

Arg16Gly β 2-Adrenergic Receptor Gene Polymorphism in Japanese Patients with Aspirin-Exacerbated Respiratory Disease

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Key Words

β 2-Adrenergic receptor • ADRB2 • Gene polymorphism • Aspirin-exacerbated respiratory disease • Aspirin-tolerant asthma

Abstract

Background: There has been no report that investigated β 2-adrenergic receptor (ADRB2) gene polymorphism in patients with aspirin-exacerbated respiratory disease (AERD).

Methods: DNA in the specimens in three groups of study subjects classified patients with AERD, patients with aspirin-tolerant asthma (ATA) and normal controls was extracted, and the target DNA sequence of the ADRB2 was amplified using a set of primers to generate an amplicon of 219 bp in length. Allelic discrimination assay for single nucleotide polymorphisms relating to the ADRB2 gene expression was carried out by using a previously described single nucleotide polymorphism detective system, sequence-specific thermal-elution chromatography. **Results:** The frequency of the Gly variant allele in patients with AERD was significantly lower than that in patients with ATA ($p = 0.007$), and the odds ratio (OR) of AERD to ATA associated with wild-type ArgArg homozygote was 3.300. Frequencies of wild-type ArgArg ho-

mozygote are significantly higher than those of variant-type ArgGly/GlyGly genotype in patients with AERD compared with those with ATA ($p < 0.001$, OR = 3.153). In patients with AERD, frequencies of wild-type ArgArg homozygote in both female and male patients are significantly higher than those of variant-type ArgGly/GlyGly genotype in male patients compared with those with ATA ($p < 0.001$, OR = 5.128 and $p = 0.007$, OR = 4.367, respectively). Also, in patients with AERD, frequencies of wild-type ArgArg homozygote in female patients are significantly higher than those of variant-type ArgGly/GlyGly genotype in female patients compared with those with ATA ($p = 0.002$, OR = 2.825). **Conclusions:** We were the first to analyze Arg16Gly ADRB2 gene polymorphism in Japanese patients with AERD, and showed that Arg-16Gly ADRB2 gene polymorphism in Japanese patients with AERD is different from that in the patients with ATA.

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Introduction

Aspirin-exacerbated respiratory disease (AERD), so-called aspirin-intolerant asthma, which affects 10–20% of adult asthmatics, is known to represent acute asthmatic

attacks by means of ingestion of aspirin and other non-steroidal anti-inflammatory drugs (NSAID) [1]. Individuals with AERD are more commonly found in middle-aged female, and their asthmatic symptoms tend to be chronic, perennial and severe [2–5]. However, the pathophysiologic mechanisms underlying the development of this specific asthma phenotype have not yet been fully understood.

Recent studies indicated that asthma is due to a strong combination of genetic factors [6]. The inhibitory action of aspirin and NSAID on cyclooxygenase (COX) activity may cause diversion to the 5-lipoxygenase pathway, which leads to the overproduction of cysteinyl leukotrienes (LTs) [7]. Therefore, genetic association studies of LT-related genes have been undertaken to explore the genetic determinants of AERD. In fact, LTC₄ synthase promoter polymorphism has been reported to be associated with AERD [8, 9]. Recent investigations have shown that the genetic polymorphisms of 5-lipoxygenase promoter [10] and cysteinyl LT receptor 1 promoter [11] influence the susceptibility to AERD as risk factors. However, conflicting results have also been reported [12, 13].

As current asthma treatments are effective for most but not all patients and LT modifiers may be preferred to AERD, pharmacogenomics offers the opportunity to ascertain associations between genetic variability and response to various asthma medications [14]. β_2 -Adrenergic bronchodilators act directly on β_2 -adrenergic receptor (ADRB2), which is encoded by an intronless gene, located on chromosome 5q31–32 [15]. It contains several reported single nucleotide polymorphisms (SNPs) [16], and three nonsynonymous polymorphisms are present in the coding region of ADRB2 gene, including Arg16Gly (A46G, rs1042713), Gln27Glu (C79G, rs1042714) and Thr164Ile (C491T, rs1800888) [17–19]. Although the ADRB2 gene is not considered to be a major susceptibility gene for asthma, it has been suggested that its variant alleles may play a role in intermediate or asthma-associated phenotypes [20], such as airway hypersensitivity [21], asthma severity [22] and response to medications [23].

