The Significance of the Trp 64 Arg Mutation of the β_3-Adrenergic Receptor Gene in Impaired Glucose Tolerance, Non–Insulin-Dependent Diabetes Mellitus, and Insulin Resistance in Japanese Subjects

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It has been reported that the Trp 64 Arg mutation of the human β_3-adrenergic receptor (β_3-AR) gene is related to an earlier age of onset of non-insulin-dependent diabetes mellitus (NIDDM) and features of insulin resistance and weight gain in morbidly obese patients. However, such findings have not been consistent in varying ethnic populations. In the present study, we investigated the frequency of the Trp 64 Arg mutation of the human β_3-AR gene in Japanese control subjects (n = 253) and in NIDDM (n = 314) and impaired glucose tolerance (IGT) patients (n = 100). We compared the frequency of the mutation with the body-mass index (BMI) in these groups and with the metabolic clearance rate (MCR) of glucose in the NIDDM patients. A Trp 64 Arg mutation was observed in 36.7%, 31.6%, and 37.0% of the control, NIDDM, and IGT subjects, respectively. The frequency of the homozygotes for the mutation was 4.3%, 4.8%, and 3.0%, respectively. Neither the genotype frequency (Trp/Arg, Arg/Arg) nor the frequency of the mutated allele was significantly different among the three groups. The BMI of the subjects with the mutation was not significantly higher than that of the subjects without the mutation in each group. Furthermore, the allele frequency (A) was not different among the subjects with different BMIs (BMI $<$ 22.0, 22.0 $\leq$ BMI $\leq$ 26.4, BMI $>$ 26.4) in each group. In a separate group of NIDDM patients, the MCR of the subjects with intermediate BMIs (22.0 $\leq$ BMI $\leq$ 26.4) with the mutation tended to be lower than that of those without the mutation. In addition, the MCR of the subjects with the mutation in this group was significantly lower compared with that of those with a BMI less than 22. These results indicate that the Trp 64 Arg mutation of the β_3-AR gene may not contribute to the development of NIDDM or be a determinant of obesity in the Japanese population. However, the mutation may contribute to insulin resistance in NIDDM patients with an intermediate BMI.

ADIPOSE TISSUE PLAYS a crucial role in energy homeostasis. Obesity, which results from the imbalance between caloric intake and energy expenditure, is often associated with hyperinsulinemia, glucose intolerance, and dyslipidemia.[1,2] The β_3-adrenergic receptor (β_3-AR) is predominantly expressed in brown and white adipose tissue in rodents and involved in the regulation of lipolysis and thermogenesis.[3,4] In humans, β_3-AR is expressed exclusively in white adipose tissue, especially that lining the gastrointestinal tract,[5-8] and is also important for energy metabolism by regulating the resting metabolic rate and lipolysis.[9,10] Thus, abnormalities of β_3-AR are thought to lead to obesity and obesity-related disorders.[11,12] A missense mutation of the human β_3-AR gene (Trp 64 Arg) has recently been reported to be implicated in obesity, insulin resistance, and non–insulin-dependent diabetes mellitus (NIDDM). It has been reported that among Pima Indians, subjects with this mutation have an earlier age of onset of NIDDM and a tendency toward a lower metabolic rate.[13] The mutation is also associated with clinical features of the insulin-resistance syndrome in nondiabetic Finns.[14] In French Caucasians, this genetic variation is reported to be related to an increased capacity to gain weight in patients with morbid obesity.[15] In addition, it has been reported that the β_3-AR gene may be one of the loci contributing to obesity and hyperinsulinemia in Japanese subjects.[16-18]

However, findings that are inconsistent with these observations have been reported in different, as well as the same ethnic populations.[19-23] This implies that the abnormalities of the β_3-AR gene may be less important to predispose subjects to obesity and insulin resistance in some ethnic groups. Alternatively, the discrepancy of the results may reflect differences in the study subjects, in terms of subject numbers, sex, and age.

