Gln27Glu and Arg16Gly Polymorphisms of the β_2-Adrenergic Receptor Gene Are Not Associated With Obesity in Japanese Men

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The β_2-adrenergic receptor (β_2AR), β_3AR, or uncoupling protein 1 (UCP1) may play a pathogenic role in obesity. In Swedish Caucasians, a polymorphism at codon 27 (Gln27Glu) of the β_2AR gene was shown to be associated with obesity, but no such association was shown for a polymorphism of codon 16 (Arg16Gly). Thus, we investigated whether these polymorphisms contribute to obesity in 210 Japanese men. The frequencies of the Gln27Glu and Arg16Gly polymorphisms were 0.05 and 0.48, respectively, and there was no association with obesity. A strong linkage disequilibrium between the Gln27Glu and Arg16Gly polymorphisms was shown, but there was no apparent additive effect on the clinical or metabolic characteristics. Our results suggest that the Gln27Glu and Arg16Gly polymorphisms of the β_2AR gene are not a major contributing factor to obesity in Japanese men.

AS AN IMPORTANT REGULATOR of energy expenditure and fuel metabolism, the sympathetic nervous system plays an important role in controlling body weight. The β_3-adrenergic receptor (β_3AR) is expressed in visceral fat in humans[1] and is thought to act on increases in lipolysis.[2] Uncoupling protein 1 (UCP1) is a specific intramitochondrial component of brown adipose tissue that uncouples the respiration as heat,[3] but the volume of brown adipose tissue is lower in the adult than in the infant. The β_2AR coexists with β_3AR in abdominal subcutaneous adipose tissue and seems to be of greater importance than β_3AR for the mobilization of lipids.[4] It has also been shown that the sympathetically mediated thermogenic response to stimuli is related to the stimulation of both β_1AR and β_2AR, but not β_3AR.[5] Thus, β_2AR, as well as β_3AR or UCP1, may play pathogenic roles in obesity.

Genetic influences have an important role in determining obesity.[6,7] Associations between obesity and a missense mutation of the β_3AR gene have been reported.[8-13] An A to G polymorphism at position −3826 base pairs (bp) in the 5′ flanking domain of the UCP1 gene was shown to be associated with an increased propensity for weight gain,[14] and we previously reported that the UCP1 polymorphism might be a weak contributing factor to obesity in Japanese men.[13] In a recent study of Swedish Caucasians, obesity was shown to be associated with a glutamine (Gln) to glutamic acid (Glu) polymorphism at codon 27 of the β_2AR gene (Gln27Glu), but not with an arginine (Arg) to glycine (Gly) polymorphism at codon 16 (Arg16Gly) of the same gene.[15] The effects of Gln27Glu and Arg16Gly on the development of obesity have been shown to differ among the races.[16-21]

Thus, we investigated the associations of Gln27Glu and Arg16Gly polymorphisms in the β_2AR gene with obesity, insulin resistance, dyslipidemia, and type 2 diabetes in Japanese men.

SUBJECTS AND METHODS

A total of 264 unrelated Japanese men who were genetically homogeneous and aged 30 to 71 years (mean ± SD, 51.9 ± 7.3) were recruited from individuals who underwent general health examinations at Kanazawa Municipal Hospital from December 1995 to April 1997, as previously described.[13] After excluding 50 subjects with prior treatment for diabetes, hyperlipidemia, hypertension, or hyperuricemia, DNA was available from 210 of 214 subjects. These subjects were aged 30 to 68 years (51.1 ± 7.3). All subjects underwent a 75-g oral glucose tolerance test after an overnight fast, and 149 were classified as having normal glucose tolerance (NGT), 47 as impaired glucose tolerance (IGT) or impaired fasting glycemia (IFG), and 14 as type 2 diabetes by World Health Organization criteria.[22] Informed consent was obtained from all subjects.

Blood samples were obtained in the morning after an overnight fast. The body mass index (BMI), waist to hip ratio, systolic and diastolic blood pressure, serum total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, uric acid, free fatty acid (FFA), fasting plasma glucose, and fasting serum insulin were determined in all subjects. The waist to hip ratio was determined as the ratio of the waist circumference at the umbilical level to the maximum hip circumference. The serum FFA level was measured by an enzymatic method based on the activity of acyl-coenzyme A synthetase with a NEFA-HR kit (Wako Pure Chemical, Osaka, Japan). Serum insulin was determined by radioimmunoassay with a Phadeseph Insulin RIA kit (Pharmacia Diagnostics, Uppsala, Sweden). Insulin resistance was assessed by homeostasis model assessment (HOMA).[23]

Genomic DNA was extracted from peripheral blood leukocytes. To detect the β_2AR polymorphism, we performed a polymerase chain reaction (PCR) with primers as previously described.[24] The Gln27Glu polymorphism was verified by digestion of the 168-bp PCR product using restriction enzyme *Bbv*I and electrophoresis on a 3% agarose gel. The PCR products containing an intact *Bbv*I site are cleaved into 105- and 63-bp fragments. In the presence of the polymorphism, the restriction site is lost. The Arg16Gly polymorphism was verified by digestion of the PCR product using restriction enzyme *Nco*I and electrophoresis on a 3% agarose gel. The 168-bp PCR products containing an intact *Nco*I site are cleaved into 146- and 22-bp fragments. In the presence of the polymorphism, the new restriction site appears, and the 146-bp fragments are cleaved into 128- and 18-bp fragments.

