

Original

Gender-Dependent Effect of AGT M235T Polymorphisms on Glycated Hemoglobin in Japanese Females with Type 2 Diabetes

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We investigated to determine how AGT (M235T), β 3AR (Trp64Arg), and UCP1 (-3826 A/G) polymorphisms effect on blood pressure, body mass index (BMI), glycemic control and serum lipids levels in Japanese patients with type 2 diabetes mellitus (T2DM). A total of 157 patients were screened for the variants of SNPs. The AGT (M235T), β 3AR (Trp64Arg), and UCP1 (-3826A/G) polymorphisms were analyzed by PCR-RFLP, and BMI, blood pressure, HbA1c, and serum lipids were examined. The patients who carry either or both β 3AR TrpArg/ArgArg and UCP1 AG/GG genotypes demonstrated the higher BMI than patients with β 3AR TrpTrp and UCP1 AA genotypes. Male patients with β 3AR TrpArg/ArgArg and/or UCP1 AG/GG genotypes had more frequently had high systolic blood pressure than those with β 3AR TrpTrp (wild) and UCP1 AA (wild) genotypes (133 ± 2 vs. 122 ± 3 mmHg, $p < 0.01$). Age and the existence of β 3AR TrpArg/ArgArg and/or UCP1 AG/GG genotypes were independent factors significantly associated with systolic blood pressure by multivariate analysis in male patients with T2DM. Blood pressure did not show AGT genotype-specific difference. However, female patients with AGT TT genotype showed higher mean HbA1c levels than females with AGT MM/MT genotypes (8.6 ± 1.8 vs. $7.6 \pm 1.4\%$, $p < 0.001$). The AGT TT genotype was an independent factor significantly associated with HbA1c levels in female patients by multivariate analysis. Thus, gender is a very important factor in the effects of the AGT (M235T) polymorphism on HbA1c levels in Japanese patients with T2DM.

Key words: diabetes; SNPs; AGT; β 3AR; UCP1; HbA1c

1 Introduction

Obesity and metabolic disorders have been associated with mutations called the single nucleotide polymorphism (SNPs) in the thrifty genotypes, such as β 3-adrenergic receptor (β 3AR) Trp64Arg or uncoupling protein 1 (UCP1) -3826 A/G polymorphisms.¹ The condition is brought on by larger visceral fat mass accompanied by insulin resistance. It has been suggested that these SNPs were associated with an increased tendency to gain weight and a low basal metabolic rate, and as a result causes with type 2 diabetes mellitus (T2DM) or hyperlipidemia.^{2,3} One of brown adipose tissue's specific inner-mitochondrial components is UCP1 which varies respiration coupling and dissipates oxidation energy as heat. An

association between the polymorphic Bcl I site (A-G polymorphism at position -3826 bp in the 5' flanking domain) of the UCP1 gene and fat gain has been reported.⁴ Its dysfunction also leads to obesity and insulin resistance. There are several reports in which the frequency of the β 3AR (Trp64Arg) or the UCP1 (-3826 A/G) SNPs in obese people, especially Japanese women, was found to be approximately two times higher than levels found in Finns or Canadians.^{5,6}

The angiotensinogen gene (AGT) Met235Thr (M235T) polymorphism has been shown to be associated with essential hypertension in Caucasian populations.⁷ This polymorphism was found to be in the tight linkage disequilibrium with a molecular variant of the gene promoter, which was shown to affect the transcription rate of the gene.⁸ Some researchers demonstrated a positive

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association between the AGT T235 allele and hypertension.⁹ However, the reports are controversial.¹⁰ The factors such as age, sex, and race explain the different results. Particularly, ethnic differences in the frequencies of these genotypes have been reported.¹¹

In this study we investigated to determine how AGT (M235T), β 3AR (Trp64Arg), and UCP1 (-3826 A/G) polymorphisms effect on blood pressure, glycemic control and body mass index (BMI) in Japanese patients with T2DM.

2 Subjects and methods

2.1 Subjects

Patients diagnosed with T2DM who fulfilled the WHO criteria for diabetes were recruited from outpatients visiting Kajiyama Clinic, specializes in diabetes treatment (Kyoto city, Japan), between 2008 and 2009. Patients were excluded if they had any significant diseases that were likely to affect the outcome or compliance with this study. The exclusion criteria were as follows: heart failure, hepatic dysfunction, renal failure, or serious physical or mental conditions. The study protocol was approved by the Ethics Committee of the School of Comprehensive Rehabilitation at Osaka Prefecture University. Informed consent was obtained from all the subjects before enrollment in the study. A total of 157 adults with T2DM were assigned to the study group.

