Prevalence of the Trp64Arg Missense Mutation of the β_3-Adrenergic Receptor Gene in Japanese Subjects

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Prompted by the recent findings that a tryptophan to arginine (Trp64Arg) mutation in the β_3-adrenergic receptor gene was associated with an earlier onset of non–insulin-dependent diabetes mellitus (NIDDM) in Pima Indians, with abdominal obesity and insulin resistance in Finns, and with an increased capacity to gain weight in French whites, we studied the prevalence of this mutation in 231 diabetic and 95 nondiabetic Japanese subjects and assessed its contribution to the development of obesity and NIDDM. The allelic frequencies of the mutation were 0.18 in diabetic and 0.23 in nondiabetic subjects, showing no significant difference between the two groups ($P = .067$). In nondiabetic subjects, body mass index (BMI) did not differ between those with and without the mutation (22.2 ± 3.5 *v* 21.4 ± 3.2 kg/m^2, $P = .252$). In NIDDM subjects, BMI at the time of study and maximal BMI before the start of treatment did not differ between those with and without the mutation (22.8 ± 2.6 *v* 23.2 ± 3.7 kg/m^2, $P = .678$, and 24.7 ± 2.6 *v* 24.9 ± 3.1 kg/m^2, $P = .277$). Homozygotes for the mutation did not have trends to have increased BMI in either diabetic or nondiabetic subjects. The age at diagnosis of NIDDM also did not differ between the two groups (48.8 ± 9.9 *v* 47.8 ± 12.5 years, $P = .796$). Fasting serum cholesterol and triglyceride levels and systolic and diastolic blood pressure before the start of treatment did not differ between NIDDM subjects with and without the mutation. In conclusion, although the Trp64Arg mutation is not uncommon in Japanese, it does not appear to be associated with obesity, NIDDM, age at diagnosis of NIDDM, or dyslipidemia. Our results suggest that the mutation has minor effects, if any, on the development of obesity and NIDDM in Japanese.

NON–INSULIN-DEPENDENT diabetes mellitus (NIDDM) is a polygenic multifactorial disorder characterized by defects in insulin secretion from the pancreatic islet β cells and impaired insulin action on target tissues.[1] Obesity is a well-known risk factor for the development of NIDDM,[2] and impaired insulin action has often been attributed to concomitant obesity, particularly abdominal obesity.[3,4] In humans, obesity is a familial disorder that may be genetically determined, and a low resting metabolic rate leads to excess accumulation of energy, weight gain, and obesity.[5,6] In rodents, resting metabolic rate is regulated by the sympathetic nervous system acting through the modulation of lipolysis and thermogenesis in brown adipose tissue.[7]

The β_3-adrenergic receptor, a G protein–coupled receptor that crosses the cell membrane seven times, is predominantly expressed in brown and white adipose tissues, being more abundant in the former.[8] Stimulation of the receptor by β-adrenergic agonists results in increased lipolysis and thermogenesis through the activation of adenyl cyclase followed by an increase in the intracellular concentrations of cyclic adenosine monophosphate. It exerts minimal lipolytic effects on subcutaneous fat cells, but exhibits a marked lipolytic function in fat cells from the visceral region.[8,9] The major role of the receptor is thought to be regulation of the resting metabolic rate.[10] In β_3-adrenergic receptor–deficient mice created by targeted disruption of the gene, body weight was increased in female mice and fat stores were increased in both males and females.[11] Thus, the gene encoding the β_3-adrenergic receptor is a candidate gene for obesity and insulin resistance in humans.

Recently, it was reported that among Pima Indians, homozygotes for a missense mutation that replaced tryptophan with arginine at position 64 (Trp64Arg) in the first intracellular loop of the receptor had an earlier onset of NIDDM and tended to have a lower metabolic rate.[12] In Finns,[13] the mutation was associated with abdominal obesity and insulin resistance, and was speculated to accelerate the onset of NIDDM. In French whites,[14] subjects with the mutation had an apparently increased capacity to gain weight.