In the present study, we studied the frequency of the Trp 64 Arg mutation of the β_3-AR gene in nondiabetic Japanese subjects (n = 253) and Japanese patients with impaired glucose tolerance (IGT) (n = 100) and NIDDM (n = 314). We investigated the association of the mutation with variables, including body-mass index (BMI), blood pressure, and serum lipids. In addition, we examined the association of the mutation with the metabolic clearance rate (MCR) of glucose determined by a hyperinsulinemic-euglycemic glucose clamp in a separate group of NIDDM subjects.

SUBJECTS AND METHODS

Study Subjects

Two hundred fifty-three control subjects were recruited from the General Health Check-Up Center of Osaka Kita Posts and Telecommunications Hospital and Takeda Hospital. One hundred IGT subjects and 314 NIDDM subjects were recruited from the inpatient and outpatient populations of Kyoto University Hospital, Hamamatsu University School of Medicine Hospital, and Shizuoka General Hospital. The

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NIDDM and IGT subjects were diagnosed by the criteria of the World Health Organization (WHO). Control subjects were defined as individuals with normal glucose tolerance according to WHO criteria and without any laboratory findings indicative of systemic disease. BMI was calculated as weight (in kilograms) divided by height (in meters squared). According to criteria of the Japan Society for the Study of Obesity, a BMI less than 22.0 is considered to be non-obese and a BMI greater than 26.4 (>120% of BMI 22.0) to be obese in Japanese. Blood pressure, serum total cholesterol, and triglyceride levels were measured. Informed consent was obtained from the subjects.

Hyperinsulinemic-Euglycemic Glucose Clamp

A hyperinsulinemic clamp was performed using the method of DeFronzo et al.[24] Briefly, in a euglycemic clamp test, the plasma insulin concentration was rapidly raised by a priming injection of short-acting insulin and maintained by a continuous infusion of short-acting insulin at 40 mU $\cdot$ m^{-2} $\cdot$ min^{-1}. The plasma glucose concentration was simultaneously kept constant at a fasting glucose level by a variable glucose infusion. The glucose infusion rate was determined for the last 30 minutes during the steady-state period. The MCR of glucose (mL $\cdot$ kg^{-1} $\cdot$ min^{-1}) was calculated by dividing the glucose infusion rate by the plasma glucose concentration.

Detection of the Trp 64 Arg Mutation of β_3-AR

Genomic DNA was extracted from peripheral blood leukocytes obtained from the subjects.[25] A polymerase chain reaction (PCR) was performed using 50 ng of genomic DNA as the template with a forward primer (5′-CGC CCA ATA CCG CCA ACA C-3′) and a reverse primer (5′-CCA CCA GGA GTC CCA TCA CC-3′) under the same conditions as previously described.[14] PCR products were digested by a restriction endonuclease, *Bst*OI, and by incubating the reaction mixture at 60°C for 2 hours. Digested samples were subjected to 3% agarose gel electrophoresis, and the gel was stained with ethidium bromide to determine the presence of a mutation.

Statistical Analysis

Clinical data were expressed as means ± SD. Differences in clinical characteristics between groups were tested by Mann-Whitney *U* test. The frequencies of the alleles and genotypes among the patients and control subjects were counted and were compared by the chi-square test, with the values predicted by the assumption of Hardy-Weinberg equilibrium in the sample. MCR data are presented as means ± SEM. Differences in the values of MCR between genotypes were tested by the Mann-Whitney *U* test because they are not normally distributed. *P* values less than .05 were considered significant.