2.2 Methods

Laboratory data, body weight and body mass index (BMI) were collected of all participants. BMI was calculated as weight in kilograms divided by height in meters squared. Fasting blood samples were collected in the morning after an overnight fast from all participants. HbA1c levels (JDS) were determined by a latex cohesion method (JCA-BM2250, KYOWA MEDEX, Co. Ltd., Tokyo, Japan). The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula $\text{HbA1c (\%)} = \text{HbA1c (JDS)}(\%) + 0.4\%$, considering the relational expression of HbA1c (JDS)(%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP).¹² Total cholesterol and triglyceride levels were determined by enzyme assay. High density lipoprotein cholesterol (HDL-cholesterol) levels were determined by a direct method (Labospect 008K, Bio Majesty JCA-BM 8060, JEOL, Ltd., Tokyo, Japan) and low density lipoprotein cholesterol (LDL-cholesterol) levels by an enzymatic method (Bio Majesty JCA-BM 8060, JEOL, Ltd., Tokyo, Japan). Blood pressure was measured from the right arm using a mercury sphygmomanometer in a sitting position after 5 minutes of rest.

A total of 157 patients were screened for the variants of β 3AR (Trp64Arg), UCP1 (-3826 A/G), and AGT (M235T) SNPs. Genomic DNA was extracted from peripheral blood leukocytes of the subjects with DNA Extractor WB Kit (Wako. Pure Chem., Osaka, Japan). The variants of the genes were detected using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.¹³ The amplification protocol and method of measurement were the same as previously reported for β 3AR (Trp64Arg),¹⁴ UCP1 (-3826 A/G),⁵ and AGT (M235T).¹⁵

All continuous variables are reported as mean \pm SD unless otherwise indicated. Comparisons of clinical and laboratory characteristics between the patients with SNPs and without SNPs were done by unpaired Student's *t*-test or chi-square test as appropriate using SPSS version 15.0 for Windows (SPSS Inc, Chicago, IL). Multivariate analysis was conducted using a stepwise method by JMP version 9.0 for Windows (SAS Inc. Cary, NC). SNPs, Age, BMI, and duration of diabetes were incorporated as independent variables for analysis of HbA1c, systolic blood pressure levels. A two-tailed value of $p < 0.05$ was considered significant.

3 Results

The characteristics of the patients are shown in Table 1. The baseline biophysical parameters show no gender differences in age, duration of T2DM, BMI, HbA1c, blood pressure, and LDL-cholesterol. The frequencies of the β 3AR (Trp64Arg) genotypes in the 157 subjects with T2DM were TrpTrp (wild) type, 69%; TrpArg type, 27%; and ArgArg type, 4%. The frequencies of the UCP1 (-3826 A/G) AA (wild), AG, and GG genotypes were 28, 47, and 25%. The patients who carry both or either genotype of β 3AR TrpArg/ArgArg or UCP1 AG/GG

Table 1 Clinical characteristics of patients with T2DM.

	Total <i>n</i> = 157	Male <i>n</i> = 85	Female <i>n</i> = 72
Age (yrs)	61.3 \pm 12.4	59.9 \pm 12.8	63.0 \pm 11.7
Duration of diabetes (yrs)	9.1 \pm 8.9	8.8 \pm 9.4	9.6 \pm 8.3
Therapy (<i>n</i>) (diet/OHA/insulin)	82/68/7	48/35/2	34/33/5*
Antihypertensive agents (<i>n</i>)	36	19	16
Lipid-lowering agents (<i>n</i>)	39	10	28*
BMI (kg/m ²)	24.6 \pm 4.2	24.6 \pm 3.5	24.3 \pm 5.0
HbA1c (%)	8.6 \pm 2.2	8.8 \pm 2.4	8.2 \pm 1.7
Plasma glucose (mg/dl)	204 \pm 101	228 \pm 119	173 \pm 60*
T-C (mg/dl)	222 \pm 37	217 \pm 33	229 \pm 41*
HDL-C (mg/dl)	57 \pm 15	53 \pm 14	63 \pm 15*
LDL-C (mg/dl)	136 \pm 32	133 \pm 31	140 \pm 33
TG (mg/dl)	163 \pm 121	183 \pm 144	139 \pm 80*
SBP (mmHg)	133 \pm 16	132 \pm 18	134 \pm 15
DBP (mmHg)	78 \pm 11	79 \pm 10	77 \pm 11

Data are expressed as mean \pm SD or *n*.

* $p < 0.05$, values are different from male and female.

