including nocturnal symptoms, history of hospitalization in the past year, emergency visits or ICS/LABA usage were examined, by comparing the haplotype with the presence of Glycine to that of Arginine at amino acid postion 16. *Arg16* is exclusively linked to *Gln27*, thus it was compared with either *Gly16-Gln27* (OR_1*) or *Gly16-Glu27* (OR_2**) haplotypes. No patient with history of hospitalization in the past year was found in haplotype *Gly-Glu*, so it was combined with haplotype *Gly-Gln* and compare with *Arg-Gln* (OR_1***).

There are interethnic variations in the frequencies of ADRB2 polymorphisms. As compared with Caucasians, Thai asthmatic patients exhibit an approximate four-fold lower allele frequency of the *Glu27* substitution (8.8% versus 41%) and a slightly lower frequency for *Gly16*, (43.1%versus 58%). This is consistent with previous studies conducted in Oriental populations [15]. No homozygous *Glu27* patients were detected in our study, which is similar to previous studies which reported that the *Glu- Glu* genotype was very rare (e.g., 0-1% in Chinese[26,27], and 3% in Mexican[17]).

The LD between the *Arg16Gly* and *Gln27Glu* alleles was lower in Thais than in the Caucasian population (D' = 0.36 versus 0.46)[15]. This can be interpreted that alleles of the two polymorphisms were less correlated in Asian than Caucasians. As a result, the *Gly-Glu* haplotype frequency observed in our study was lower than the frequency reported in Caucasians (i.e., 12% versus 28.8%[16], respectively), but this was not much different compared with the frequency in African-Americans (8.9%)[16,25].

The clinical implications of these findings remain unclear. Firstly, the study should be re-tested using larger datasets. Should the results be confirmed on a larger scale, the genetic effects may be more important in the context of environmental variables, such as smoking status and sex, as well as variations between racial groups. As an example, one study suggests that the genotype effect may be more apparent among nonsmokers[28].

In summary, our results suggest that there is a relationship between the presence of the *Glu27* allele and a protective effect on asthma phenotypes in Thai asthmatic patients. This area of investigation requires larger-scale studies, particularly across multiple ethnic groups.

Acknowledgements

This study was supported by Research Grant from the Faculty of Medicine, Ramathibodi Hospital, Mahidol University No 09-48-292/2006.

References

1 Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of beta 2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. Am J Respir Cell Mol Biol 1995; 13: 25-33.

2 Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. Biochemistry 1994; 33: 9414-9.

3 Contopoulos-Ioannidis DG, Manoli EN, Ioannidis JP. Meta-analysis of the association of beta2-adrenergic receptor polymorphisms with asthma phenotypes. J Allergy Clin Immunol 2005; 115: 963-72.

4 D'Amato M, Vitiani LR, Petrelli G, *et al*. Association of persistent bronchial hyperresponsiveness with beta2-adrenoceptor (ADRB2) haplotypes. A population study. Am J Respir Crit Care Med 1998; 158: 1968-73.

5 Holloway JW, Dunbar PR, Riley GA, *et al*. Association of beta2-adrenergic receptor polymorphisms with severe asthma. Clin Exp Allergy 2000; 30: 1097-103.

6 Reihsaus E, Innis M, MacIntyre N, Liggett SB. Mutations in the gene encoding for the beta 2-adrenergic receptor in normal and asthmatic subjects. Am J Respir Cell Mol Biol 1993; 8: 334-9.

7 Turki J, Pak J, Green SA, Martin RJ, Liggett SB. Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. J Clin Invest 1995; 95: 1635-41.

8 Hall IP, Wheatley A, Wilding P, Liggett SB. Association of Glu 27 beta 2-adrenoceptor polymorphism with lower airway reactivity in asthmatic subjects. Lancet 1995; 345: 1213-4.

9 Dewar JC, Wheatley AP, Venn A, Morrison JF, Britton J, Hall IP. Beta2-adrenoceptor polymorphisms are in linkage disequilibrium, but are not associated with asthma in an adult population. Clin Exp Allergy 1998; 28: 442-8.

10 Weir TD, Mallek N, Sandford AJ, *et al*. Beta2-Adrenergic receptor haplotypes in mild, moderate and fatal/near fatal asthma. Am J Respir Crit Care Med 1998; 158: 787-91.

11 Tan S, Hall IP, Dewar J, Dow E, Lipworth B. Association between beta 2-adrenoceptor polymorphism and susceptibility to bronchodilator desensitisation in moderately severe stable asthmatics. Lancet 1997; 350: 995-9.

12 Israel E, Chinchilli VM, Ford JG, *et al.* Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. Lancet 2004; 364: 1505-12.

13 Israel E, Drazen JM, Liggett SB, *et al.* The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma. Am J Respir Crit Care Med 2000; 162: 75-80.

14 Taylor DR, Drazen JM, Herbison GP, Yandava CN, Hancox RJ, Town GI. Asthma exacerbations during long term beta agonist use: influence of beta(2) adrenoceptor polymorphism. Thorax 2000; 55: 762-7.

15 Thakkinstian A, McEvoy M, Minelli C, *et al.* Systematic review and meta-analysis of the association between {beta}2-adrenoceptor polymorphisms and asthma: a HuGE review. Am J Epidemiol 2005; 162: 201-11.

16 Drysdale CM, McGraw DW, Stack CB, *et al.* Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci U S A 2000; 97: 10483-8.

17 Santillan AA, Camargo CA Jr, Ramirez-Rivera A, *et al*. Association between beta2-adrenoceptor polymorphisms and asthma diagnosis among Mexican adults. J Allergy Clin Immunol 2003; 112: 1095-100.

18 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. Eur Respir J 2005; 26: 319-38.

19 Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J Clin Invest 1997; 100: 3184-8.

20 Attia J, Thakkinstian A, D'Este C: Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. J Clin Epidemiol 2003, 56:297-303.

21 Chapman NH, Thompson EA. Linkage disequilibrium mapping: The role of population history, size, and structure. In: Rao DC, Province MA, eds. Genetic dissection of complex traits, New York, Academic press, 2001; pp. 417-28.

22 Thakkinstian A, D'Este C, Attia J. Haplotype analysis of VDR gene polymorphisms: a meta-analysis. Osteoporos Int 2004; 15: 729-34.

23 McCaskie PA, Carter KW, Palmer LJ. SimHap: A comprehensive modelling framework and a simulation-based approach to haplotypic analysis of population-based data 2004.

24 Hancox RJ, Sears MR, Taylor DR. Polymorphism of the beta2-adrenoceptor and the response to long-term beta2-agonist therapy in asthma. Eur Respir J 1998; 11: 589-93.

25 Hawkins GA, Tantisira K, Meyers DA, *et al*. Sequence, haplotype, and association analysis of ADRbeta2 in a multiethnic asthma case-control study. Am J Respir Crit Care Med 2006; 174: 1101-9.

26 Leung TF, Tang NL, Chan IH, *et al*. Distribution in allele frequencies of predisposition-to-atopy genotypes in Chinese children. Pediatr Pulmonol 2002; 34: 419-24.

27 Wang Z, Chen C, Niu T, *et al*. Association of asthma with beta(2)-adrenergic receptor gene polymorphism and cigarette smoking. Am J Respir Crit Care Med 2001; 163: 1404-9.

28 Litonjua AA, Silverman EK, Tantisira KG, Sparrow D, Sylvia JS, Weiss ST. Beta 2-adrenergic receptor polymorphisms and haplotypes are associated with airways hyperresponsiveness among nonsmoking men. Chest 2004; 126: 66-74.