A Trp 64 Arg Mutation in the β_3-Adrenergic Receptor Gene Is Not Associated With Moderate Overweight in Japanese Workers

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The aim of the present study was to investigate the trend for obesity and the relationship between Trp64Arg mutation in the β_3-adrenergic receptor gene and obesity-related phenotypes in Japanese workers. A total of 658 workers, 483 women and 175 men, responded to this survey. Genomic DNA was prepared from leukocytes, and DNA amplification by polymerase chain reaction was performed using 100 ng genomic DNA. Amplified fragments were digested with *Bst*NI and analyzed by 3% agarose gel electrophoresis. The body mass index (BMI) increased from 21.1 ± 2.6 to 22.0 ± 3.0 kg/m^2 for women and from 20.9 ± 2.1 to 22.1 ± 2.6 kg/m^2 for men from 1982 to 1997. Body weight increased 63.3% for women and 67.4% for men over 15 years. The frequency of the Trp64Arg allele was 19.9%, and the frequency of heterozygote and homozygote carriers of the mutation was 32.9% and 3.4%, respectively. Changes in the BMI and body weight over 15 years from 1982 to 1997 did not differ significantly between subjects with or without the mutant allele in either women or men. The BMI was not significantly higher in subjects who were homozygous or heterozygous for the Trp64Arg mutation versus subjects who were homozygous for the normal allele. This mutation of the β_3-adrenergic receptor gene is not likely a major determinant of moderate obesity in the Japanese population.

OBESITY IS A MAJOR health problem throughout the world and is associated with diabetes mellitus, hypertension, cardiovascular disease, certain cancers, osteoarthritis, and other health conditions.[1] Obesity may be less prevalent in the Japanese compared with other ethnic populations.[2] Goto and Yamaguchi[3] reported that the prevalence of a body mass index (BMI) greater than 27 kg/m^2 was 6.9% in the Japanese.[3] Although the rapid economic development in Japan has caused changes in the health-supportive environment and life-style and therefore might affect the prevalence of obesity, the trend for obesity is still unclear in the Japanese population.

Adipose tissue, which plays a crucial part in regulating the storage and mobilization of energy, has been the focus of efforts to identify candidate genes for obesity. One such candidate is the gene for the β_3-adrenergic receptor, the main receptor involved in the regulation of thermogenesis and lipolysis in adipose tissue. There is some evidence that genetic variation in the β_3-adrenergic receptor may predispose subjects to abdominal obesity and insulin resistance by decreasing energy expenditure.[4-6]

However, such findings have not been consistent and, when positive, are generally characterized by marginal levels of significance.[7] The study that found an association between the mutant allele and BMI was conducted with Japanese subjects, and the allele was twice as common in obese individuals versus control subjects.[8] Trp64Arg mutation in the β_3-adrenergic receptor gene could be predictive of some difficulties in the increased BMI in the Japanese.[9-10] It is not clear that the genetic variation in the β_3-adrenergic receptor may play a pathogenic role in the development of obesity in the Japanese. Therefore, the aim of the present study was to investigate the trend for obesity and the relationship between Trp64Arg mutation in the β_3-adrenergic receptor gene and obesity-related phenotypes in Japanese workers.

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SUBJECTS AND METHODS

Subjects

Workers aged 20 to 62 years at a medical equipment factory in Izumo City underwent a physical examination in April 1997. A total of 658 workers, 483 women (41.5 ± 8.9 years) and 175 men (34.8 ± 10.4 years), responded to the survey. Forty-seven workers (39 women and eight men) in the factory did not participate in this study because of a long absence from work. The BMI in 1982 and 1997 was obtained from 357 medical records (311 women and 46 men), calculated as weight in kilograms divided by height in meters squared. Overweight was defined as a minimum of 27.3 kg/m^2 for women and 27.8 kg/m^2 for men according to NHANES III.[11]

Three hundred nineteen workers (272 women and 47 men) participated voluntarily in the analysis of genetic variation of the β_3-adrenergic receptor from a total of 352 workers (305 women and 47 men) aged 35 and over 40 years who underwent blood sampling for regular health examinations. The study protocol was approved by the factory ethics committee, and all subjects provided written informed consent.