OHA; oral hypoglycemic agents, BMI; body mass index, T-C; total cholesterol, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, TG; triglyceride, SBP; systolic blood pressure, DBP; diastolic blood pressure.

were 79% in the subjects. The patients who carry genotype of β 3AR TrpArg/ArgArg showed higher BMI than those with β 3AR wild genotypes (24.7 vs. 23.1 kg/m², $p < 0.05$), although there were no differences between UCP1 wild and AG/GG genotypes with respect of BMI, blood pressure, HbA1c, or serum lipids concentrations. However, the patients who carry both or either β 3AR TrpArg/ArgArg and/or UCP1 AG/GG genotypes demonstrated the higher BMI than subjects with β 3AR wild and UCP1 wild genotypes (Fig. 1). Male patients with β 3AR TrpArg/ArgArg and/or UCP1 AG/GG genotypes showed significant higher systolic blood pressure than males with β 3AR wild and UCP1 wild genotypes when analyzed separately in gender (133 ± 2 vs. 122 ± 3 mmHg, $p < 0.01$, Fig. 1). As shown in Table 2, age and β 3AR TrpArg/ArgArg and/or UCP1

AG/GG genotypes were independent factors significantly associated with increased systolic blood pressure levels by multivariate analysis in male patients with T2DM. No difference was observed in HbA1c or serum lipids in patients with or without β 3AR or UCP1 SNPs.

The frequencies of the AGT (M235T) MM (wild), MT, and TT genotypes were 3, 35, and 61%. Because of the low frequency of the MM genotype ($n = 5$), we combined the patient groups with the MM/MT genotypes

Table 2 Multivariate analysis of systolic blood pressure in male patients with T2DM.

Variables	β	SEM	p
β 3AR and UCP1 genotypes			
TrpArg/ArgArg and/or AG/GG vs. TT/AA (Wild)	-4.635	2.201	0.038
BMI	0.674	0.571	0.241
Age	0.369	0.154	0.018

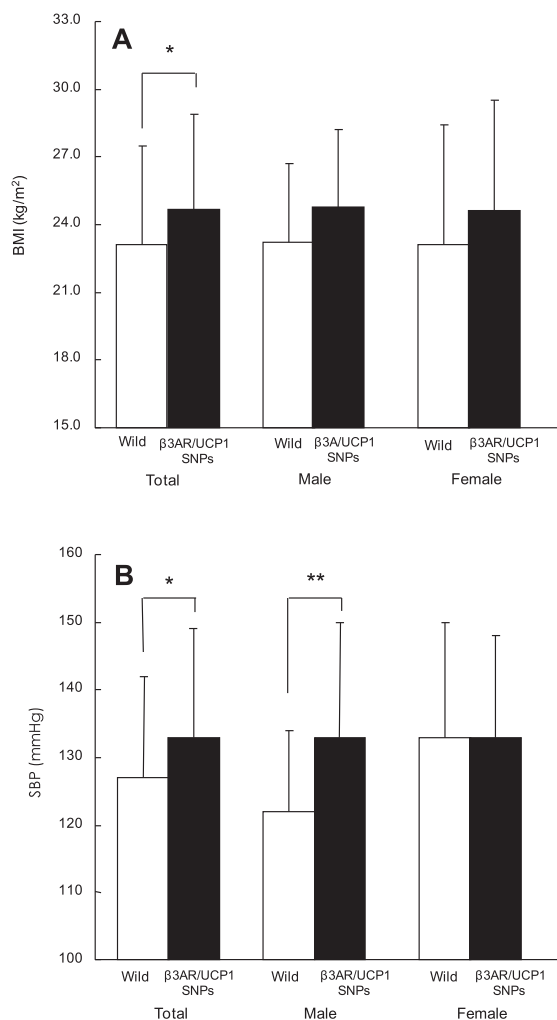


Fig. 1 Comparison of (A) BMI or (B) systolic blood pressure with or without thrifty SNPs β 3AR (Trp64Arg) and/or UCP1 -3826 A/G polymorphisms. Values are mean \pm SD. Differences between with and without thrifty SNPs were examined by Student's t -test. * $p < 0.05$, ** $p < 0.01$. Significant differences in each genotype. BMI; body mass index, SBP; systolic blood pressure.

