Original Article

β2- and β3-Adrenoceptor Polymorphisms Relate to Subsequent Weight Gain and Blood Pressure Elevation in Obese Normotensive Individuals

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High blood pressure (BP) is a major determinant of cardiovascular events in obesity. The β2- and β3-adrenoceptor polymorphisms are associated with obesity and hypertension. In the present study, we examine the relationships of β2- and β3-adrenoceptor polymorphisms with further weight gain–induced BP elevation in obese subjects. Changes in BP, body weight, total body fat-mass, waist-to-hip ratio, plasma norepinephrine (NE) and leptin levels, and β2(Arg16Gly)- and β3(Trp64Arg)-adrenoceptor polymorphisms were measured periodically over a 5-year period in 55 entry obese (body mass index [BMI]≥25.0 kg/m²) normotensive (BP<140/90 mmHg) men. BP elevation and weight gain were defined as ≥10% increases from entry levels over 5 years in mean BP or BMI. Obese subjects with weight gain, BP elevation or weight gain–induced BP elevation had higher frequencies of the Gly16 allele of Arg16Gly and Arg64 allele of Trp64Arg. Subjects carrying the Gly16 or Arg64 alleles had significantly greater total fat-mass and waist-to-hip ratio at entry and over a 5-year period compared to the subjects who did not carry these polymorphisms. Subjects carrying the Gly16 allele had similar levels of plasma NE, higher levels of plasma leptin and a lower slope of the regression lines between plasma leptin and NE levels. Those carrying the Arg64 allele had higher plasma NE levels at entry and over a 5-year period compared to the subjects without the Arg64 allele, but plasma leptin levels and slopes were similar. The findings demonstrate that the Arg64 allele of the β3-adrenoceptor polymorphisms relates to weight gain–induced BP elevation accompanying high plasma NE (heightened sympathetic activity) in obese men. The Gly16 allele of the β2-adrenoceptor polymorphisms links to weight gain–induced BP elevation associated with leptin resistance. β2- and β3-adrenoceptor polymorphisms could predict the future BP elevation and further weight gain–induced BP elevation in originally obese subjects. (*Hypertens Res* 2006; 29: 951–959)

***Key Words*: β2-adrenoceptor polymorphisms, β3-adrenoceptor polymorphisms, obesity, sympathetic nerve activity, leptin**

Introduction

Obesity and obesity-related cardiovascular disease are a rapidly growing public health problem (*1*, *2*), and there is evidence that human obesity and hypertension have strong genetic as well as environmental determinants (*3*–*5*). High blood pressure (BP) is a significant determinant factor for cardiovascular events in obese subjects, but the mechanisms of high BP in obesity have not been fully clarified. The ther-

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Table 1. Characteristics of Obese Normotensive Subjects at Entry and at Year 5

Variable	With BP elevation				Without BP elevation			
	With weight gain		Without weight gain		With weight gain		Without weight gain	
	At entry	At year 5	At entry	At year 5	At entry	At year 5	At entry	At year 5
Subjects (*n*)	17	17	10	10	16	16	12	12
Smoker/non-smoker (*n*/*n*)	5/12	3/14	3/7	2/8	5/11	3/13	4/8	2/10
Age (years)	37±5	42±5¶	38±4	43±4¶	36±4	41±4¶	37±4	42±4¶
BMI (kg/m^2)	27.5±2.0	31.8±2.2¶	27.7±2.3	27.5±1.7†	27.6±1.5	30.7±2.3¶	27.3±2.1	27.5±1.3†
Total fat-mass (kg)	25.0±3.7	27.5±3.6$^{\|}$	22.7±3.4*	23.8±4.3*	23.1±2.7‡	26.8±3.3$^{\|}$	20.5±3.5*,‡	21.8±4.2†,‡
Waist-to-hip ratio	1.17±0.09	1.26±0.08$^{\|}$	1.07±0.09*	1.09±0.10†	1.10±0.10‡	1.17±0.09$^{\ddagger,\|}$	1.06±0.11	1.07±0.08*
Systolic BP (mmHg)	135±4	153±5¶	128±7†	139±5†,¶	130±7‡	137±7$^{\S,\|}$	126±7*	130±8*,‡
Diastolic BP (mmHg)	83±6	93±7¶	78±6*	87±7¶	82±6	85±6‡	80±6	81±8‡
Mean BP (mmHg)	102±7	113±8¶	94±7*	104±7†,¶	99±7	102±6§	96±6	97±7‡
Heart rate (bpm)	75±6	77±5	74±5	75±4	72±6	75±6	74±7	75±6
Norepinephrine (pmol/ml)	2.13±0.51	2.44±0.55$^{\|}$	1.90±0.45*	2.28±0.54$^{\|}$	1.85±0.46‡	2.09±0.47$^{\ddagger,\|}$	1.71±0.31‡	1.98±0.37‡
Leptin (ng/ml)	6.1±2.4	7.8±2.6$^{\|}$	6.2±2.3	7.5±4.3	5.5±2.4	7.7±3.6$^{\|}$	6.4±2.1	6.9±3.0

