

ORIGINAL ARTICLE

UGT1A1*28 is associated with greater decrease in serum K⁺ levels following oral intake of procaterolNorihito Yokoe, MD¹, Etsuro Yamaguchi, MD, PhD¹, Masaki Nishimura, MD¹, Hiroyuki Tanaka, MD¹, Ayumu Takahashi, MD, PhD¹, Kenji Baba, MD, PhD², Masahiko Goshō, PhD³, and Shoshiro Okada, MD, PhD⁴¹Division of Respiratory Medicine and Allergology, Department of Internal Medicine, Aichi Medical University, Nagakute, Japan, ²Medical Clinic, Aichi Medical University, Nagakute, Japan, ³Advanced Medical Research Center, Aichi Medical University, Nagakute, Japan, and ⁴Department of Pharmacology, School of Medicine, Aichi Medical University, Nagakute, Japan**Abstract**

Background and objective: Procaterol is a potent β_2 -agonist frequently used for the management of asthma and chronic obstructive pulmonary disease. The efficacy and adverse effects of β_2 -agonists are heterogeneous in individual patients, which may be partly caused by genetic variations in metabolizing enzymes and receptor molecules. The present study was designed to analyze the relationship between gene polymorphisms and physiological effects of procaterol in healthy subjects. **Methods:** Ninety-two non-smoking healthy volunteers were given 1 μ g/kg body weight (max 50 μ g) of procaterol as a dry syrup preparation, and the serum concentrations of procaterol, serum K⁺, and the physical responses were monitored for 240 min. We genotyped β_2 -adrenergic receptor (*ADRB2*) (Arg16Gly and Gln27Glu), cytochrome P450 3A4 (rs2246709, rs4646437), and uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) (rs4148323 [allele A, *6], rs12479045, rs4148328, rs4663971, rs12052787, rs4148329, A (TA)_{6/7} TAA [seven-repeat allele, *28]). Procaterol concentrations in serum were measured by liquid chromatography-tandem mass spectrometry. **Results:** No gene polymorphisms affected serum procaterol concentrations. Meanwhile, overall serum K⁺ level changes were significantly lower in carriers of *UGT1A1**28 than in non-carriers after correcting for strong effects of serum procaterol concentrations and baseline K⁺ levels. No other polymorphisms were associated with serum K⁺ levels. None of polymorphisms of *ADRB2* were associated with any physical responses. **Conclusion:** The present study indicates that significant hypokalemia may occur in carriers of *UGT1A1**28 by systemic administration of procaterol and potentially by other β_2 -agonists metabolized in the liver.

Keywords β_2 -adrenergic receptor, cytochrome P450, uridine diphosphate glucuronosyltransferase 1A1, single nucleotide polymorphism**History**

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Introduction

Bronchial asthma is characterized by variable airflow obstruction due to airway inflammation and hyperresponsiveness. Anti-inflammatory treatments with inhaled corticosteroids (ICS) are recommended as first-line maintenance therapy [1]. For patients whose asthma is poorly controlled by ICS therapy alone, the addition of β_2 -agonist yields better control of asthma symptoms [2]. β_2 -agonists are also the mainstay of pharmacotherapy for chronic obstructive pulmonary disease [3].

Procaterol (Meptin[®]) is a potent, orally and inhalationally active β_2 -agonist used to treat reversible bronchospastic disease [4]. It was first launched in Japan in 1980, and has been widely approved in Japan and other Asian countries. The action of procaterol is more selective on bronchial smooth muscle than on cardiac muscle [5]. β_2 -agonists have adverse

effects such as tachycardia and tremor. In addition, they decrease serum K⁺ levels by activation of the Na⁺-K⁺ pump on cell membranes, which results from increased adenylyl cyclase activity by β_2 -agonists [6–8]. The efficacy and adverse effects of β_2 -agonists are heterogeneous in patients, which might be partly caused by genetic variations in the β_2 -adrenergic receptor gene (*ADRB2*) responsible for function [9]. *ADRB2* is located on chromosome 5q31-q32 and encodes the β_2 -adrenergic receptor which is a G-protein coupled receptor expressed in airway smooth muscle and induces bronchial relaxation [10]. Variable results have been reported on the association between the functional polymorphisms Arg16Gly and Gln27Glu of *ADRB2* and the response to β_2 -agonist inhalation or other phenotypes of asthma patients [11–15].