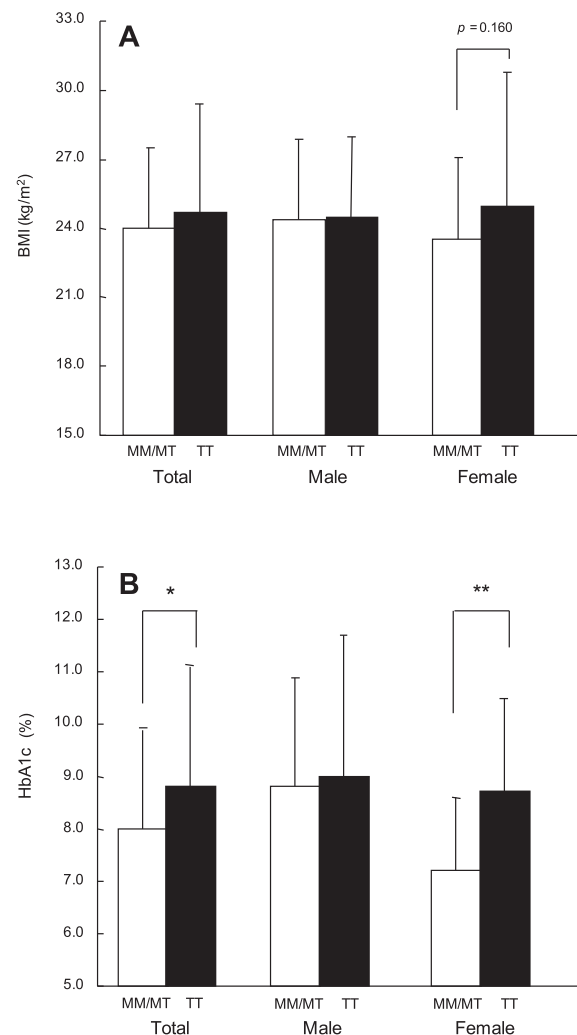


Fig. 2 Comparison of (A) BMI or (B) HbA1c with or without SNPs of AGT M235T genotypes. Values are mean \pm SD. Differences between with and without SNPs were examined by Student's t -test. * $p < 0.05$, ** $p < 0.001$. Significant differences in each genotype.

Table 3 Multivariate analysis of HbA1c levels in female patients with T2DM.

Variables	β	SEM	<i>p</i>
AGT genotypes			
MM/MT vs. TT	-0.599	0.194	0.002
BMI	-0.020	0.039	0.605
Age	0.001	0.016	0.929

for comparison with patients who carry the AGT TT genotype. No association was found in hypertension between the AGT TT and MM/MT genotypes (132 ± 15 vs. 133 ± 18 mmHg, $p = 0.686$). However, the females with AGT TT genotype demonstrated significant higher HbA1c values than females with the MT/MM genotypes (8.6 ± 1.8 vs. $7.6 \pm 1.4\%$, $p < 0.001$, Fig. 2), whereas this association was not found in male patients. By multivariate analysis AGT TT genotype was independently associated with increased HbA1c levels in female patients with T2DM (Table 3). No difference was observed in blood pressure or serum lipids in patients with or without AGT SNPs.

4 Discussion

In this study the genotype frequencies of $\beta 3$ AR (Trp64Arg), UCP1 (-3826A/G), or AGT (M235T) polymorphisms did not differ the reports of Japanese subjects in the previous reports.^{5, 6, 10, 14, 16} The $\beta 3$ AR (Trp64Arg) or UCP1 (-3826A/G) gene did not have an independent effect on the blood pressure, but its existence with $\beta 3$ AR TrpArg/ArgArg and/or UCP1 AG/GG genotypes and age resulted in higher systolic blood pressure in Japanese male patients with T2DM.

The AGT TT genotype is found in low frequency in the Caucasian,¹⁷ in contrast to its high prevalence in east Asians,^{13, 18} and African-American populations.^{11, 19} It is generally believed that AGT M235T polymorphism itself does not cause hypertension but it is rather a marker of the DNA segment that actually is responsible for susceptibility to hypertension.²⁰ However, the reports are controversial.^{10, 21-23} The inconsistency among the previous studies might be due to differences in the criteria for subject selection. Especially, differences in age and ethnic background play an important role. The informative value of this AGT (M235T) polymorphism may be sensitive to phylogenetic distance and the heterogeneity of the genetic background. Iwai et al. indicated that the TT genotype of the angiotensinogen gene was a predictor of blood pressure in a subpopulation less than 50 years of age of Japanese subjects.²⁴ In contrast, BMI was reported to be a strong predictor of blood pressure in a subpopulation more than 50 years of age.

In our study the AGT TT genotype was not associat-

ed with a hypertension, however, the female diabetic carriers of the AGT TT genotype was associated the higher glycated hemoglobin levels, which might be related to visceral obesity and hyperinsulinemia.²⁵ Because AGT is regarded as one of the cytokines produced from adipocytes and serum AGT concentrations are reported to be positively correlated with BMI. The mean BMI of female patients tended to be higher than that of female patients with AGT AA/AG genotypes, although it was not statistically significant (Fig. 2A). However, larger and further investigation requires more detail information.

The $\beta 3$ AR TrpArg/ArgArg or UCP1 AG/GG genotypes did not have an independent effect on the blood pressure, but with the $\beta 3$ AR TrpArg/ArgArg and/or UCP1 AG/GG genotypes resulted in higher systolic blood pressure in male patients with T2DM. The female diabetic carriers of the AGT TT genotype might associate the higher glycated hemoglobin levels. Thus, gender is a very important factor in the effects of AGT TT genotypes on glycated hemoglobin levels in Japanese patients with T2DM. Since the number of patients in this study is small, these conclusions must be interpreted with caution.

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Conflict of interest

The authors declared no conflict of interest.

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