In Japanese, it was reported recently that individuals homozygous for the mutation had a higher body mass index (BMI)[15,16] and elevated serum insulin after an oral insulin load.[15] Although one report suggested that homozygotes for the mutation tended to have an earlier age of onset of NIDDM,[16] the frequency of the mutation did not differ between subjects with and without NIDDM.[15-17]

Since the associations reported previously between the mutation and some markers of obesity and an earlier age of onset of NIDDM are weak, further study is necessary to confirm or reject the observations. Herein, we have further evaluated the contribution of this mutation to the development of obesity and NIDDM in Japanese, in whom insulin secretory defects have been suggested to play a more significant role than insulin resistance in the development of NIDDM.[18]

SUBJECTS AND METHODS

Subjects

A total of 231 patients with NIDDM (147 men and 84 women) and 95 nondiabetic subjects (38 men and 57 women) were studied after informed consent had been obtained. The subjects were all Japanese and were not first-degree relatives (ie, siblings, parents, or children) or second-degree relatives (ie, grandparents, grandchildren, uncles, aunts, nephews, or nieces) based on history. One hundred one NIDDM subjects were recruited from Yamaguchi University Hospital and

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affiliated facilities,[19] 69 from Tokyo University Hospital and affiliated facilities, and 61 from factory employees who received an annual health examination at the factory-owned medical facility, Toyokohan Hospital. Individuals with NIDDM met the diagnostic criteria of the World Health Organization. We excluded subjects with other conditions affecting glucose tolerance, such as severe liver diseases or endocrine disorders; otherwise, we chose the subjects randomly based on disease status and willingness to enter the study. Nondiabetic control subjects were recruited among patients and staff members at Yamaguchi University Hospital and affiliated facilities.[19] They were randomly selected solely on the basis of two criteria: (1) random plasma glucose levels less than 6.7 mmol/L, and (2) absence of personal or family history of diabetes in second-degree relatives. We did not perform an oral glucose tolerance test on the control subjects. Clinical data were collected from medical records, records of physical examinations, or interview. For participants recruited at Toyokohan Hospital, BMI, lipid values after overnight fasting, and blood pressure before the start of treatment were used for the analyses. Clinical characteristics of the subjects are summarized in Table 1. All values are presented as the mean ± SD.

Genomic DNA was extracted from peripheral blood leukocytes as reported previously.[19]

Restriction Fragment Length Polymorphism Analysis

A 5′ primer (5′-CGCCCAATACCGCCAACAC-3′) and a 3′ primer (5′-CCACCAGGAGTCCCATCACC-3′) were synthesized using a DNA synthesizer (model 392; Applied Biosystems Japan, Urayasu, Japan). A 210-bp DNA fragment containing a polymorphic *Bst*NI site was amplified by polymerase chain reaction (PCR) in a volume of 10 μL containing 100 ng genomic DNA, 10 pmol of each primer, 200 μmol/L of each dNTP, 1.0 mmol/L $MgCl_2$, 10 mmol/L Tris hydrochloride (pH 9.0), 50 mmol/L KCl, 0.1% Triton X-100, 4% formamide (pH 8.0), and 0.5 U Taq polymerase (Promega, Madison, WI). PCR were initiated with denaturation at 94°C for 3 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes in a thermocycler (Program Temp Control System PC-700; ASTEC, Fukuoka, Japan). After the amplification, 10 μL of a mixture containing 10 mmol/L NaCl, 2 mmol/L Tris hydrochloride, 2 mmol/L $MgCl_2$, 0.2 mmol/L dithiothreitol, and 5 U *Bst*NI (New England Biolabs, Beverly, MA) was added and further incubated at 60°C for 16 hours to digest the products. The digested samples were analyzed by electrophoresis through a 3% low–melting-point agarose gel and ethidium bromide staining.

Statistical Analysis

The statistical significance of differences between groups in quantitative variables was tested using unpaired *t* tests or Mann-Whitney tests when appropriate. Associations between the genetic variation in the β_3-adrenergic receptor and NIDDM were assessed by Fisher's exact test.