In addition to receptor function, individual variation in metabolic rate might be an important determinant of adverse effects. Cytochrome P450 3A4 (*CYP3A4*) and uridine diphosphate glucuronosyltransferase (*UGT*) 1A1 are responsible for the metabolism of procaterol [16,17]. Similarly, other β -agonists are metabolized by these enzymes [18]. *CYP3A4* is the most abundant P-450 enzyme in the human liver, and

Correspondence: Etsuro Yamaguchi, MD, PhD, Division of Respiratory Medicine and Allergology, Department of Internal Medicine, School of Medicine, Aichi Medical University, Yazako 1-1, Karimata, Nagakute, Aichi 480-1195, Japan. Tel: +81-561-62-3311. E-mail: etsuro@aichi-med-u.ac.jp

CYP3A4 enzymes metabolize more than 50% of prescription drugs [19]. *CYP3A4* activity varies widely in humans, and many DNA sequence polymorphisms influence patient's responses to commonly prescribed drug classes [20]. Most studies on genetic variation of *UGT1A1* have focused on the *UGT1A1**28 variant (a common polymorphism in the *UGT1A1* promoter TATA box). Since this variant confers slow metabolizing activity, patients either heterozygous or homozygous for the *UGT1A1**28 allele (known as the 6/7 or 7/7 genotype) are at greater risk for severe diarrhea or neutropenia induced by irinotecan, an antineoplastic agent [21].

The major limitation of the variable results on *ADRB2* gene polymorphism from previous studies may be that the study subjects were all asthma patients in whom the effects of other drugs and the disease itself cannot be excluded; such factors may confound the results of all types of genetic studies. Therefore, the present study was designed to analyze the relationship between gene polymorphisms of *ADRB2*, *CYP3A4*, and *UGT1A1*, and the serum concentrations and physiological effects of procaterol in healthy subjects.

Materials and methods

Subjects, drug administration and monitoring

The subjects were 92 non-smoking students (52 men and 40 women; mean age, 23.3 [SD, 2.5] years) without past history of major diseases. They were all Japanese and recruited at Aichi Medical University. They had no abnormalities on annual health checkup. This study was planned based on the ethical guidelines of human genome and gene analysis and approved by the Institutional Review Board of Aichi Medical School (No. 261). All the subjects gave written informed consent for collecting their peripheral blood samples and use of medical information for this study.

Procaterol as a dry syrup preparation was given at a dose of 1 µg or less/kg body weight (BW), since the maximum dose per subject was limited to 50 µg because of the regulation by the Japanese Pharmaceutical Affairs Law. The drug was administered orally after more than 12 h of fasting. Venous blood for the measurement of procaterol concentrations and serum K⁺ levels was collected immediately before taking procaterol and at 60, 120, and 240 min after taking the drug. Blood pressure, pulse rate, and intensity of hand tremor were recorded before taking the drug and at the times of blood collection. Tremor was graded as 0 (no visible tremor), 0.5 (subtle tremor), 1 (apparent but mild tremor without difficulty in writing), or 2 (difficult in writing because of tremor).

Measurement of procaterol concentration and K⁺ levels in serum

Chemicals and reagents

Procaterol hydrochloride and ¹³C-labelled procaterol hydrochloride were provided by Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan).

Instrumentation

The LC/MS/MS system consisted of an Agilent 1200 and an HTS-PAL auto-sampler. Mass spectrometric detection was

conducted on an AB/MDS Sciex API5000 system (ABI, Foster City, CA) equipped with Turbolon Spray interface. The data acquisition software used was Analyst version 1.4.2 (AB Ciex, Framingham, MA).

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) conditions

The analytes were separated on an L-column ODS (2.1 mm × 150 mm, 5 µm, Chemicals Evaluation and Research Institution, Sugito, Japan) and maintained at 40 °C. The mobile phase flow rate was 0.2 ml/min. The eluents were (A) 10 mmol/L ammonium acetate and (B) methanol, with gradient 20% B to 70% B in 5 min, hold 20% B to 9 min. The mass spectrometer was operated in positive ion mode with an electrospray ionization source for the analytes. Quantification was performed using multiple reactions monitoring of the m/z 291→231 transition for procaterol and the m/z 294→234 transition for ¹³C-procaterol with a scan time of 500 ms per transition. The source desolvation temperature was 700 °C.