The most commonly cited SNPs in the ADRB2 gene occur at nucleotide position 46 (guanine/adenine) and 79 (cytosine/guanine), and result in the substitution of glycine (Gly) for arginine (Arg) at codon 16 (Arg16Gly) and glutamic acid (Glu) for glutamine (Gln) at codon 27 (Gln27Glu). Following the identification of SNPs in the ADRB2 gene that affect cell-surface expression or ligand-binding activity of the receptor, there has been particular interest in pharmacogenetic associations related to this receptor [17–19, 24]. Currently, only the pharmacogenetic effects on the Arg16Gly polymorphism appear to be of

sufficient magnitude to affect asthma therapy, although whether the ADRB2 gene polymorphisms are associated with airway responsiveness to β_2 -adrenergic bronchodilators remains controversial [25–29].

Because the asthmatic symptoms of AERD tend to be chronic, perennial and severe [3–5], we hypothesized that ADRB2 gene polymorphisms might differ between patients with AERD and those with ATA. To the best of our knowledge, there has been no report that investigated the ADRB2 gene polymorphism in patients with AERD. As shown in the previous reports about the genotype frequencies of the ADRB2 gene in Asian populations [30–32], position 491 is not polymorphic in Japanese. The allelic frequency of Gln27Glu polymorphism of the ADRB2 gene is less prevalent among Japanese than white populations, and only 7.5% of the subjects carried the polymorphism [33]. In fact, the frequency of GluGlu in a Japanese population is 2.3% in the dbSNP database of the National Center for Biotechnology Information. On the other hand, the frequency of Arg16 allele is 53.8% in a Japanese population, which is similar to that observed in white populations [33]. So, in the present study, Arg16Gly polymorphism was regarded as representative SNP for the analyses. This is the first report of the Arg16Gly ADRB2 gene polymorphism in Japanese patients with AERD.

Subjects and Methods

Subjects

This study was performed with the approval of the Institutional Ethics Committee of the Gunma Institute for Allergy and Asthma, Gunma Hospital for Allergic and Respiratory Diseases, and written informed consent was obtained from each individual before the study commenced.

All subjects were nonsmoking Japanese and were recruited from the outpatient clinic of Gunma Hospital for Allergic and Respiratory Diseases, Yukawa Clinic of Internal Medicine and Hiroshima Allergy and Respiratory Clinic in Japan. Smoking habit was ascertained by means of a questionnaire. Characteristics of the study population are shown in table 1.

Diagnosis of bronchial asthma was confirmed using the Global Initiative for Asthma guidelines. All patients showed clinical symptoms that met the criteria for asthma, such as cough, wheeze and shortness of breath, and they were diagnosed by experienced pulmonologists. Forced expiratory volume in 1 s (FEV₁) was measured with a spirometer, and airway reversibility was defined as a >12% and >200 ml increase in volume in the first second of forced expiration from baseline after inhalation of short-acting β_2 -adrenergic bronchodilators. The diagnosis of AERD was made on the basis of either a positive result on lysine-aspirin challenge test [34] or an apparent history of more than one self-reported episode of bronchial response to aspirin or NSAID ingestion. The provocation test could not be applied to the subjects who did not give writ-

Table 1. Clinical characteristics of the study subjects

	AERD	ATA	NC
Number of subjects	95	300	100
Age, years	51.4 ± 13.4	49.8 ± 15.9	47.1 ± 13.6
Male gender	26 (27.4%)	107 (35.7%)	34 (34.0%)
FEV ₁ , % predicted	73.7 ± 11.5	75.5 ± 24.5	NA
Serum total IgE, IU/ml ^a	206.6 ± 232.9	458.5 ± 651.5	NA
Atopy ^b	21 (22.1%)	129 (43.0%)	NA
Pollinosis ^c	40 (42.1%)	179 (59.7%)	NA

NC = Normal controls; NA = not applicable.

^a $p < 0.001$ for AERD patients versus ATA patients by the Welch t test.

^b $p < 0.001$ for AERD patients versus ATA patients by the χ^2 test.