Data are mean±SD. Obese normotensive subjects: $n=55$. *$p<0.05$, $^{\dagger}p<0.01$ *vs*. subjects with significant weight gain; $^{\ddagger}p<0.05$, $^{\S}p<0.01$ *vs*. subjects with significant BP elevation. $^{\|}p<0.05$, $^{\P}p<0.01$ *vs*. value at entry. BP, blood pressure; BMI, body mass index.

mogenic effects in obesity have been mainly attributed to the activity of the β2- and β3-adrenergic receptors in humans (*6–12*), but these findings have not been confirmed in other studies (*13*, *14*).

We have previously reported in nonobese, normotensive individuals that the Gly16 allele of the β2-adrenoceptor polymorphisms is related to greater weight gain and BP elevation, whereas the Glu27 allele of the β2-adrenoceptor polymorphisms and Trp64 allele of the β3-adrenoceptor polymorphisms are linked to BP elevation, but not to weight gain (*15*). Several studies have shown that the Trp64Arg of the β3-adrenoceptor polymorphisms may be strongly associated with obesity (*9–11*), however, this finding has not been confirmed in other studies (*13*, *14*, *16*). Thus, we could speculate that the mechanisms by which β2- and β3-adrenoceptor polymorphisms cause weight gain–induced BP elevation differ between nonobese normotensive subjects and obese normotensive subjects.

Reports of an association of β2- and β3-adrenergic receptor polymorphisms with obesity and hypertension in obesity have been discordant (*4–14*). The discordance might be caused by differences in the degree of obesity, gender, or ethnicity in the studied cohorts. Additionally, few studies have simultaneously taken into account obesity and hypertension as related to polymorphisms of β2- and β3-adrenoceptor genes in the same study population followed longitudinally for several years. Plasma norepinephrine (NE) levels, as an index of sympathetic nerve system activity, and plasma leptin levels, both of which have been reported to have strong associations with obesity, are also examined in the present study.

Thus, the present study examines the associations of the β2- and β3-adrenoceptor polymorphisms with BP elevation as well as plasma NE level as an index of sympathetic nerve activity and plasma leptin level in originally obese normotensive men over 5 years to further evaluate the mechanisms of obesity-related hypertension.

Methods

Subjects

Subjects were recruited from a cohort of 1,121 men who work in a single company in Osaka, Japan as part of their annual medical evaluation. Subjects at study entry were excluded who were >50 years of age, had diabetes mellitus (fasting glucose level>110 mg/dl), or had hypertension (BP≥140/90 mmHg). Only overweight or obese men (body mass index [BMI] ≥25 kg/m^2) (*17*, *18*) were enrolled in the present study, because the goal of the present study was to evaluate the mechanisms of BP elevation in obese subjects. Additional exclusions were subjects who were taking medications for hypertension, hyperlipidemia, hyperuricemia or other illness, and those who were enrolled in a weight loss program (*19*). Only subjects who had steady body weight and BP levels (weight and mean BP had not changed significantly [<5%] over the year prior to the entry period) were enrolled in this study (*20*, *21*). After exclusion, 55 overweight or obese (BMI≥25 kg/m^2) normotensive (<140/90 mmHg) young men on no medications were enrolled in the present study. Informed consent was obtained from each subject, as approved by the Ethics Committee of Osaka University Graduate School of Medicine, Japan.