DNA Analysis

Genomic DNA was prepared from leukocytes using the DNA Extractor WB Kit (Wako Pure Chemical, Osaka, Japan). Amplification of DNA by the polymerase chain reaction was performed using 100 ng genomic DNA as a template with the primers (upstream, 5′-CGCCCAATACCGCCAACAC-3′; downstream, 5′-CCACCAGGAG-TCCCATCACC-3′) under the same conditions used by Kadowaki et al.[8] The amplified fragments of 210 base pairs (bps) were digested with *Bst*NI and analyzed by 3% agarose gel electrophoresis. The appearance of a 161-bp fragment instead of 99-bp and 62-bp fragments indicates the presence of the Trp64Arg mutation in the β_3-adrenergic receptor gene.

Statistical Analysis

Statistical analyses were performed with the chi-square test for qualitative variables and Student's *t* test for quantitative variables. All data are expressed as the mean ± SD.

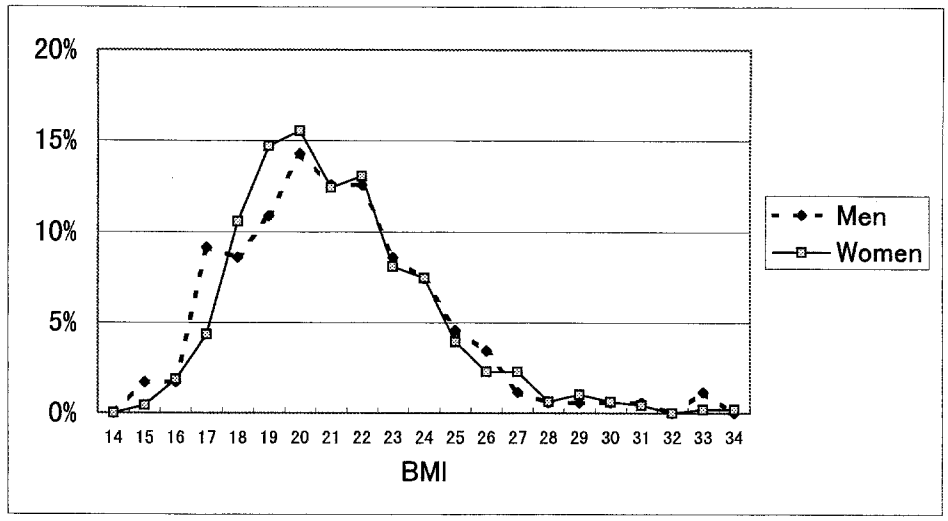

Fig 1. BMI distribution of Japanese workers by sex in 1997.

RESULTS

Trend for Overweight

The BMI of the workers in 1997 is presented by sex in Fig 1. The most frequent range for the BMI was 20.0 to 20.9 kg/m^2 in both sexes, and the mean BMI was 21.6 ± 2.9 for women and 21.6 ± 3.2 for men. The prevalence of overweight was estimated at 4.6% for women and 4.0% for men in the present population. Although there was not a significant difference in the BMI by sex, a greater proportion of women were classified as overweight versus men. In cross-sectional data for the various age groups in 1997, significant increases in the BMI were present for the groups aged 40 to 49 and 50 to 62 years in women and 20 to 29 and 30 to 39 years in men (Table 1).

BMI values in 1982 and 1997 were available for 357 workers (311 women and 46 men). To examine trends over time, changes in the body weight and BMI for 15 years were analyzed. The workers showed significant changes in the BMI and body weight from 1982 to 1997 (Table 2). The most frequent range for the BMI change was −0.5 to 2.5 kg/m^2 for men and 0 to 1.5 kg/m^2 for women, with 67.4% of men and 63.3% of women showing increased body weight over 15 years.

Mutation of the β_3-Adrenergic Receptor Gene and BMI

Results of Trp67Arg mutation in the β_3-adrenergic receptor gene are listed in Table 3. The frequency of the Trp64Arg allele was 19.9% (127 of 638), with the frequency of heterozygote and homozygote carriers of the mutation being 32.9% (105 of 319) and 3.4% (11 of 319), respectively. There was not a significantly higher frequency of the mutant allele by sex and age. The BMI in 1997 and changes in the BMI and body weight over 15 years from 1982 to 1997 did not differ significantly between workers with and without the mutant allele in either women or men. Heterozygotes for the Trp67Arg mutation did not differ from homozygotes in the BMI.

Table 1. BMI of Workers by Sex and Age in 1997

Group	Age Group (yr) 20-29	30-39	40-49	50-62
Women				
No.	50	140	211	82
Mean ± SD	21.0 ± 2.9	21.6 ± 3.1	21.5 ± 2.7	22.4 ± 3.2
Men			*	
No.	71	61	27	16
Mean ± SD	20.8 ± 2.9	22.0 ± 3.5	22.4 ± 3.2	22.1 ± 2.6
	*			

*$P < .05$. Student's t test.