Sample extraction

An aliquot of 500 µl of serum (calibrator, QC, subject) was spiked with 25 µl of working internal standard (¹³C-labeled procaterol hydrochloride, 12 ng/ml in water) and then diluted with 500 µl of water. Sample extraction was performed by solid-phase extraction (SPE) with ISOLUTE C18 EC cartridges, 500 mg/3 ml format from International Solvent Technologies (Mid Glamorgan, UK). After conditioning and equilibrating of SPE columns with 2 ml of methanol and 2 ml of water, the whole samples were loaded on SPE columns. After the samples were passed through, SPE columns were washed with 2 ml of water and 2 ml of 20/80 methanol/water. The procaterol samples were eluted slowly with 2 ml 60/40/1 water/methanol/acetic acid, dried in a centrifuge evaporator, and reconstituted in 200 µl of 90/10/1 water/methanol/acetic acid. An aliquot of 10 µl was injected into the LC/MS/MS system for analysis.

Serum K⁺ levels were measured by the electrode method used for routine clinical laboratory assay.

Genotyping

Two tag single nucleotide polymorphisms (SNPs), rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu), were selected for *ADRB2*. Likewise, rs2246709 and rs4646437 were selected for *CYP3A4*. These SNPs were genotyped using TaqMan probes. *UGT1A1* A (TA)_{6/7}TAA was determined by direct sequencing. Four tag SNPs of *UGT1A1* (rs4148323, rs4148328, rs4663971 and rs12479045) were genotyped by the amplification refractory mutation system (ARMS) [22]. Primer sequences are presented in Supplementary Table 1. TaqMan probes were used for genotyping other tag SNPs of *UGT1A1* (rs4148329 and rs12052787). Three subjects could not be genotyped for either one of polymorphisms, rs2246709, rs1042714 and rs12479045.

Statistical analysis

Simple comparison of serum K⁺ levels at each time point with the baseline levels was made by repeated measure

analysis of variance. More precise analysis according to genotypes of *CYP3A4*, *ADRB2*, and *UGT1A1* was performed using the repeated measure analysis of covariance (ANCOVA). Multiple regression analysis was also used to further examine the effects of *UGT1A1* A (TA)_{6/7}TAA. The effect of polymorphisms on serum procaterol concentrations and physical responses were adjusted by the dose of procaterol/kg BW as a covariate, since some volunteers took procaterol of less than 1 µg/kg BW because of the fixed maximum dose of 50 µg/body. The effect of polymorphisms on serum K⁺ levels was adjusted by serum K⁺ levels before taking procaterol and procaterol concentration at each time point as covariates. In post-hoc comparisons, the nominal α level was adjusted by Bonferroni's correction. Correlation between procaterol concentration and serum K⁺ levels at each time point was assessed by the Pearson's correlation coefficient. The *p* value of 0.05 was set as a significant level. All statistical analyses were made using IBM SPSS Statistics ver 22 (Tokyo, Japan). Data are presented as mean [SD] in the text and mean $\pm$ SE in the figures.

The strength of linkage disequilibrium estimated by the squared-allele frequency correlations (*r*²) was calculated using SNPalyze (DYNACOM, Chiba, Japan). Haplotypes were inferred by SNPalyze, too.

Results

Genotypes of polymorphisms

Genotype frequencies are presented in Supplementary Table 2. The genotype distributions of all polymorphisms were mostly consistent with those posted by JSNP DATABASE (<http://snp.ims.u-tokyo.ac.jp/>) and were not significantly deviated from the Hardy–Weinberg equation. Regarding linkage disequilibrium, *r*² between rs2246709 and rs4646437 of *CYP3A4* was 0.1285 and that between rs1042713 and rs1042714 of *ADRB2* was 0.0742. Those for *UGT1A1* are presented in Supplementary Table 3.