Measurements

After an overnight fast of >12 h, BMI, total body fat-mass, the ratio of waist circumference to hip circumference (waist-to-hip ratio), BP, heart rate, and venous sampling for plasma

Table 2. Frequencies of Genotype and Alleles between Subjects with and without Significant BP Elevation and between Subjects with and without Significant Weight Gain

		Genotypes		χ^2 test for 3 genotypes*	χ^2 test for allele*
Arg16Gly, β2-adrenoceptor gene	Arg16/Arg16	Arg16/Gly16	Gly16/Gly16		
With BP elevation ($n=27$)	0 (0.0%)	11 (40.7%)	16 (59.3%)	$\chi^2=21.80$, $p<0.001$	$\chi^2=19.91$, $p<0.001$
Without BP elevation ($n=28$)	10 (35.7%)	16 (57.1%)	2 (7.1%)		
With weight gain ($n=33$)	3 (9.1%)	14 (42.4%)	16 (48.5%)	$\chi^2=10.76$, $p=0.005$	$\chi^2=9.18$, $p=0.002$
Without weight gain ($n=22$)	7 (31.8%)	13 (59.1%)	2 (9.1%)		
Gln27Glu, β2-adrenoceptor gene	Gln27/Gln27	Gln27/Glu27	Glu27/Glu27		
With BP elevation ($n=27$)	20 (74.1%)	7 (25.9%)	0 (0.0%)	—, —	[$\chi^2=1.11$, $p=0.291$]
Without BP elevation ($n=28$)	25 (89.3%)	3 (10.7%)	0 (0.0%)		
With weight gain ($n=33$)	26 (78.8%)	7 (21.2%)	0 (0.0%)	—, —	[$\chi^2=0.12$, $p=0.735$]
Without weight gain ($n=22$)	19 (86.4%)	3 (13.6%)	0 (0.0%)		
Trp64Arg, β3-adrenoceptor gene	Trp64/Trp64	Trp64/Arg64	Arg64/Arg64		
With BP elevation ($n=27$)	13 (48.1%)	12 (44.4%)	2 (7.4%)	$\chi^2=6.30$, $p=0.043$	$\chi^2=5.02$, $p=0.025$
Without BP elevation ($n=28$)	22 (78.6%)	6 (21.4%)	0 (0.0%)		
With weight gain ($n=33$)	17 (51.5%)	14(42.4%)	2 (6.1%)	$\chi^2=5.61$, $p=0.061$	$\chi^2=4.38$, $p=0.036$
Without weight gain ($n=22$)	18 (81.8%)	4 (18.2%)	0 (0.0%)		

*Between subjects with and without BP elevation or weight gain. BP elevation, subjects with significant mean BP elevation (≥10%) over 5 years; weight gain, subjects with significant increases in body mass index (≥10%) over 5 years. Values in brackets show results that the power of the performed test is below the desired power of 0.800. BP, blood pressure.

NE, leptin and extraction of genomic DNA from leukocytes were taken every year for 5 years. BP and heart rate were measured in the recumbent position using an automated sphygmomanometer (TM-2713, A&D Co., Tokyo, Japan) with an adjusted cuff size, which had been standardized against a mercury sphygmomanometer. The percentage body fat-mass was determined with impedance measurements (BF-102, Tanita Co Ltd., Tokyo, Japan), and total body fat-mass (kg) was calculated according to the following equation:

Total body fat-mass
= [percentage body fat-mass (%)/100] × body weight (kg).

Significant BP elevation and weight gain over 5 years were defined as a ≥10% increase in mean BP or BMI compared with the values at entry (*15*, *20–22*).

Laboratory Determinations

Plasma NE was measured by high-performance liquid chromatography by a fluorometric method as previously described for this laboratory (intra-assay coefficient of variation [CV] = 2.1%; inter-assay CV = 3.6%; sensitivity = 0.06 to 120 nmol/l) (*20*). Plasma leptin was measured by radioimmunoassay using a human leptin RIA kit (Linco Research, St. Charles, USA) as previously described for this laboratory (*20*).

Genotyping

Genotyping was performed by the TaqMan assay as previously described (Applied Biosystems, Foster City, USA) (*23*). Two polymorphisms in the β2-adrenergic receptors (arginine/glycine substitution, Arg16Gly; and glutamine/glutamate substitution, Gln27Glu) of the β2-adrenoceptor gene were studied (*6*, *7*). One polymorphism (tryptophan/arginine substitution, Trp64Arg) of the β3-adrenoceptor gene was also studied (*10*).