Table 1. Clinical Characteristics of the Study Subjects

	Nondiabetic	NIDDM		
Characteristic	Yamaguchi (n = 95)	Yamaguchi (n = 101)	Tokyo (n = 69)	Toyokohan (n = 61)
Sex (F/M)	57/38	55/46	24/45	5/56
Age at study (yr)	56.4 ± 14.8	55.7 ± 10.3	54.5 ± 13.1	52.4 ± 11.6
BMI (kg/m²)	21.6 ± 3.4*	23.0 ± 3.6*	22.3 ± 3.4*	24.8 ± 2.9†
Age at onset of NIDDM (yr)	—	49.7 ± 12.1	42.7 ± 12.6	48.2 ± 9.1

*BMI at study.

†Maximal BMI before treatment.

Table 2. Genotypic and Allelic Frequencies

	NIDDM				
Parameter	Total	Yamaguchi	Tokyo	Toyokohan	Nondiabetic
Genotype	(N = 231)	(n = 101)	(n = 69)	(n = 61)	(n = 95)
Trp/Trp	0.67	0.69	0.64	0.71	0.62
Trp/Arg	0.30	0.29	0.32	0.26	0.30
Arg/Arg	0.03	0.02	0.04	0.03	0.08
Allele	(N = 462)	(n = 202)	(n = 138)	(n = 122)	(n = 190)
Trp	0.82	0.82	0.80	0.84	0.77
Arg	0.18	0.18	0.20	0.16	0.23

RESULTS

Allelic Frequency of the Trp64Arg Mutation in NIDDM and Nondiabetic Subjects

Digestion of 210-bp PCR products with *Bst*NI yielded fragments of the following sizes: 99, 62, 30, 12, and seven bp in Trp64 homozygotes; 161, 99, 62, 30, 12, and seven bp in Trp64/Arg64 heterozygotes; and 161, 30, 12, and seven bp in Arg64 homozygotes. The allelic frequency of the Arg64 was 0.23 in nondiabetic subjects (Table 2). In subjects with NIDDM, allelic frequencies were 0.18, 0.20, and 0.16 for those recruited from the Yamaguchi area, Tokyo area, and Toyokohan Hospital, respectively. Allelic frequencies did not differ among the three groups of NIDDM subjects. The overall allelic frequency of Arg64 in Japanese NIDDM subjects was 0.18, not significantly different from that in the nondiabetic subjects ($P = .067$, Fisher's exact test). No genotypic frequency differences were detected between NIDDM and nondiabetic subjects (Table 2).

Clinical Characteristics of Individuals With the Trp64Arg Mutation

In the nondiabetic subject group, BMI was compared between those with and without the Arg64 allele (Table 3). Data were available for 66 individuals (33 men and 33 women). BMI was 22.2 ± 3.5 kg/m² in subjects with the mutant allele and 21.4 ± 3.2 kg/m² in those without the allele ($P = .311$, unpaired *t* test). BMI did not differ significantly between those with and without the allele in either men or women (data not shown).

BMI values were available at the time of study for 178 NIDDM patients recruited from Yamaguchi University Hospital, Tokyo University Hospital, their affiliates, and Toyokohan Hospital. BMI was 22.8 ± 2.6 and 23.2 ± 3.7 kg/m² in those with and without the mutation, respectively ($P = .678$; Table 3). Maximal BMI data before the start of treatment were available for 46 diabetic patients recruited from Toyokohan Hospital, because they had annual physical examinations since beginning employment at Toyokohan factory at a relatively young age. Maximal BMI was 24.7 ± 2.6 and 24.9 ± 3.1 kg/m² for those with and without the mutation, respectively ($P = .173$, unpaired *t* test; Table 3). BMI was also compared among the genotypes (Fig 1). Homozygotes for the mutation (Arg/Arg) did not appear to have a higher BMI, although the number of subjects studied was small.

The mean age at diagnosis of NIDDM was 47.8 ± 12.5 years in those with the mutation and 48.8 ± 9.9 years in those without it (Table 4). The age difference was not significant ($P = .796$, unpaired *t* test).