^c $p = 0.003$ for AERD patients versus ATA patients by the χ^2 test.

ten informed consent mainly because of the risk of significant occult disease, although the likelihood of severe reaction was considered very low [4]. Patients with AERD combined with aspirin-intolerant urticaria were excluded from this study. Aspirin-tolerant asthma (ATA) was defined as bronchial asthma with no history of NSAID-induced asthma attack. A total of 100 nonsmoking subjects with no history of bronchial asthma or other respiratory symptoms were selected from healthy volunteers who visited our clinic for annual routine physical examinations, and comprised normal controls. The serum levels of total IgE were measured by the CAP system (Phadia, Uppsala, Sweden). Also, serum specific IgE antibodies were measured, and the patients with positive radioallergosorbent test scores for house dust mite and Japanese cedar were classified as atopy and pollinosis, respectively. The presence of rhinosinusitis and nasal polyps could not be determined because of the absence of otolaryngologists at the hospital who were specialized in using paranasal sinus X-ray and rhinoscopy.

Genotyping of ADRB2 Polymorphism

DNA in the specimens obtained by rubbing buccal mucosa with a cotton swab was extracted by using QIAamp 96 DNA blood kits (Qiagen, Hilden, Germany). The target DNA sequence of the ADRB2 NM_000024.4 was amplified using a set of primers that were previously described [35, 36] (forward, nucleotides 188–212: 5'-AGCCAGTGCGCTCACCTGCCAGACT-3'; reverse, nucleotides 406–383: 5'-GCTCGAACTTGGCAATGGCTGTGA-3') to generate an amplicon of 219 bp in length. Allelic discrimination assay for SNPs relating to the ADRB2 expression (rs 1042713) was carried out by using a previously described SNPs detective system, sequence-specific thermal-elution chromatography [37]. All subjects and investigators remained unaware of the genotype until the final analysis.

Statistical Analysis

Data are presented as means ± SD or numbers (with percentages in parentheses) of observations, unless stated otherwise. Differences in the clinical characteristics among the three participant groups (patients with AERD, patients with ATA and normal controls) were compared using either the ANOVA test or t test, and qualitative data were compared by the χ^2 test. Allele frequen-

cies were estimated by gene counting method. Significant departures of genotype frequency from the Hardy-Weinberg equilibrium were tested by the χ^2 analysis. Differences in minor allele (Gly) frequency in patients with AERD and control subjects were compared with that in patients with ATA by means of the χ^2 test and calculation of odds ratio (OR) with 95% confidence interval (CI). OR with 95% CI associated with ArgArg of patients with AERD was compared with that of patients with ATA. Polymorphisms related to the asthma phenotype were further examined by multivariable logistic regression models with adjustment for covariates, namely with the asthma phenotype as dependent variable and independent variables including age (continuous value), gender (male = 0, female = 1), two alternatives genotype models that were wild-type allele (ArgArg) and variant-type alleles (ArgGly/GlyGly), and interaction between gender and genotype (male with ArgGly/GlyGly = 0, female with ArgGly/GlyGly = 1, male with ArgArg = 2, female with ArgArg = 3; female with ArgGly/GlyGly = 0, male with ArgGly/GlyGly = 1, male with ArgArg = 2, female with ArgArg = 3). Differences in the mean value of the phenotypic characteristics within the patients with AERD were compared using the t test. Statistical analyses were undertaken using SPSS for Windows version 17 (SPSS Inc., Chicago, Ill., USA). $p < 0.05$ were considered to be significant.

Results

The clinical characteristics of the study subjects are summarized in table 1. The levels of serum total IgE in the patients with AERD were significantly lower than those in the patients with ATA ($p < 0.001$). There were significant differences in prevalence of atopy and pollinosis between the patients with AERD and the patients with ATA ($p < 0.001$ and $p = 0.003$, respectively).