RESULTS

Frequency of the Trp 64 Arg Mutation of the β_3-AR Gene in Control Subjects and Patients With NIDDM or IGT

We investigated 253 control subjects, 314 NIDDM patients, and 100 IGT patients for the presense of the Trp 64 Arg mutation of the β_3-AR gene. The characteristics of the subjects in the three groups are shown in Table 1. BMI did not significantly differ among the groups. Ages of NIDDM and IGT subjects were significantly higher than those of control subjects. Systolic blood pressure values of NIDDM and IGT subjects were significantly higher than that of control subjects. The diastolic blood pressure of IGT subjects was also significantly higher than that of the control subjects, as well as the NIDDM subjects. Triglyceride levels of the NIDDM and IGT subjects were significantly higher than that of the control subjects. As shown in Table 2, a Trp 64 Arg mutation was observed in 36.7%, 31.6%, and 37.0% of control, NIDDM, and IGT subjects, respectively. Frequencies of subjects homozygous for the mutation were 4.3%, 4.8%, and 3.0%, respectively. The genotype frequency (Trp/Arg, Arg/Arg) was not significantly different among the three groups. We also compared frequencies of the mutated allele in these groups and found no significant difference among them (Table 2).

Table 1. Clinical Characteristics of Control Subjects and Patients With NIDDM and IGT

Characteristics	Control Subjects (n = 253)	NIDDM Subjects (n = 314)	IGT Subjects (n = 100)
Sex (male/female)	190/63	165/149	76/24
Age (years)	45 ± 12 (250)	53 ± 14† (300)	52 ± 11† (100)
BMI*	23.8 ± 4.3 (232)	23.0 ± 4.0 (285)	22.9 ± 3.0 (97)
Blood pressure (mm Hg)			
Systolic	118 ± 17 (218)	132 ± 22† (177)	130 ± 22† (95)
Diastolic	74 ± 11 (218)	75 ± 12 (177)	81 ± 12†‡ (95)
Serum lipids (mg/dL)			
Total cholesterol	196 ± 32 (214)	198 ± 38 (182)	201 ± 38 (96)
Triglycerides	108 ± 59 (203)	117 ± 53§ (167)	119 ± 54§ (93)

NOTE. Data are means ± SD (n).
*Calculated as weight (kg) divided by height (m^2).
†$P < .0001$ *v* control subjects.
‡$P < .0005$ *v* NIDDM subjects.
§$P < .05$ *v* control subjects.

Trp 64 Arg Mutation of the β_3-AR Gene in Relation to BMI, Blood Pressure, and Serum Lipids

We investigated the association of the Trp 64 Arg mutation with BMI, blood pressure, and serum lipids in control, NIDDM, and IGT subjects. As shown in Fig 1, in control subjects, the BMI of subjects with the mutation in heterozygous and homozygous form was not significantly higher than that of those without the mutation. Similar results were observed in the NIDDM and IGT groups. The allele frequency of the β_3-AR gene was examined with respect to BMI, as shown in Table 3. The frequency of the mutated allele (A) was not significantly different among the subjects with different BMIs (BMI $<$ 22.0, 22.0 $\leq$ BMI $\leq$ 26.4, BMI $>$ 26.4) in the control, NIDDM, and

Table 2. Genotype Frequency and Allele Frequency of the Trp 64 Arg Mutation of the β_3-AR Gene in Control Subjects and Patients With NIDDM and IGT

	Control Subjects (n = 253)		NIDDM Subjects (n = 314)		IGT Subjects (n = 100)	
Variable	No.	%	No.	%	No.	%
Genotype						
Arg 64 Arg	11	4.3	15	4.8	3	3.0
Trp 64 Arg	82	32.4	84	26.8	34	34.0
Trp 64 Trp	160	63.2	215	68.5	63	63.0
Allele frequency	104/506	20.6	114/628	18.2	40/200	20.0