DISCUSSION

The frequency of the Trp64Arg allele was 0.199 in the present Japanese population. The frequency of this mutation was reported to be 0.18 to 0.23 in other areas of Japan,[8-10,12] and the mutation is more common in the Japanese than in Caucasians, African-Americans, and Mexican-Americans, but less common than in Pima Indians.[4-6]

Changes in the BMI and body weight over 15 years did not differ significantly between workers with and without the mutant allele in the present study. Heterozygotes for the Trp67Arg mutation also did not differ from homozygotes in the BMI in the present Japanese population. Kadowaki et al[8] reported that the BMI was significantly higher in subjects with the mutation versus those without it. Pima Indians with the mutation tended to have a lower metabolic rate and a higher BMI.[5] Individuals with the mutation apparently had an increased capacity to gain weight in French Caucasians,[4] and it

Table 2. Change in BMI and Body Weight for Workers Over 15 Years From 1982 to 1997

Group	1997	1982	Change
Women			
No.	311	311	
Age (yr)	45.5 ± 6.2	30.5 ± 6.2	
BMI (kg/m^2)	22.0 ± 3.0	21.1 ± 2.6*	0.8 ± 1.7
Body weight (kg)	52.6 ± 7.9	50.6 ± 6.9*	2.0 ± 4.2
Men			
No.	46	46	
Age (yr)	42.7 ± 7.1	27.7 ± 7.1	
BMI (kg/m^2)	22.1 ± 2.6	20.9 ± 2.1*	1.2 ± 1.7
Body weight (kg)	63.1 ± 8.8	59.6 ± 7.1*	3.6 ± 5.0

*$P < .001$, 1997 v 1982 by paired Student's t test.

Table 3. Obesity Parameters of Workers by Sex and Genotype

Group	1997			Changes Over 15 Years			
	No. of Subjects	Age (yr)	BMI (kg/m²)	No. of Subjects	Age (yr)	BMI (kg/m²)	Body Weight (kg)
Women							
Homozygotes	9	44.0 ± 5.0	20.8 ± 1.9	6	45.7 ± 4.3	0.3 ± 1.0	0.8 ± 2.4
Heterozygotes	91	45.3 ± 5.3	22.1 ± 2.8	76	45.6 ± 5.4	0.9 ± 1.7	2.1 ± 3.8
Without mutation	172	46.3 ± 6.0	21.9 ± 2.8	142	46.9 ± 6.0	0.8 ± 1.8	1.9 ± 4.4
Men							
Homozygotes	2	40.8 ± 4.9	25.1 ± 1.9	1	35.4	−0.2	−0.5
Heterozygotes	14	42.9 ± 10.2	22.7 ± 3.2	7	42.3 ± 8.2	1.5 ± 1.8	4.5 ± 5.3
Without mutation	31	42.7 ± 6.9	22.0 ± 2.0	17	44.7 ± 7.0	1.1 ± 1.8	3.2 ± 4.9

NOTE. No significant difference was observed between genotypes using Student's *t* test.

was associated with abdominal obesity in Finns.[6] In contrast, the BMI did not differ between subjects with and without the mutation in the present study and a few other studies in Japanese[9] and Canadian and Swedish[13] subjects. Since these studies that did not find an association between the mutation of the β_3-adrenergic receptor gene and obesity were conducted to investigate both simple obesity,[13] as in the present study, and morbid obesity,[9,13] the reason for the inconsistent findings regarding the mutation and obesity does not seem to be due to the degree of obesity and ethnicity.

The base mutation produces a replacement of tryptophan by arginine at position 64, which is located in the first intracellular loop of the β_3-adrenergic receptor and is thought to be important for binding to noradrenaline and coupling to Gs proteins in the adipose cell.[5] The mutant β_3-adrenergic receptor may lead to a decrease of thermogenesis and lipolysis in brown and white adipose tissue. Pharmacological investigations with highly selective β_3-adrenergic antagonists considered to be potent antiobesity and antidiabetic drugs have confirmed the involvement of β_3-adrenoceptors in both adipocyte lipolysis and thermogenic activity in various laboratory animals.[13-14] However, the lack or the presence of only a weak proportion of β_3-adrenoceptors in intraabdominal fat cells has been reported.[15,16] Therefore, the Trp64Arg mutation of the β_3-adrenergic receptor gene is not likely a major determinant of moderate obesity, as shown by the present investigation.

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