Table 2 shows the frequencies of the genotype and the minor Gly allele in each group. The genotype distribution fulfills the Hardy-Weinberg equilibrium except for the

Table 2. Genotype frequencies of the ADRB2 gene in each group

Genotype	ArgArg	ArgGly	GlyGly	Allele frequency	p value	OR	HWE p value
AERD (n = 95)	32 (33.7%)	33 (34.7%)	30 (31.6%)	0.489	0.007	3.300 (2.252–4.831)	<0.05
ATA (n = 300)	40 (13.3%)	160 (53.4%)	100 (33.3%)	0.600	–	1	0.054
NC (n = 100)	15 (15.0%)	50 (50.0%)	35 (35.0%)	0.600	1.000	–	0.676

Minor Gly alleles in patients with AERD and control subjects were compared with that in patients with ATA by means of the χ^2 test. OR 95% CI (in parentheses) associated with ArgArg of patients with AERD was compared with that of patients with ATA.

HWE = Hardy-Weinberg equilibrium.

Table 3. Multivariable logistic regression analysis of genotype of the ADRB2 gene in Japanese patients with AERD compared with those with ATA

Value	OR	p value
Age	0.996 (0.980–1.013)	0.661
Gender		
Male	1.000	
Female	1.287 (0.748–2.214)	0.362
Genotype		
ArgGly/GlyGly	1.000	
ArgArg	3.153 (1.789–5.558)	<0.001
Gender $\times$ (Genotype) ¹		
Male (ArgGly/GlyGly)	1.000	
Female (ArgGly/GlyGly)	1.815 (0.960–3.436)	0.067
Male (ArgArg)	4.367 (1.495–12.821)	0.007
Female (ArgArg)	5.128 (2.331–11.236)	<0.001
Female (ArgGly/GlyGly)	1.000	
Male (ArgGly/GlyGly)	1.815 (0.960–3.436)	0.067
Male (ArgArg)	2.404 (0.901–6.410)	0.080
Female (ArgArg)	2.825 (1.453–5.495)	0.002

Figures in parentheses are 95% CI.

¹ The interaction between gender and genotype.

AERD group ($p < 0.05$). Samples were re-genotyped and all these results were replicated according to previously reported recommendations [38]. The frequencies of the Gly allele in patients with ATA [frequency of allele (q) = 0.600] and control subjects (q = 0.600) were the same, whereas the frequency in patients with AERD was decreased (q = 0.489). The frequency of ArgArg homozygote in patients with AERD was significantly higher than that in patients with ATA (p = 0.007, OR = 3.300, 95% CI = 2.252–4.831).

Table 3 shows the results of multivariable logistic regression analysis of the genotype controlling age and gen-

der in patients with AERD compared with those with ATA. Frequencies of wild-type ArgArg homozygote are significantly higher than those of variant-type ArgGly/GlyGly genotype in patients with AERD compared with those with ATA ($p < 0.001$), and the OR of patients with AERD associated with wild-type ArgArg homozygote to those with variant-type ArgGly/GlyGly genotype was 3.153 (95% CI = 1.789–5.558). In patients with AERD, frequencies of wild-type ArgArg homozygote in both female and male patients are significantly higher than those of variant-type ArgGly/GlyGly genotype in male patients compared with those with ATA ($p < 0.001$, OR = 5.128, 95% CI = 2.331–11.236, and p = 0.007, OR = 4.367, 95% CI = 1.495–12.821, respectively). Also, in patients with AERD, frequencies of wild-type ArgArg homozygote in female patients are significantly higher than those of variant-type ArgGly/GlyGly genotype in female patients compared with those with ATA (p = 0.002, OR = 2.825, 95% CI = 1.453–5.495).

Then we assessed the clinical characteristics of patients with AERD in relation to genotypes between wild-type ArgArg and variant-type ArgGly/GlyGly. FEV₁ (% predicted) in the patients with ArgArg and ArgGly/GlyGly were 71.4 ± 13.6 and 74.9 ± 10.1 , respectively, and there was no significant difference between them (p = 0.171). Serum total IgE levels in the patients with ArgArg and ArgGly/GlyGly was 170.5 ± 188.5 IU/ml and 209.4 ± 246.4 IU/ml, respectively, and there was no significant difference between them.

Discussion

Although the ADRB2 gene is not considered to be a major susceptibility gene for asthma, it has been suggested that its variant alleles may play a role in intermediate or asthma-associated phenotypes [20], such as airway hy-

persensitivity [21], asthma severity [22] and response to medications [23]. However, to the best of our knowledge, there has been no report that investigated the ADRB2 polymorphism in patients with AERD.