Table 3. BMI in Nondiabetic and NIDDM Subjects (kg/m²)

	Nondiabetic	NIDDM*				NIDDM†
Parameter	Yamaguchi (n = 66)	Total (N = 178)	Yamaguchi (n = 97)	Tokyo (n = 35)	Toyokohan (n = 46)	Toyokohan (n = 46)
Without Arg64	21.4 ± 3.2	23.2 ± 3.7	23.1 ± 4.1	22.0 ± 3.2	23.8 ± 3.0	24.9 ± 3.1
With Arg64	22.2 ± 3.5	22.8 ± 2.6	22.7 ± 2.2	23.4 ± 4.5	22.6 ± 3.1	24.7 ± 2.6
P	.311	.678	.944	.170	.406	.173

*BMI at study.
†Maximal BMI before treatment.

Hypertension and dyslipidemia are often associated with obesity, especially abdominal obesity.[20,21] We also compared these parameters in subjects with NIDDM who had received health examinations at Toyokohan Hospital (Table 5). The respective values for fasting total cholesterol for those with and without the mutation were 208.1 ± 37.8 and 215.7 ± 38.7 mg/dL, fasting triglycerides 160.4 ± 104 and 121.6 ± 70.0 mg/dL, systolic blood pressure 133.7 ± 22.2 and 127.4 ± 20.4 mmHg, and diastolic blood pressure 77.6 ± 9.5 and 76.7 ± 11.1 mmHg. None of these parameters differed significantly between the two groups (P = .954, .431, .293, and .798 for cholesterol, triglycerides, and systolic and diastolic blood pressure, respectively, by unpaired t test).

DISCUSSION

We examined the prevalence of the Trp64Arg missense mutation of the β_3-adrenergic receptor gene in Japanese subjects with and without NIDDM (Table 2). The allelic frequency of the Arg64 mutation was 0.18 in NIDDM patients and 0.23 in nondiabetic subjects. This mutation was more common in Japanese than in whites, African-Americans, and Mexican-Americans, but less common than in Pima Indians.[12-14]

In Pima Indians,[12] homozygotes for the mutation had an earlier onset of NIDDM and tended to have a lower metabolic rate. In addition, BMI tended to be greater in those with the mutation. In Finns,[13] the Trp64Arg mutation was associated with abdominal obesity and insulin resistance, and was postulated to accelerate the onset of NIDDM. In French whites,[14] individuals with the mutation had an apparently increased capacity to gain weight.

In contrast, BMI did not differ between those with and without the mutation in diabetic or nondiabetic Japanese subjects (Table 3). To eliminate the potential effect of diabetes treatment, we also studied maximal BMI before the start of treatment in a subset of NIDDM subjects and obtained similar results. Widén et al[13] reported that in nondiabetic subjects, siblings with the mutated allele had a higher waist to hip ratio despite having a similar BMI. The waist to hip ratio may be a better marker than BMI for the obesity associated with this mutation, since visceral fat deposition, in which predominantly β_3-adrenergic receptors are expressed, is more closely related to insulin resistance. However, data for the waist to hip ratio were not available in the present study. The age at diagnosis of NIDDM did not differ between Japanese subjects with and without the Arg64 mutation.

Recently, Kadowaki et al[15] reported that in nondiabetic Japanese subjects, BMI was significantly higher in those homozygous for the mutant allele than in those homozygous for the normal allele. In the population, the frequency of the Trp64Arg mutation was higher in obese subjects (BMI > 26.4) than in non-obese subjects (BMI < 22). Fujisawa et al[16] also suggested that homozygotes for the mutation had a marginally higher BMI in the Japanese population including both diabetic and nondiabetic subjects. However, in our study population, we did not observe any trends for an increase in BMI in either diabetic or nondiabetic subjects with the mutation, even in the homozygous state (Table 3 and Fig 1). The different results may be due to the small number of individuals homozygous for the mutant allele in our study subjects, especially in the nondiabetic group, since the effect of the mutation may be more prominent in nondiabetic subjects.[15] However, of note is that Awata and Katayama[17] reported results similar to ours. These results