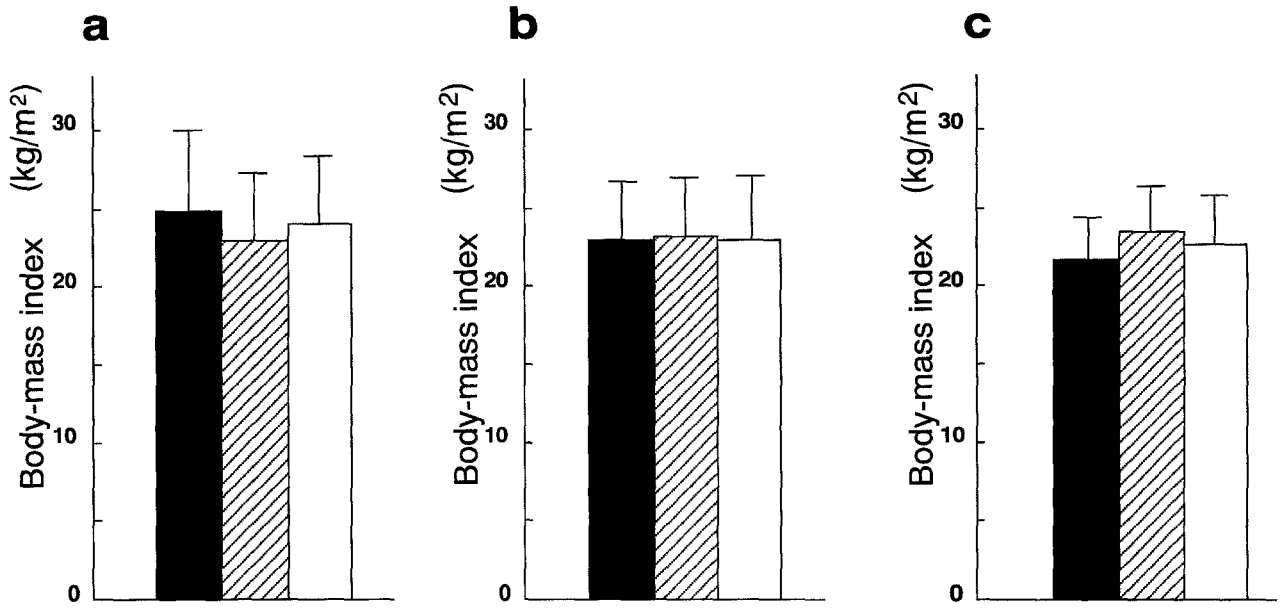

Fig 1. BMI of control, NIDDM, and IGT subjects according to genotype of β_3-AR gene. Among controls (a), the BMI of the Trp 64 Arg heterozygote subjects (▨) and the Arg 64 Arg homozygote subjects (■) was not significantly higher than that of Trp 64 Trp normal subjects (□). Similar results were obtained in NIDDM (b) and IGT (c) subjects.

IGT subjects. Blood pressure, total cholesterol, and triglyceride values were also not significantly different among those with or without the mutation in each group (data not shown).

Trp 64 Arg Mutation of the β_3-AR Gene in Relation to Insulin Resistance in NIDDM Patients

We examined whether the Trp 64 Arg mutation is associated with insulin resistance in NIDDM patients. To assess insulin resistance in NIDDM patients, we determined the MCR of glucose using a hyperinsulinemic-euglycemic glucose clamp in 59 NIDDM patients and examined the relationship of mutation incidence with MCR. NIDDM patients in this study had stable glycemic control and did not show a significant variation of BMI during the study. Because it is a well-known fact that BMI influences MCR, we divided the subjects into three groups according to BMI (BMI $<$ 22.0, 22.0 $\leq$ BMI $\leq$ 26.4, and BMI $>$ 26.4). We also selected subjects from 40 to 65 years of age and divided them similarly with respect to BMI. Because the number of subjects with the mutation was limited in each group, both the homozygotes and heterozygotes for the mutation were considered for comparison. As shown in Table 4, in subjects of all ages with an intermediate BMI (22.0 $\leq$ BMI $\leq$ 26.4), the MCR of subjects with the mutation tended to be lower than that of those without the mutation, although not significantly (P = .179). However, the MCR of intermediate BMI subjects with the mutation was significantly lower than that of those with the mutation and with a BMI less than 22 (P = .031). Because the MCR of subjects without the mutation was not different between intermediate BMI subjects and subjects with a BMI less than 22, it is likely that the decreased MCR of subjects with the mutation and an intermediate BMI is the influence of the mutation, rather than BMI itself. Similar results were obtained when 40- to 65-year-old subjects were examined (Table 5), further indicating that the Trp 64 Arg mutation of the β_3-AR gene has an influence on MCR in subjects with an intermediate BMI.