Procaterol concentrations and gene polymorphisms

The mean [SD] serum procaterol concentrations for all study subjects at 60, 120, and 240 min after taking procaterol were 111 [46] pg/ml, 100 [38] pg/ml, and 71 [22] pg/mL (Figure 1), respectively. These values are similar to a previous study [23]. *p* Values by repeated measure ANCOVA to test association between serum procaterol concentrations and each polymorphism are presented in Table 1. No polymorphisms were associated with procaterol concentration.

K⁺ levels and gene polymorphisms

The mean [SD] K⁺ levels of procaterol for all study subjects at the baseline, 60, 120, and 240 min after taking procaterol were 3.9 [0.2], 3.6 [0.3], 3.4 [0.3], and 3.5 [0.3] mEq/L, respectively. All serum K⁺ levels after taking procaterol were significantly decreased as compared with the baseline levels (baseline vs. 60 min, *p* = 1.0 $\times$ 10^{−18}; vs. 120 min, *p* = 3.3 $\times$ 10^{−23}, vs. 240 min, *p* = 2.4 $\times$ 10^{−21}). Meanwhile, there was a strong correlation between procaterol concentrations and serum K⁺ levels (Figure 2). To correct this strong effect of procaterol and to clarify the independent effect of gene polymorphisms,

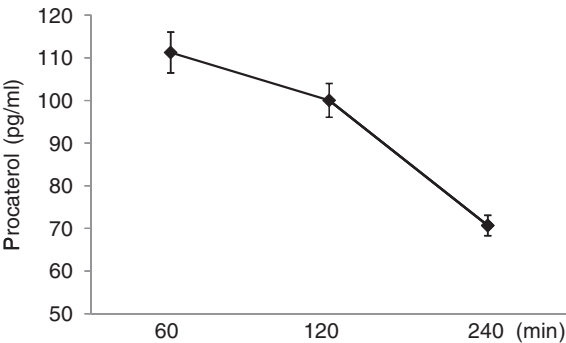


Figure 1. Mean serum procaterol concentrations after taking the drug. Data are mean $\pm$ SE.

Table 1. Association between serum procaterol concentrations and genotypes of each polymorphism.

Polymorphism	<i>df</i>	<i>F</i>	<i>p</i> Value
<i>CYP3A4</i>			
rs2246709	(2, 87)	0.355	0.702
rs4646437 ^a	(1, 89)	0.483	0.489
<i>UGT1A1</i>			
A(TA) _{6/7} TAA ^{a,b}	(1, 89)	0.088	0.767
rs4148323 ^c	(2, 88)	0.182	0.834
rs4148328	(2, 88)	1.083	0.343
rs4148329	(2, 88)	0.152	0.859
rs4663971	(2, 88)	0.430	0.652
rs12052787 ^a	(1, 89)	0.484	0.488
rs12479045 ^a	(1, 88)	0.031	0.861

Association between the temporal changes in serum procaterol concentrations and each polymorphism were assessed by repeated measure ANCOVA with the dose of procaterol/BW as a covariate, and *p* values are presented with degrees of freedom (*df*) and *F* values.

^aThree genotypes were grouped into homozygote of major allele and the other genotype, because only a few homozygotes of minor allele were present for these polymorphisms.

^bSeven-repeat allele is *UGT1A1**28.

^cThe allele A of this SNP is *UGT1A1**6. For definition of abbreviations, see text.

procaterol concentrations at each time point were included as a covariate as well as baseline serum K⁺ levels. Only *UGT1A1* A (TA)_{6/7}TAA polymorphism was significantly associated with post-dose changes of K⁺ levels (Table 2, Figure 3) even after further correcting multiple testing by Bonferroni's method. Serum K⁺ levels in subjects with (TA)_{6/6} genotype at baseline, 60, 120, and 240 min were 3.91 [95% C.I., 3.86–3.97] mEq/L, 3.60 [3.51–3.68] mEq/L, 3.47 [3.38–3.55] mEq/L, and 3.53 [3.45–3.61] mEq/L, respectively, and those in subjects with (TA)_{6/7} or (TA)_{7/7} genotype at baseline, 60, 120, and 240 min were 3.91 [3.78–4.03] mEq/L, 3.43 [3.31–3.56] mEq/L, 3.28 [3.18–3.37] mEq/L, and 3.40 [3.31–3.49] mEq/L, respectively. If we choose the differences in serum K⁺ levels between the baseline and individual time points as a dependent variable, and performed repeated measure ANCOVA with procaterol concentrations as covariates, similar result regarding the polymorphism was obtained (*df* [1, 87], *F* = 7.9, *p* = 0.006).