For TaqMan assay of single-nucleotide polymorphisms (SNPs) in the β2-adrenergic receptor gene, the following probes were used: for Arg16Gly, CGCATGGCTTCC ATTGGGTGC and CGCATGGCTTCTATTGGGTGC; and for Gln27Glu, CTCGTCCCTTTCCTGCGTGACGT and CTCGTCCCTTTGCTGCGTGACGT. The primers used for both Arg16Gly and Gln27Glu were GGAACGGCAGCG CCTTCT and CAGGACGATGAGAGACATGACGAT. For the Trp64Arg SNP in the β3-adrenergic receptors, the probes were TCTCGGAGTCCAGGCGATGGCCA and CTCGGA GTCCGGGCGATGGCC, and the primers were GGAGGC AACCTGCTGGTCAT and CACGAACACGTTGGTCAT GGT.

Statistical Analysis

Genotype frequencies and Hardy-Weinberg equilibrium were

Table 3. Characteristics of Subjects According to Gly16 Allele

Variables	With Gly16 allele		Without Gly16 allele	
	At entry	At year 5	At entry	At year 5
Subjects (*n*)	45	45	10	10
Smoker/non-smoker (*n*/*n*)	16/29	8/37	1/9	2/8
Age (years)	37±8	42±8§	37±5	42±5§
BMI (kg/m^2)	27.4±2.0	30.0±2.1§	27.7±2.2	28.4±2.6
Total body fat-mass (kg)	23.9±3.3*	26.8±3.7†,‡	19.4±3.5	20.8±4.2
Waist-to-hip ratio	1.12±0.07*	1.18±0.08*	1.04±0.11	1.08±0.09
Systolic BP (mmHg)	130±6	142±7*,‡	131±7	135±7
Diastolic BP (mmHg)	82±7	88±6*,‡	80±8	82±6
Mean BP (mmHg)	98±6	106±7*,‡	97±8	100±7
Heart rates (beats/min)	74±8	77±6*	73±9	72±5
Plasma norepinephrine (pmol/ml)	1.90±0.71	2.17±0.69	1.95±0.39	2.38±0.47
Plasma leptin (ng/ml)	6.3±2.6*	8.0±2.4†,‡	4.7±1.3	5.4±1.8

Data are mean±SD. *$p<0.05$, †$p<0.01$ *vs*. values in subjects without Gly16 allele. ‡$p<0.05$, §$p<0.01$ *vs*. value at entry. BMI, body mass index; BP, blood pressure.

estimated with the χ^2 test. Values are shown as the mean±SD. All data analyses were performed with SPSS 8.0 for Windows programs (SPSS Inc., Chicago, USA). Changes in measured parameters within each group and differences among groups were examined by 2-way analysis of variance. When these differences were significant, the Dunnett test was used to determine whether the differences of the mean measured variables at entry and 5 years were significant within the groups and among the groups compared with baseline. Relationships between BP changes, hormonal measurements and anthropometric measurements were assessed by multiple linear regressions. Analysis of covariance was applied to evaluate the interactions between plasma NE as a dependent variable and leptin as an independent variable in alleles of β2- and β3-adrenoceptor polymorphisms to compare the relationships between those parameters. Values of $p<0.05$ were considered significant.

Results

Prevalence of Weight Gain and Blood Pressure Elevation over a 5-Year Period

Twenty-seven subjects had a significant BP elevation, and 33 obese subjects had significant weight gain over 5 years. Seventeen obese subjects had both significant weight gain and BP elevation, and 12 subjects had steady BP levels and weight over 5 years (Table 1). The prevalence of BP elevation was higher in obese subjects compared to nonobese subjects in our previous study (25.6% in nonobese subjects, 49.1% in obese subjects, $\chi^2=4.35$, $p=0.037$) (*15*).

Characteristics of the 4 Study Groups According to Weight Gain and Blood Pressure Elevation

Obese normotensive subjects with a significant BP elevation had greater total body fat-mass, waist-to-hip ratio, systolic BP and plasma NE levels at entry and at 5 years, especially in subjects with significant weight gain. Subjects who significantly gained body weight (BMI), especially in the group with a significant BP elevation, had significantly greater total body fat-mass, waist-to-hip ratio, systolic, diastolic and mean BP, and plasma NE levels at entry and at 5 years compared to subjects who did not gain weight, but BMI and plasma leptin levels at entry were similar. In subjects who had both significant weight gain and BP elevation over 5 years, total body fat-mass, waist-to-hip ratio, systolic, diastolic and mean BP levels and plasma NE levels at entry and over a 5-year period were greatest among the 4 study groups according to weight gain and BP elevation (Table 1). Frequencies of smokers were not different among the 4 study groups at entry and at the 5-year period (Table 1).