Table 3. Allele Frequency of the Trp 64 Arg Mutation of the β_3-AR Gene in Control Subjects and Patients With NIDDM and IGT According to BMI

	Control Subjects (n = 232)		NIDDM Subjects (n = 285)		IGT Subjects (n = 97)	
BMI Group	No.	%	No.	%	No.	%
Non-obese (BMI $<$ 22)	38/160	23.8	40/234	17.1	10/62	16.1
Intermediate (22 $\leq$ BMI $\leq$ 26.4)	42/222	18.9*	45/252	17.9*	24/124	19.4*
Obese (BMI $>$ 26.4)	14/82	17.1*	17/84	20.2*	3/8	37.5*

*Difference not significant *v* non-obese subjects in each group.

Table 4. MCR of Glucose for the Genotype of the β_3-AR Gene According to BMI

BMI Group	No.	MCR
BMI $<$ 22		
Arg 64 Arg¶/Trp 64 Arg	9	7.3 ± 0.8
Trp 64 Trp	24	6.8 ± 0.6*
22 $\leq$ BMI $\leq$ 26.4		
Arg 64 Arg‖/Trp 64 Arg	4	4.2 ± 1.1†
Trp 64 Trp	17	5.8 ± 0.6‡§
BMI $>$ 26.4		
Arg 64 Arg/Trp 64 Arg	1	4.04
Trp 64 Trp	4	4.3 ± 0.7

NOTE. Data are mean ± SEM.

*Difference not significant *v* BMI $<$ 22, Arg 64 Arg/Trp 64 Arg.

†$P <$ .05 *v* BMI $<$ 22, Arg 64 Arg/Trp 64 Arg.

‡Difference not significant *v* 22 $\leq$ BMI $\leq$ 26.4, Arg 64 Arg/Trp 64 Arg.

§Difference not significant *v* BMI $<$ 22, Trp 64 Trp.

¶The number of homozygotes was 2 and the values of MCR were 7.49 and 8.92, respectively.

‖The number of homozygotes was 2 and the values of MCR were 1.83 and 7.01, respectively.

Table 5. MCR of Glucose for the Genotype of the β_3-AR Gene According to BMI

BMI Group	No.	MCR
BMI < 22		
Arg 64 Arg£/Trp 64 Arg	6	7.9 ± 0.7
Trp 64 Trp	14	6.1 ± 0.6*
22 ≤ BMI ≤ 26.4		
Arg 64 Arg‖/Trp 64 Arg	3	4.2 ± 1.5†
Trp 64 Trp	12	6.0 ± 0.6‡§
BMI > 26.4		
Arg 64 Arg/Trp 64 Arg	1	4.04
Trp 64 Trp	1	2.43

NOTE. Data are means ± SEM, and are only for subjects aged 40 to 65.

*Difference not significant *v* BMI < 22, Arg 64 Arg/Trp 64 Arg.

†$P < .05$ *v* BMI < 22, Arg 64 Arg/Trp 64 Arg.

‡Difference not significant *v* 22 ≤ BMI ≤ 26.4, Arg 64 Arg/Trp 64 Arg.

§Difference not significant *v* BMI < 22, Trp 64 Trp.

£The number of homozygotes was 1 and the value of MCR was 8.92.

‖The number of homozygotes was 2 and the values of MCR were 1.83 and 7.01, respectively.