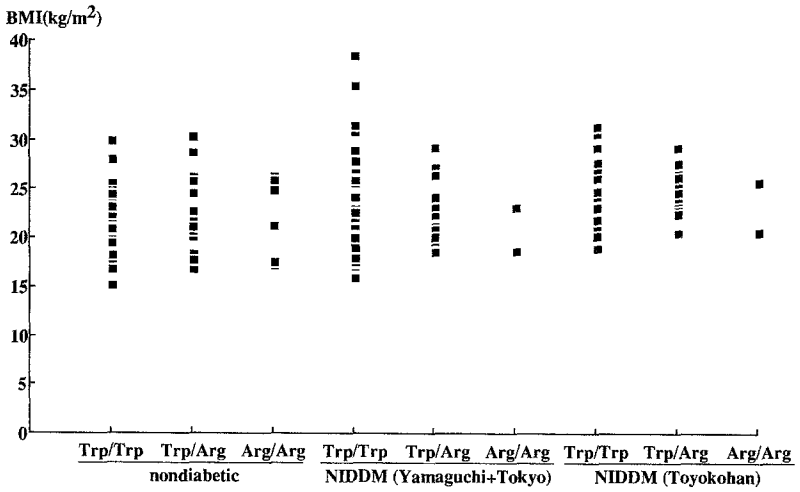

Fig 1. Distribution of BMI by Trp64Arg missense mutation genotypes. BMIs were plotted by genotype, ie, according to the Trp64Arg mutation of the β_3-adrenergic receptor gene. The values are BMIs at the time of study for nondiabetic and NIDDM subjects recruited from Yamaguchi and Tokyo University Hospitals and affiliates, and maximal BMIs before the treatment of diabetes for subjects recruited from Toyokohan Hospital.

Table 4. Age at Diagnosis of NIDDM (yr)

Parameter	Yamaguchi (n = 96)	Tokyo (n = 29)	Toyokohan (n = 57)	Total (N = 182)
Without Arg64	50.1 ± 13.1	41.8 ± 13.2	47.3 ± 8.4	47.8 ± 12.5
With Arg64	48.6 ± 9.7	46.0 ± 10.3	50.1 ± 10.6	48.8 ± 9.9
P	.298	.311	.119	.796

Table 5. Fasting Serum Lipids and Blood Pressure in NIDDM Subjects

Parameter	Total Cholesterol (mg/dL)*	Triglyceride (mg/dL)*	Systolic Pressure (mm Hg)†	Diastolic Pressure (mm Hg)†
Without Arg64	215.6 ± 39.6	175.8 ± 146.0	129.4 ± 23.9	78.2 ± 12.9
With Arg64	214.9 ± 29.9	172.5 ± 102.8	136.9 ± 22.8	77.3 ± 8.7
P	.954	.431	.293	.798

*n = 58.
†n = 54.

suggest that the Trp64Arg mutation of the β_3-adrenergic receptor gene may have weak effects, if any, on the development of obesity in the Japanese.

The impact of the mutation on the development of NIDDM appeared to be even weaker in the Japanese. Our observation of no association with the mutation and NIDDM is in agreement with other studies in Japanese subjects.[15-17] Although Fujisawa et al[16] suggested that the frequencies of the mutated Arg allele and Arg/Arg genotype tended to be higher in NIDDM subjects, the differences are not significant when compared even after the available data are pooled from our study and other studies in Japanese subjects[16,17] (data not shown).

The Trp64Arg mutation of the β_3-adrenergic receptor may play a less significant role in the Japanese than in whites or Pima Indians[12-14] in the development of obesity and NIDDM, despite the higher prevalence of this mutation in the population than in whites. The Japanese may not have sufficient genetic/environmental backgrounds leading to obesity development and insulin resistance in the presence of the mutated β_3-adrenergic receptor, in contrast to Pima Indians and whites, and factors other than the β_3-adrenergic receptor mutation may have a stronger influence on the susceptibility to obesity and NIDDM.