When we performed multiple linear regression analysis using changes in mean BP as a dependent variable, total body fat-mass ($p<0.05$), waist-to-hip ratio ($p<0.05$) and plasma NE ($p<0.01$) at entry were significant determinant variables ($r^2=0.31$, $F=5.28$, $p=0.001$).

Relationships between Genotypes of the β2- and β3-Adrenoceptor Polymorphisms, Significant Blood Pressure Elevation and Significant Weight Gain

Obese normotensive subjects who had a significant BP elevation or significant weight gain carried higher frequencies of the Gly16 allele of the Arg16Gly and Arg64 allele of the Trp64Arg polymorphisms compared to those without a sig-

Table 4. Characteristics of Subjects According to Arg64 Allele

Variables	With Arg64 allele		Without Arg64 allele	
	At entry	At year 5	At entry	At year 5
Subjects (*n*)	20	20	35	35
Smoker/non-smoker (*n*/*n*)	6/14	3/17	11/24	7/28
Age (years)	37±7	42±7§	37±9	42±9§
BMI (kg/m^2)	28.0±2.7	30.4±2.9‡	27.4±2.1	29.3±2.9
Total body fat-mass (kg)	24.9±3.8†	28.2±3.9†,‡	21.9±3.6	24.3±3.9‡
Waist-to-hip ratio	1.16±0.09*	1.23±0.08†,‡	1.07±0.09	1.09±0.10
Systolic BP (mmHg)	134±5*	151±8†,§	128±6	135±8
Diastolic BP (mmHg)	81±6	89±7‡	81±6	86±7
Mean BP (mmHg)	99±6	109±8*,§	97±6	102±7
Heart rates (beats/min)	79±8*	80±9*	71±7	74±9
Norepinephrine (pmol/ml)	2.30±0.54†	2.61±0.56†,‡	1.71±0.65	1.94±0.64
Leptin (ng/ml)	5.8±1.9	7.0±2.5‡	6.1±2.6	8.0±2.5‡

Data are mean±SD. *$p<0.05$, †$p<0.01$ *vs*. values in subjects without Arg64 allele. ‡$p<0.05$, §$p<0.01$ *vs*. value at entry. BMI, body mass index; BP, blood pressure.

nificant BP elevation or a significant weight gain (Table 2). Those who had both weight gain and BP elevation had higher frequencies of the Gly16 and Arg64 alleles compared to those in subjects without either weight gain or BP elevation ($\chi^2=10.62$, $p=0.001$, $\chi^2=6.14$, $p=0.013$, respectively). The Glu27 allele, which was related only to BP elevation in our previous report on nonobese subjects over a 5-year period (*14*), was not linked to either significant weight gain or BP elevation in obese normotensive subjects in the present study (Table 2).

The allele frequencies of Gly16 and Glu27 of the β2-adrenoceptor polymorphisms were 57.3% and 9.1%, and that of Arg64 of the β3-adrenoceptor polymorphism was 20.0%, but all studied loci allele and genotype frequencies were in accordance with the Hardy-Weinberg equilibrium (Arg16Gly, $p=0.982$; Gln27Glu, $p=0.458$; Trp64Arg, $p=0.866$). The allele frequencies of Gly16, Glu27 and Arg64 in obese subjects were higher than those in our nonobese normotensive cohort (*15*). The allele distributions for the Glu27 and the Arg64 in our subjects were similar to those in previous studies in Japanese cohorts, but lower than studies in Caucasians (*6*, *7*, *10–13*, *24*, *25*).

Relationships between the Gly16 Allele of the β2-Adrenoceptor Polymorphisms, the Arg64 Allele of the β3-Adrenoceptor Polymorphisms, Plasma Norepinephrine and Leptin

The findings in the relationships between frequencies of the Gly16 and Arg64 alleles, BP elevations and weight gain suggest that the Gly16 and Arg64 alleles relate to BP elevation or weight gain in obese normotensive subjects. Thus, we compared the parameters between subjects with and without the Gly16 and Arg64 alleles.

Obese subjects who carried the Gly16 allele had significantly greater total body fat-mass, waist-to-hip ratio and plasma leptin levels at entry and at year 5 compared to those without the Gly16 allele, but the BMI and plasma NE levels were similar between the two groups (Table 3). Subjects who carried the Arg64 allele had significantly greater total body fat-mass, waist-to-hip ratio, systolic BP, heart rates, and plasma NE levels at both entry and year 5 compared to those without the Arg64 allele, but the BMI and plasma leptin levels were similar between the two groups (Table 4).