To further confirm these results, we performed multiple regression analysis with serum K⁺ levels at each time point as dependent variables, and *UGT1A1* A (TA)_{6/7}TAA ([TA]_{6/6} genotype vs [TA]_{6/7} or [TA]_{7/7} genotype), procaterol

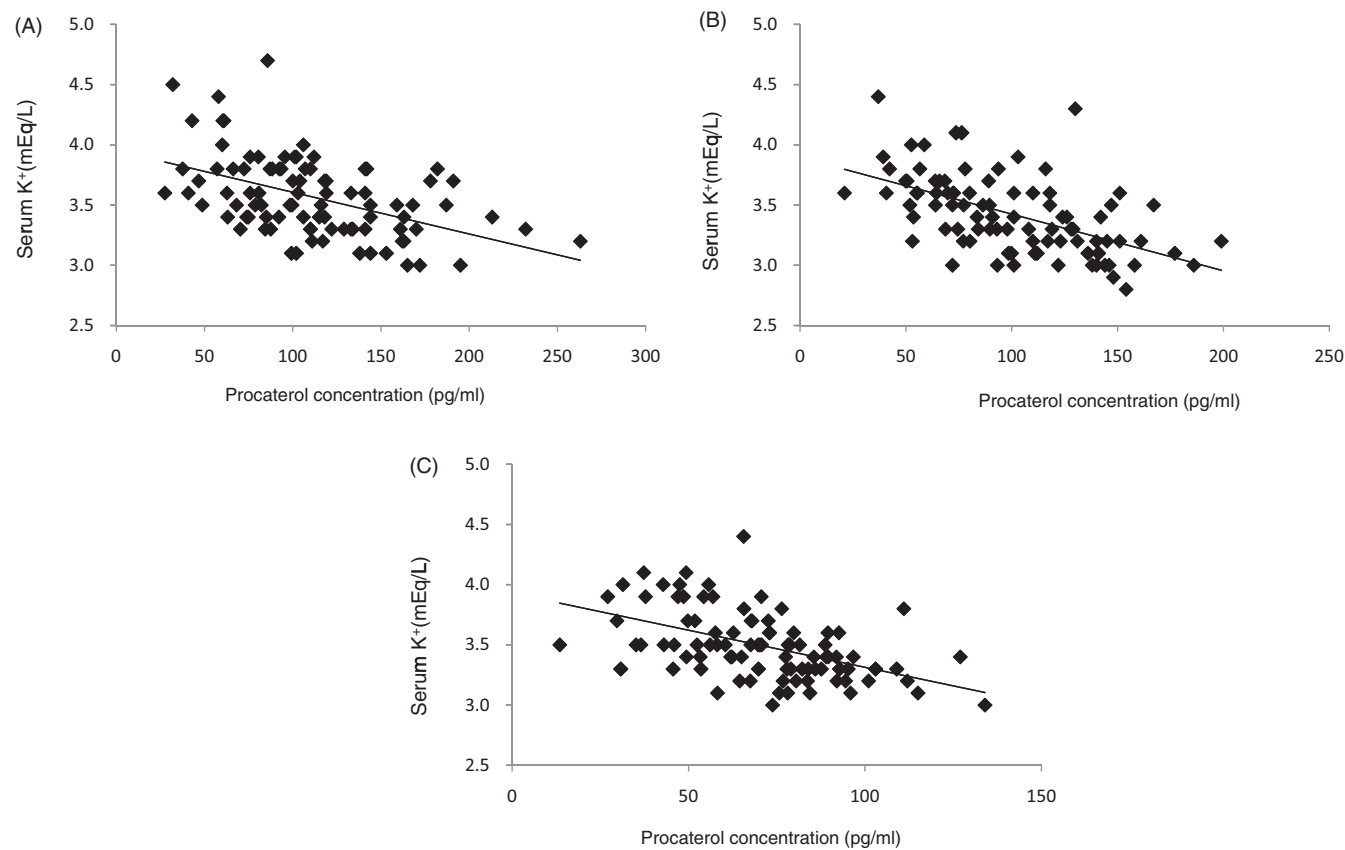


Figure 2. Correlation between procaterol concentrations and serum K⁺ at 60 min ($r = -0.469$, $p = 2.7 \times 10^{-6}$) (A), 120 min ($r = -0.554$, $p = 1.8 \times 10^{-8}$) (B), and 240 min ($r = -0.500$, $p = 4.6 \times 10^{-6}$) (C).