Alternatively, the present results may be compatible with an earlier report that β-cell insulin secretory defects play more important roles in the development of NIDDM in the Japanese than in other ethnic groups.[18]

In conclusion, the Trp64Arg mutation in the β_3-adrenergic receptor gene is not uncommon in the Japanese. This mutation appears to have minor effects on the development of obesity, and no association is found between the mutation and the development of NIDDM, age at diagnosis of NIDDM, dyslipidemia, or hypertension in the Japanese. Factors other than the β_3-adrenergic receptor mutation appear to play more important determinant roles in the development of obesity and NIDDM, possibly reflecting the major contribution of an insulin secretory defect in the development of NIDDM in the Japanese population.

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REFERENCES

1. DeFronzo RA: The triumvirate: β-Cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37:667-687, 1988

2. Barrett-Connor E: Epidemiology, obesity, and non–insulin-dependent diabetes mellitus. Epidemiol Rev 11:172-181, 1989

3. Pouliot M-C, Despres J-P, Nadeau A, et al: Visceral obesity in men: Associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 41:826-834, 1992

4. Caro JF: Insulin resistance in obese and nonobese man. J Clin Endocrinol Metab 73:691-695, 1991

5. Griffiths M, Payne PR, Stunkard AJ, et al: Metabolic rate and physical development in children at risk of obesity. Lancet 336:76-78, 1990

6. Ravussin E, Lillioja S, Knowler WC, et al: Reduced rate of energy expenditure as a risk factor for body-weight gain. N Engl J Med 318:467-472, 1988

7. Arch JRS, Kauman AJ: β_3 and atypical β-adrenoceptors. Med Res Rev 13:663-729, 1993

8. Krief S, Lonnqvist F, Raimbault S, et al: Tissue distribution of β_3-adrenergic receptor mRNA in man. J Clin Invest 91:344-349, 1993

9. Emorine LJ, Marullo S, Briend-Sutren M-M, et al: Molecular characterization of human β_3-adrenergic receptor. Science 245:1118-1121, 1989

10. Lonnqvist F, Thorne A, Nilsell K, et al: A pathogenic role of visceral fat β_3-adrenergic receptors in obesity. J Clin Invest 95:1109-1116, 1995

11. Susulic VS, Frederick RC, Lawitts J, et al: Targeted disruption of the beta 3-adrenergic receptor gene. J Biol Chem 270:29483-29492, 1995

12. Walston J, Silver K, Bogardus C, et al: Time of onset of non–insulin-dependent diabetes mellitus and genetic variation in the β_3-adrenergic receptor gene. N Engl J Med 333:343-347, 1995

13. Widén E, Lehto M, Kanninen T, et al: Association of a polymorphism in the β_3-adrenergic receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 333:348-351, 1995

14. Clement K, Vaisse C, Manning B, et al: Genetic variation in the β_3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 333:352-354, 1995

15. Kadowaki H, Yasuda K, Iwamoto K, et al: A mutation in the β_3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 215:555-560, 1995

16. Fujisawa T, Ikegami H, Yamato E, et al: Association of Trp64Arg mutation of the β_3-adrenergic-receptor with NIDDM and body weight gain. Diabetologia 39:349-352, 1996

17. Awata T, Katayama S: Genetic variation in the β_3-adrenergic receptor in Japanese NIDDM patients. Diabetes Care 19:271-272, 1996

18. Kosaka K, Hagura R, Kuzuya T: Insulin responses in equivocal and definite diabetes, with special reference to subjects who had mild glucose intolerance but later developed definite diabetes. Diabetes 26:944-952, 1987

19. Noda K, Matsutani A, Tanizawa Y, et al: Polymorphic microsatellite repeat markers at the glucokinase gene locus are positively associated with NIDDM in Japanese. Diabetes 42:1147-1152, 1993

20. Kaplan NM: The deadly quartet; Upper body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch Intern Med 149:1514-1520, 1989

21. Fujioka S, Matsuzawa Y, Tokunaga K, et al: Contribution of intraabdominal visceral fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 36:54-59, 1987