Relationships between Plasma Leptin and Norepinephrine Levels

Plasma NE levels at entry correlated significantly with plasma leptin levels in subjects with the Gly16 allele ($r=0.32$, $F=4.29$, $p=0.045$) and those without the Gly16 allele ($r=0.78$, $F=12.71$, $p=0.007$) (Fig. 1A). These correlations were also observed in subjects with the Arg64 allele ($r=0.52$, $F=6.81$, $p=0.018$) and those without the Arg64 allele ($r=0.47$, $F=9.28$, $p=0.005$) (Fig. 1B). The slope in subjects carrying the Gly16 allele at entry tended to be lower compared to that in subjects without the Gly16 allele ($F=2.06$, $p=0.0516$) (Fig. 1A), whereas that in subjects carrying the Arg64 allele was similar to that in subjects without the Arg64 allele ($F=0.35$, $p=0.7269$) (Fig. 1B). The correlations between plasma leptin and NE levels over a 5-year period were similar to those at entry.

Discussion

The present longitudinal study over a 5-year period shows that the Arg64 allele of Trp64Arg of the β3-adrenoceptor gene has a substantial influence on future BP elevation, further weight gain and weight gain–induced BP elevations in originally overweight or obese (BMI≥25 kg/m^2) (*17*, *18*) nor-