Interestingly, previous studies have found that ADRB2 polymorphisms are of functional importance for the receptor [39], and the association between ArgArg homozygote of the Arg16Gly polymorphism and decreased lung function in asthma at age 11 years has been reported [40]. On the other hand, medications for asthma such as β_2 -adrenergic bronchodilators used for the treatment of AERD may affect the features because it has been suggested that a subpopulation of patients with asthma could be at risk for severe exacerbations dealt with by the use of β_2 -adrenergic bronchodilators [41, 42]. Because the asthmatic symptoms of AERD tend to be chronic, perennial and severe [3–5], we hypothesized that the ADRB2 gene polymorphisms might differ between patients with AERD and those with ATA.

The present study, in the initial screen, showed the genotype frequencies of the Arg16Gly polymorphism in the three groups (patients with AERD, patients with ATA and normal controls). The genotype distribution fulfills the Hardy-Weinberg equilibrium except for the AERD group. So, samples were re-genotyped and all these results were replicated according to previously reported recommendations [38]. The frequencies of the Gly allele in patients with ATA and control subjects were the same, whereas the frequency in patients with AERD was decreased. The frequency of ArgArg homozygote in patients with AERD was significantly higher than that in patients with ATA. In the second screen of the present study, the polymorphisms related to AERD were further examined by multivariable logistic regression analysis with adjustment for covariates. As the results, frequencies of wild-type ArgArg homozygote are significantly higher than those of variant-type ArgGly/GlyGly genotype in patients with AERD compared with those with ATA. In patients with AERD, frequencies of wild-type ArgArg homozygote in both female and male patients are significantly higher than those of variant-type ArgGly/GlyGly genotype in male patients compared with those with ATA. Also, in patients with AERD, frequencies of wild-type ArgArg homozygote in female patients are significantly higher than those of variant-type ArgGly/GlyGly genotype in female patients compared with those with ATA.

The present study has certain limitations. First, the number of study subjects was limited. Second, patients with AERD were diagnosed by experienced pulmonolo-

gists, and the diagnosis of AERD was made on the basis of either a positive result on lysine-aspirin challenge test [34] or an apparent history of more than one self-reported episode of bronchial response to aspirin or NSAID ingestion. However, some patients, who did not give written informed consent, did not undergo the provocation test because of the risk of significant occult disease, suggesting a presence of some false positives in the patients. Also in the present study, ATA was defined as bronchial asthma with no history of NSAID-induced asthma attack. If possible, asthmatic patients who not only had no history of aspirin and/or NSAID intolerance but also showed a negative response in the aspirin challenge test should be classified as having ATA. Ideally everyone in the study should have the challenge test to be certain about aspirin intolerance. Finally, although the association between ArgArg homozygote of the Arg16Gly polymorphism and decreased lung function has been reported [40] and it has also been suggested that a subpopulation of patients with asthma could be at risk for severe exacerbations dealt with use of β_2 -adrenergic bronchodilators [41, 42], the relation among the genotype, lung function status and the medications including β_2 -adrenergic bronchodilators in the asthmatic patients was not investigated as the patients' questionnaire about the use of the drugs, such as whether they took them repeatedly or not, were not recruited enough to the Gunma Institute for Allergy and Asthma. The present study showed that FEV₁ (% predicted) in AERD patients with ArgArg and ArgGly/GlyGly were 71.4 ± 13.6 and 74.9 ± 10.1 , respectively, and there was no significant difference between them ($p = 0.171$). Therefore, the results of the present study should be interpreted with caution. However, a good number of the patients with AERD were recruited from the outpatient clinics of experienced pulmonologists in Japan. Interestingly, a recent study indicated a possible interaction of four-locus genes including Arg16Gly genotype and cysteinyl LT receptor 1 promoter genotype in Korean subjects with AERD [43], suggesting the possible interactions with ADRB2 and overproduction of cysteinyl LTs in pathobiology of AERD. Further studies are required.

In conclusion, this study showed that the Arg16Gly ADRB2 gene polymorphism in Japanese patients with AERD is different from that in the patients with ATA, suggesting that sequence variations in the ADRB2 gene could contribute to the development of AERD. Further studies are required including a replication study in another population.

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