Table 2. Association between serum K⁺ levels and genotypes of each polymorphism.

Polymorphism	df	F	p Value
<i>CYP3A4</i>			
rs2246709	(2, 86)	2.873	0.062
rs4646437 ^a	(1, 89)	1.087	0.300
<i>ADRB2</i>			
rs1042713 (Arg16Gly)	(2, 87)	1.143	0.324
rs1042714 (Gln27Glu) ^a	(1, 86)	0.004	0.952
<i>UGT1A1</i>			
A(TA) _{6/7} TAA ^{a,b}	(1, 87)	10.884	0.001 ^c
rs4148323 ^d	(2, 86)	0.547	0.581
rs4148328	(2, 88)	2.105	0.128
rs4148329	(2, 87)	0.039	0.962
rs4663971	(2, 87)	2.624	0.078
rs12052787 ^a	(1, 89)	1.041	0.310
rs12479045 ^a	(1, 88)	1.104	0.296

Association between the temporal changes in serum K⁺ levels and each polymorphism were assessed by repeated measure ANCOVA with serum procaterol concentrations and pre-dose serum K⁺ levels as covariates. *p* Values are presented with *df* and *F* values.

^aThree genotypes were grouped into homozygote of major allele and the other genotype, because only a few homozygotes of minor allele were present for these polymorphisms.

^bSeven-repeat allele is *UGT1A1**28.

^c*p* Value after Bonferroni's correction is 0.015.

^dThe allele A of this SNP is *UGT1A1**6. For definition of abbreviations, see text and Table 1.

concentrations at each time point and baseline K⁺ levels as independent variables. The polymorphism was a significant independent variable ($\beta = -0.206$ [95% confidence interval, -0.264 to -0.490], $p = 0.005$ at 60 min; $\beta = -0.222$

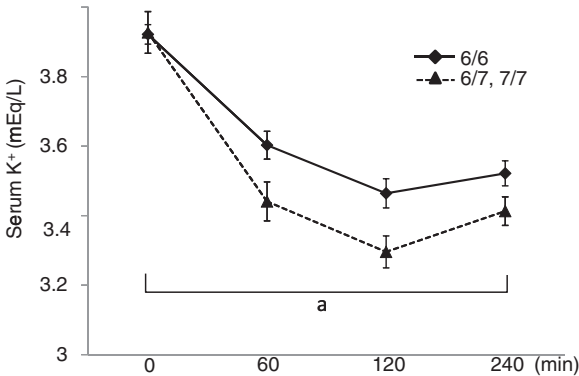


Figure 3. Mean serum K⁺ levels after taking procaterol according to *UGT1A1* A (TA)_{6/7} TAA. Data are mean $\pm$ SE. ^a $p = 1.4 \times 10^{-3}$ calculated by repeated measure ANCOVA. For definition of abbreviations, see text.

[-0.288 to -0.470], $p = 0.007$ at 120 min; $\beta = -0.174$ [-0.227 to -0.002], $p = 0.047$ at 240 min). Thus, carriers of *UGT1A1**28 (7-repeat allele) had lower serum K⁺ levels than noncarriers did.

Physical responses and gene polymorphisms

There was no significant correlation between physical responses and genotypes of two SNPs located in a coding region of *ADRB2* (Table 3). Inferred haplotypes consisted of the two SNPs were not associated with physical responses, either (Supplementary Table 4).

Table 3. Association between physical responses and genotypes of *ADRB2* polymorphism.