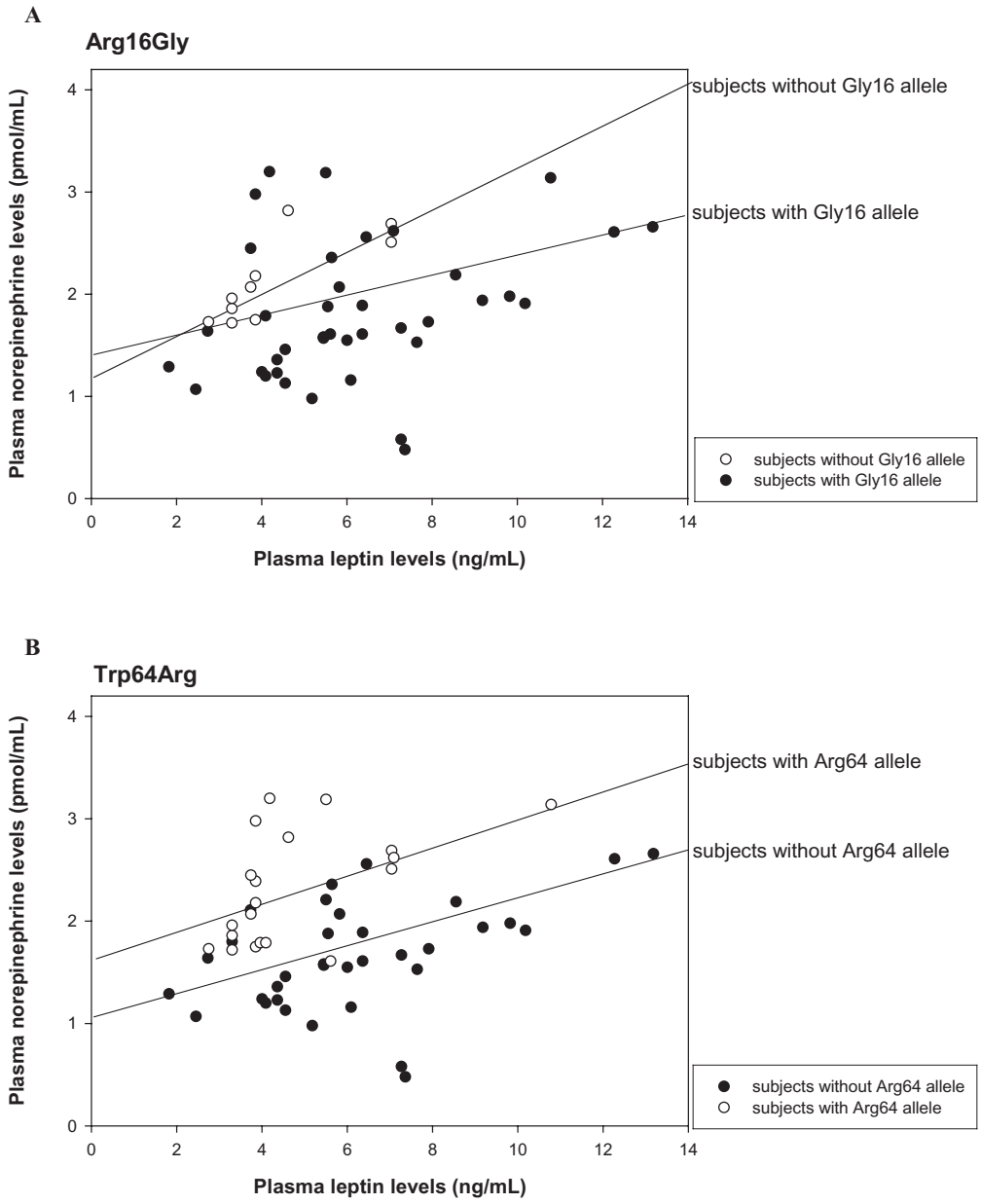

Fig. 1. *A: Correlations between plasma leptin levels at entry as an independent variable and plasma norepinephrine levels as a dependent variable in subjects with and without Gly16 allele of the β2-adrenoceptor gene. The slopes in subjects carrying the Gly16 allele tended to be lower compared to that without the Gly16 allele. B: Correlations between plasma leptin levels at entry as an independent variable and plasma norepinephrine levels as a dependent variable in subjects with and without Arg64 allele of the β3-adrenoceptor gene. The slopes were similar between subjects with and without the Arg64 allele.*

motensive (BP < 140/90 mmHg) men. It should be noted that the β3-adrenoceptor polymorphisms are linked to both weight gain and BP elevation in obese subjects, although it is related to only BP elevation in nonobese subjects, as previously reported (*15*). Further, the subjects carrying the Arg64 allele originally have a high total body fat-mass, high waist-to-hip ratio (greater abdominal obesity) and heightened sympathetic nerve activity, as seen in patients with high plasma NE. These findings demonstrate that the Arg64 allele of Trp64Arg of the β3-adrenoceptor polymorphism, which is associated with high plasma NE, is important for BP elevation, weight gain, and weight gain–related BP elevations (obesity-related hypertension) as well as original abdominal obesity in obese subjects. The Gly16 allele of the Arg16Gly polymorphism of the β2-adrenoceptor gene also had effects on BP elevation and weight gain in the obese group, which were similar to its

effects in nonobese subjects, but which in obese subjects were accompanied by high leptin levels and blunted β2-adrenoceptor function.

The β3-adrenoceptor function is important in mediating the stimulation of lipolysis by catecholamines in white adipose tissue. The decreased function of the receptor could lead to diminished lipolysis and result in the development of obesity (*9–11*). There are observations in a Japanese population that subjects homozygous for Arg64 had a greater BMI and percent fat-mass than those homozygous for Trp64 (*10, 12*). Clement *et al*. (*9*) found that obese subjects carrying Arg64 had an increased capacity for weight gain. These observations demonstrate the strong relationship of the Arg64 allele of Trp64Arg with obesity; however, other investigators (*13, 14*) have found no relationships between the β3-adrenoceptor polymorphisms and obesity. The findings on the relations of the β3-adrenoceptor polymorphisms and obesity are thus discordant. In addition, several studies have directly assessed the functional properties of the Trp64Arg, β3-adrenoceptor polymorphisms. Umekawa *et al*. (*26*) have shown that the Arg64 allele deteriorates lipolysis induced by a β3-adrenoceptor agonist in omental adipocytes, but other investigators found no differences in the lipolysis rate between those with and without the Arg64 allele in Caucasian people (*27*) and in Pima Indians (*28*). Thus, the function of the Arg64 allele of the β3-adrenoceptor polymorphisms also remains controversial.

It has been well documented that weight gain leads to BP elevation (*1, 20, 21*), but there have been few investigations on the association between β3-adrenoceptor polymorphisms such as Trp64Arg and hypertension in obese subjects (*15, 25*). Thus, the present study is one of the first to investigate the associations of the β3-adrenoceptor polymorphisms with weight gain and BP elevation while taking into account the plasma NE and leptin levels. The findings show strong associations in the Arg64 allele of the Trp64Arg in relation to weight gain, BP elevation, and weight gain–induced BP elevation accompanied by high NE. Additionally, an earlier showed that the Trp64Arg polymorphism is related to increased sensitivity to the pressure effects of NE (*29*). Thus, future weight gain and BP elevation might be caused by the Arg64 allele of the β3-adrenoceptor polymorphism through the following mechanisms: blunted β3-adrenoceptor function and resultant lower metabolic rates through sympathetic nerve activity producing future weight gain and increased sensitivity to pressure effects of NE resulting in BP elevations in the future.