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Statistical Analysis

All data are expressed as the mean ± SD. Statistical analysis was performed using the StatView II statistical package (Abacus Concepts, Berkeley, CA). Differences between group means were tested by the Mann-Whitney nonparametric test for the Gln27Glu polymorphism, or the Bonferroni *t* test after justification by 1-way ANOVA for the Arg16Gly polymorphism. The χ^2 test was used to compare frequencies. A *P* level less than .05 indicated statistical significance.

RESULTS

Among subjects with the Gln27Glu polymorphism of the β_2AR gene, 22 (10.5%) were Gln27Glu heterozygotes (Gln/Glu), 188 (89.5%) were Gln27 homozygotes (Gln/Gln), and none were Glu27 homozygotes. The frequency of the Gln27Glu allele was 0.05. The frequencies of the Gln27Glu allele in subjects with NGT and either IGT or IFG were 0.05 and 0.07, respectively, and all type 2 diabetics were Gln27 homozygotes. There were no differences in the clinical or metabolic characteristics between Gln27Glu genotypes (Table 1).

Among subjects with the Arg16Gly polymorphism of the β_2AR gene, 49 (23.3%) were Gly16 homozygotes (Gly/Gly), 104 (49.5%) were Arg16Gly heterozygotes (Arg/Gly), and 57 (27.2%) were Arg16 homozygotes (Arg/Arg). The frequency of the Arg16Gly allele was 0.48. In subjects with type 2 diabetes, NGT, and either IGT or IFG, the frequency of the Arg16Gly allele was 0.46, 0.46, and 0.55, respectively, with no significant differences between the glucose tolerance patterns. No differences in the clinical or metabolic characteristics were found between Arg16Gly genotypes (Table 2).

The combination of the Gln27Glu and Arg16Gly polymorphism was also investigated in the subjects. There was a strong linkage disequilibrium between the Gln27Glu and Arg16Gly polymorphisms (Table 3). We compared the 3 genotypes: Gln/Glu, Arg/Gly and Gly/Gly; Gln/Glu, Arg/Arg; Gln/Gln, Arg/Gly and Gly/Gly; with the wild-type genotype: Gln/Gln, Arg/Arg. There was no additive effect on the clinical or metabolic characteristics (data not shown).

Table 1. Clinical and Metabolic Characteristics According to Gln27Glu Polymorphism of the β2AR Gene

Characteristic	Gln/Glu	Gln/Gln
No. of subjects	22	188
Age (yr)	48.8 ± 6.2	51.4 ± 7.3
BMI (kg/m²)	23.6 ± 2.9	23.8 ± 2.8
Waist to hip ratio	0.88 ± 0.05	0.89 ± 0.06
Blood pressure (mm Hg)		
Systolic	112 ± 13	116 ± 14
Diastolic	73 ± 8	74 ± 10
Serum lipids (mmol/L)		
Total cholesterol	5.26 ± 1.04	5.41 ± 0.94
Triglyceride	1.54 ± 0.60	1.69 ± 0.63
HDL cholesterol	1.26 ± 0.21	1.34 ± 0.34
Serum uric acid (μmol/L)	369.6 ± 61.6	363.1 ± 70.1
Serum FFA (mEq/L)	0.48 ± 0.23	0.48 ± 0.18
Fasting plasma glucose (mmol/L)	5.39 ± 0.60	5.58 ± 0.92
Fasting serum insulin (pmol/L)	40.9 ± 18.1	36.5 ± 15.8
HOMA insulin resistance	1.61 ± 0.69	1.49 ± 0.65

NOTE. Data are the mean ± SD. All differences between subjects with the Gln/Glu genotype *v* the Gln/Gln genotype were not significant.

Table 2. Clinical and Metabolic Characteristics According to Arg16Gly Polymorphism of the β2AR Gene

Characteristic	Gly/Gly	Arg/Gly	Arg/Arg
No. of subjects	49	104	57
Age (yr)	50.2 ± 8.3	51.5 ± 7.1	51.1 ± 6.6
BMI (kg/m²)	24.2 ± 2.6	23.7 ± 2.7	23.6 ± 3.0
Waist to hip ratio	0.89 ± 0.07	0.89 ± 0.05	0.90 ± 0.05
Blood pressure (mm Hg)			
Systolic	118 ± 14	115 ± 13	113 ± 13
Diastolic	75 ± 9	74 ± 11	72 ± 10
Serum lipids (mmol/L)			
Total cholesterol	5.37 ± 0.91	5.42 ± 1.01	5.36 ± 0.90
Triglyceride	1.73 ± 0.58	1.64 ± 0.61	1.71 ± 0.70
HDL cholesterol	1.30 ± 0.25	1.35 ± 0.37	1.33 ± 0.31
Serum uric acid (μmol/L)	380.2 ± 61.4	359.7 ± 71.7	357.1 ± 69.4
Serum FFA (mEq/L)	0.47 ± 0.18	0.48 ± 0.19	0.48 ± 0.17
Fasting plasma glucose (mmol/L)	5.52 ± 0.78	5.65 ± 1.04	5.44 ± 0.64
Fasting serum insulin (pmol/L)	40.4 ± 16.6	34.7 ± 14.7	37.9 ± 17.5
HOMA insulin resistance	1.66 ± 0.73	1.46 ± 0.69	1.45 ± 0.50

NOTE. Data are the mean ± SD. All differences between subjects with the Gly/Gly or Arg/Gly genotype *v* the Arg/Arg genotype were not significant.