DISCUSSION

It was recently reported that the Trp 64 Arg mutation of the β_3-AR gene is associated with abdominal obesity and insulin resistance in nondiabetic Finnish subjects and that the mutation predisposes Pima Indian subjects to develop diabetes at an earlier age.[13,14] Since then, several investigations as to whether this mutation is associated with obesity, insulin resistance, and NIDDM in other ethnic populations, including Japanese subjects and Danish subjects, have been published.[17-20,22,23,26] These studies showed somewhat conflicting results regarding clinical variables and the characteristics of subjects associated with this mutation.

In the current study, we investigated the frequency of the Trp 64 Arg mutation in a large number of Japanese subjects, including control subjects and patients with NIDDM and IGT (667 subjects). We found that neither the frequency of the genotype nor the frequency of the mutant allele of the β_3-AR gene was significantly associated with NIDDM and IGT in Japanese subjects. This result is consistent with a previous report that showed the allele frequency of the β_3-AR gene is not associated with NIDDM in Japanese subjects.[18,22] We also found that the Trp 64 Arg mutation was not associated with the IGT subjects, which suggests this mutation may not contribute to the development of NIDDM. This contention is supported by the recent finding of the Finnish US Investigation of NIDDM (FUSION) study that the mutation was not associated with the age of onset of NIDDM in a large number of Finnish NIDDM probands.[27]

We also observed that the BMI of the subjects with the mutation was not significantly higher than that of those without the mutation in each group, indicating that the Trp 64 Arg mutation may not be a determinant of obesity in Japanese subjects. A similar result was obtained in two studies: the Québec Family Study (QFS) and the Swedish Obese Subjects (SOS).[28] However, it has recently been reported that the Arg 64 Arg genotype is somewhat associated with BMI in Japanese NIDDM subjects.[18] In addition, the allele frequency of the mutated allele (A) has been reported to be significantly higher in Japanese nondiabetic obese subjects.[17] These results seem to be inconsistent with ours and with those of another report.[23] However, the number of NIDDM patients homozygous for this mutation in the previous report[18] is relatively small. Furthermore, when our result of the allele frequency with respect to BMI is combined with a previous study,[17] there is no significant difference. Thus, a study with a greater number of subjects is needed to establish whether the Trp 64 Arg mutation of the β_3-AR gene is a determinent of obesity in Japanese subjects.

In a separate group of NIDDM subjects, we found that the MCR of intermediate BMI (22.0 ≤ BMI ≤ 26.4) subjects with the mutation tended to be lower than that of those without the mutation. The mutation significantly influenced the MCR of these subjects compared with that of subjects with a BMI less than 22, even when a modification of MCR by age was considered. This result suggests that the effect of BMI on MCR is more prominent in subjects with an intermediate BMI if the mutation exists. Although it is unclear why the mutation influenced the MCR in these subjects, it may be postulated that the mutation of the β_3-AR gene predisposes the subjects with this range of BMI to insulin resistance when it exists with some clinical conditions, such as abdominal obesity. In addition, it could be that the effect of BMI on MCR on a Trp 64 Arg background does not become evident when the subjects are clinically obese. Thus, it is worthwhile to examine the visceral fat mass in the subjects of this group.

Previous reports have suggested that the Trp 64 Arg mutation in a homozygous form may contribute to both obesity and insulin resistance.[17] We observed that the frequencies of the homozygotes for the mutation were 4.3%, 4.8%, and 3.0% of control, NIDDM, and IGT subjects, respectively. These frequencies seem to be higher than in Caucasians. We were unable to detect the association of the mutation in a homozygous form with BMI. Furthermore, since in the group of NIDDM patients whose MCR was determined, the number of homozygotes was limited (n = 2 in the group with a BMI <22 and the intermediate-BMI group, respectively), a significant difference of MCR of subjects with the mutation was obtained, particularly when both genotypes were included in the comparison. Therefore, a further study with an increased number of homozygotes is necessary to determine whether the Trp 64 Arg mutation contributes to insulin resistance in Japanese NIDDM subjects.

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