Physical response	Polymorphism	df	F	p Value
Systolic body pressure	rs1042713 (Arg16Gly)	(2, 89)	1.732	0.183
	rs1042714 (Gln27Glu) ^a	(1, 88)	0.365	0.547
Pulse rate	rs1042713 (Arg16Gly)	(2, 89)	0.467	0.629
	rs1042714 (Gln27Glu) ^a	(1, 88)	0.002	0.967
Hand tremor	rs1042713 (Arg16Gly)	(2, 74)	0.543	0.583
	rs1042714 (Gln27Glu) ^a	(1, 76)	3.817	0.054

Association between physical responses and each polymorphism of *ADRB2* were assessed by repeated measure ANCOVA with the dose of procaterol/BW as a covariate. *p* Values are presented with *df* and *F* values.

^aOnly homozygotes and heterozygotes of major allele were present for this polymorphisms. For definition of abbreviations, see text and Table 1.

Discussion

We have conducted for the first time a pharmacogenetic study of procaterol in a significant number of healthy subjects. A considerable variation in serum procaterol concentrations was observed at all time points. Among number of SNPs of genes responsible for metabolizing procaterol, only *UGT1A1* A (TA)_{6/7}TAA was associated with overall serum K⁺ changes from the baseline levels. We found that individuals with genotype 6/7 or 7/7 which includes *28 allele showed greater decrease in serum K⁺ levels than those with genotype 6/6.

Serum K⁺ levels are mainly regulated by sodium/potassium adenosine triphosphatase (Na⁺/K⁺ ATPase) [24,25]. β_2 -agonists activate Na⁺/K⁺ ATPase in the cell membrane, leading to an influx of K⁺ into cells, which results in decreased serum K⁺ levels [24,25]. Actually, our study has demonstrated a significant correlation between serum concentrations of procaterol and serum K⁺ levels. Even after correcting for such a strong effect by procaterol, we could still reveal independent effect of *UGT1A1* gene polymorphism on K⁺ levels by repeated measure ANCOVA. Given that *UGT1A1* *28 confers slow metabolizing activity, this allele was expected to be associated with increased serum procaterol concentrations. However, we could not find a significant correlation between *UGT1A1**28 and serum concentrations of procaterol at any time point. Thus, the effect of polymorphism on serum K⁺ level was not mediated by serum concentrations of procaterol regulated by the polymorphism.

The precise mechanism responsible for our finding is currently unknown. The organ mainly responsible for decreased serum K⁺ levels in response to β_2 -agonists is thought to be muscle, which has the highest organ volume in the human body [24]. However, *UGT1A1* is most abundantly expressed in the liver. Cross talk between *UGT1A1* and Na⁺/K⁺ ATPase might exist in the liver. Whatever the case may be, further biochemical studies are needed to identify the responsible mechanism.

The second intriguing finding of our study was the lack of correlation between *ADRB2* gene polymorphisms and response of physiological signs such as pulse rate, blood pressure, hand tremor, and serum K⁺ level to the administration of procaterol. Genetic studies on the putative

functional polymorphisms of *ADRB2*, Arg16Gly and Gln27Glu, have yielded inconsistent results. Three recent meta-analyses of the effects of these two polymorphisms have found no associations with asthma [26–28]. Other studies on other SNPs in this gene examined asthma-related phenotypes including response to a bronchodilator. Results from these studies, however, have also been inconsistent [9]. Our current study may represent additional findings which argue against physiologically significant roles of Arg16Gly and Gln27Glu. However, small functional effects may be observed only in patients with obstructive lung diseases after repeated administration of β_2 agonists. Physical responses to procaterol other than heart rate, pulse rate, and hand tremor may be more sensitive to the gene polymorphisms. Thus, our negative study should be confirmed by new ones of a larger sample size.

Serum procaterol concentrations were not associated with gene polymorphism of the major hepatic metabolizing enzyme *CYP3A4* as well as *UGT1A1*. Our results indicate that there is no need for concern in daily medical practice regarding the genetic regulation of serum procaterol concentrations by these genes. However, we should pay attention to decreased serum K⁺ levels associated with *UGT1A1* gene polymorphism, especially in steroid-dependent patients who may already have decreased K⁺ levels. Serum bilirubin concentrations may help to identify patients harboring *UGT1A1**28, since the bilirubin level increases with increased numbers of *UGT1A1* promoter TA repeats [29].