Fujisawa *et al*. (*25*) have reported that the allele frequency of Arg64 of Trp64Arg was similar between nonobese normotensive and hypertensive subjects. Our previous study in nonobese, normotensive men showed that the Arg64 allele of Trp64Arg is associated with a significant BP elevation, but not with weight gain (*15*). Krief *et al*. (*30*) observed that the β3-adrenoceptor mRNA levels were higher in visceral fat and lower in subcutaneous fat in humans, indicating that fat distributions might be important for the β3-adrenoceptor function. Further, Alvarez *et al*. (*31, 32*) observed that visceral obesity, but not subcutaneous obesity, is associated with heightened sympathetic nerve activity. In the present study on obese subjects, we observed significantly greater waist-to-hip ratios, total body fat-mass and plasma NE levels in subjects carrying the Arg64 allele than in those without the Arg64 allele, but there was no difference in nonobese subjects (*15*). The findings suggest that the Arg64 allele might link to abdominal obesity associated with heightened sympathetic nerve activity, especially in obese subjects who have an increased capacity to gain weight, as previously reported (*9, 11*). Thus, the relationship between the Arg64 allele and weight gain (obesity) may have differed between obese subjects and nonobese subjects because of a difference in the original degree of abdominal obesity associated with sympathetic nerve activity.

Leptin, an adipocytes-derived hormone, is important for weight loss because it reduces appetite and food intake and increases energy expenditure through sympathetic stimulation of brown adipose tissue (*33, 34*). Leptin resistance, which is defined as an impairment of the action of leptin and is often accompanied by hyperleptinemia, is a common feature of obesity (*35*). Experimental results have shown that chronic systemic and intra-cerebral administration of leptin increases BP in animal models (*36, 37*), suggesting that leptin resistance selectively affects the metabolic actions of leptin, but spares leptin-mediated sympathetic activation (*35*). It should be noted that the significant relationships observed between plasma leptin and NE (sympathetic nerve activity) in the present study differed according to the β-adrenoceptor polymorphisms (alleles) present. Obese subjects carrying the Gly16 allele of Arg16Gly of the β2-adrenoceptor gene had higher plasma leptin levels throughout the study and a lower slope between plasma leptin and NE levels, whereas the slopes were similar between those with and without the Arg64 allele of the β3-adrenoceptor gene. The findings demonstrate that the subjects carrying the Gly16 allele could have leptin resistance as well as blunted leptin-mediated plasma NE responses (sympathetic nerve activation). Further, the Gly16 allele has been described as a down-regulator of β2-adrenoceptor function (*15, 38–41*). Thus, we could propose that a blunted β2-adrenoceptor function (*15*) and leptin resistance might be possible mechanisms in weight gain and BP elevation in obese subjects carrying the Gly16 allele of Arg16Gly, β2-adrenoceptor polymorphisms.

Many epidemiological studies have concluded that obese subjects have a high prevalence of hypertension. The associations of polymorphisms linked to insulin resistance (*42–45*) or the renin-angiotensin system (*46, 47*) with hypertension and/or obesity have been investigated. Although several previous studies have described associations between the β2- and β3-adrenoceptor polymorphisms and hypertension in obesity, few studies have taken into account both variables, sympathetic nerve activity and leptin, both of which have been

shown to play a role in obesity, in the same design (*15*). The strength of the present study was the use of a longitudinal design to evaluate the associations among these polymorphisms, sympathetic nerve activity, and BP elevation in obesity and thereby help to clarify the mechanisms of obesity-related hypertension, because not all obese subjects have hypertension. Thus, we studied originally obese, but normotensive subjects in a longitudinal study. The findings showed that the Arg64 allele of the Trp64Arg, β3-adrenoceptor polymorphism was associated with weight gain, BP elevations and weight gain–induced BP elevations accompanied with high plasma NE over a 5-year period. The Gly16 allele of the Arg16Gly, β2-adrenoceptor polymorphism was also linked to further weight gain and BP elevation accompanied by leptin resistance in obese normotensive subjects. Our observations might be important to understand the mechanisms of obesity-related hypertension, and help to explain why not all obese subjects have hypertension.

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