DISCUSSION

In this study, the Gln27Glu and Arg16Gly polymorphisms of the β_2AR gene were not associated with obesity. There was a strong linkage disequilibrium between the Gln27Glu and Arg16Gly polymorphisms, but there was no apparent additive effect on the clinical or metabolic characteristics. The frequency of the Gln27Glu polymorphism was 0.05. This is slightly lower than the rate previously reported in Japanese subjects (0.07 to 0.14)[18] and much lower than the frequencies reported in African Americans (0.20),[19] Turks (0.32),[25] and Caucasians from Sweden (0.30, 0.41 to 0.48),[15,17] Denmark (0.40 to 0.44),[16] and France (0.41).[20] While β_2AR function in adipocytes has been shown not to differ between Gln27Glu genotypes,[15] the effect of the Gln27Glu polymorphism on obesity has been inconsistent.[15-20] In Swedish women, the Gln27Glu polymorphism was shown to be associated with obesity.[15] In African Americans, Danish men, and another group of Swedish subjects, no association was found between Gln27Glu and obesity.[16,17,19] In obese physically inactive French men, the wild homozygotes had a higher BMI than the other genotypes, so it has been

Table 3. Distribution of Gln27Glu and Arg16Gly Polymorphisms of the β2AR Gene

	Gln27/Gln27		Gln27/Glu27		
Genotype	No.	%	No.	%	*P*
Arg16/Arg16	54	28.7	3	13.6	
Arg16/Gly16	99	52.7	5	22.7	<.01
Gly16/Gly16	35	18.6	14	63.7	

contended that obese men with wild homozygotes may benefit from physical activity to reduce their weight.[20] In both Japanese men and women, Ishiyama-Shigemoto et al[18] reported that the Gln27Glu polymorphism was associated with obesity and hypertriglyceridemia. Our results were inconsistent with their report, but this discrepancy probably resulted from the degree of obesity. We conclude this because Ishiyama-Shigemoto et al reported that there was no significant association between the Gln27Glu polymorphism and the BMI or serum lipids in the non-obese group, while the BMI of our subjects was almost the same as that of non-obese subjects and much lower than that of the obese subjects in their report. Thus, we cannot entirely rule out the possibility of a relationship between the Gln27Glu polymorphism and obesity in obese Japanese subjects; however, Gln27Glu is not associated with obesity in Japanese subjects of normal weight. Since we did not find Glu27 homozygotes and the frequency of the Gln27Glu allele was low, it is possible that our results are false-negative.

The frequency of the Arg16Gly polymorphism was 0.48. This is almost the same as that previously reported in Japanese subjects and African Americans[18,19] and slightly lower than the frequencies reported in Swedish Caucasians (0.64, 0.66)[15] and Turks (0.60).[25] Since the Gly16 carriers showed increases in β_2AR selective agonist sensitivity, the Arg16Gly polymorphism can be assumed to alter adipocyte β_2AR function.[15] However, the Arg16Gly polymorphism was not associated with obesity in Swedish Caucasians and African Americans.[15,19] In 2 separate studies, the Arg16Gly polymorphism was associated with body weight in Japanese women. Ishiyama-Shigemoto et al[18] reported that the frequency of 16Gly homozygotes was lower in obese versus non-obese women, while no such association was present in men. Sakane et al[21] reported that the reduction in body weight was greater in obese women with the Arg16Gly polymorphism versus obese women without the polymorphism, while no difference was found in the BMI between Arg16Gly genotypes. In our study, the Arg16Gly polymorphism was not associated with obesity, possibly due to the sex difference or the degree of obesity.

According to the distribution of the Gln27Glu and Arg16Gly polymorphisms, we found a strong linkage disequilibrium between the 2 genes, consistent with the findings of Large et al[15] and Ishiyama-Shigemoto et al.[18] We did not find any additive effect on the clinical or metabolic characteristics between the Gln27Glu and Arg16Gly polymorphisms, consistent with the results of Large et al. In addition to the Gln27Glu and Arg16Gly polymorphisms, other polymorphisms at -47 and -20 in the 5′-untranslated region of the β_2AR gene may be associated with obesity and hypertriglyceridemia in Japanese subjects.[26] Therefore, further studies will be needed to clarify the relationship between these polymorphisms and obesity.

In conclusion, the Gln27Glu and Arg16Gly polymorphisms of the β_2AR gene are not a major contributing factor to obesity in Japanese men.

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