In conclusion, the present study indicates that *UGT1A1**28 may underlie the significant hypokalemia occasionally observed in patients with obstructive lung diseases treated with β_2 -agonists. The lack of robust association between the major gene polymorphisms of *ADRB2* and the effects of β_2 -agonists previously reported was supported by our negative study on healthy subjects.

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Declaration of interest

The authors report no conflicts of interest.

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Supplementary material available online

Supplementary Tables 1–4

Supplementary Table 1. Primer sequences for ARMS

	Forward primer	Reverse primer
rs4148323	5'-CCTCGTTGTACATCAGAGACA-3' 5'-CCTCGTTGTACATCAGAGACG-3'	5'-TGAGCTCCTTGTTGTGCAG-3'
rs4148328	5'-AGTGCTGAGATTACAGGCATGAG-3'	5'-ATCAGATGTCTATTATGGCAACTTTTTATA-3' 5'-ATCAGATGTCTATTATGGCAACTTTTTATG-3'
rs4663971	5'-ACACCAGGGCTTGTGGC-3' 5'-ACACCAGGGCTTGTGGG-3'	5'-TCCCCCAAGAATCTCTCAA-3'
rs12479045	5'-GGCTGGTGAGAGTGGCC-3' 5'-GGCTGGTGAGAGTGGCG-3'	5'-AGTCCTGAGGAAGCCAGGAT-3'

For definition of abbreviations, see text.

Supplementary Table 2. Genotype and allele frequencies

Polymorphism	Genotype (n, %)	Allele (n, %)
<i>CYP3A4</i>		
rs2246709	AA (13, 14), AT (36, 40), TT (42, 46)	A (62, 34), T (120, 66)
rs4646437	GG (80, 86), GA (11, 12), AA (2, 2)	G (171, 92), A (15, 8)
<i>ADRB2</i>		
rs1042713 (Arg16Gly)	AA (21, 23) AG (47, 50) GG (25, 27)	A (89, 48) G (97, 52)
rs1042714 (Gln27Glu)	CC (72, 79) CG (19, 21) GG (0, 0)	C (163, 90) G (19, 10)
<i>UGT1A1</i>		
A(TA) _{6/7} TAA*	6/6 (70, 75) 6/7 (22, 24) 7/7 (1, 1)	6 (162, 87) 7 (24, 13)
rs4148323 ⁺	GG (64, 69) GA (25, 27) AA (4, 4)	G (153, 82) A (33, 18)
rs4148328	AA (32, 34) AG (45, 49) GG (16, 17)	A (109, 59) G (77, 41)
rs4148329	AA (40, 43) AG (44, 47) GG (9, 10)	A (124, 67) G (62, 33)
rs4663971	CC (61, 65) CG (25, 27) GG (7, 8)	C (147, 79) G (39, 21)
rs12052787	CC (76, 82) CT (16, 17) TT (1, 1)	C (168, 90) T (18, 10)
rs12479045	CC (0, 0) CG (17, 19) GG (74, 81)	C (17, 9) G (165, 91)

* Seven-repeat allele is *UGT1A1**28. ⁺ The allele A of this SNP is *UGT1A1**6.

For definition of abbreviations, see text.

Supplementary Table 3. r^2 for *UGT1A1*

	rs12479045	rs12052787	rs4663971	rs4148329	rs4148328	rs4148323
A(TA) _{6/7} TAA	3.43X10 ⁻⁴	9.75X10 ⁻³	0.4377	0.0172	0.2097	0.032
rs4148323	7.50X10 ⁻³	5.80X10 ⁻³	0.0321	0.0749	0.0882	
rs4148328	0.0715	0.1154	0.3361	0.43		
rs4148329	0.0769	0.0943	0.038			
rs4663971	0.2326	0.2888				
rs12052787	0.884					

For definition of abbreviations, see text.

Supplementary Table 4. Association between physical responses and haplotypes consisted of Arg16Gly and Gln27Glu

Physical response	df	F	p value
Systolic blood pressure	(5, 180)	0.600	0.700
Pulse rate	(5, 181)	0.868	0.504
Hand tremor	(5, 111)	0.994	0.425

Six haplotypes were inferred, and association between physical responses and haplotypes were assessed by repeated measure ANCOVA with the dose of procaterol/BW as a covariate. P values are presented with df and F values.