
Association of Trp64Arg mutation of the β_3 -adrenergic receptor gene with obesity in school-age Japanese children

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Introduction

Genetic and environmental factors are involved in the pathology of obesity and its metabolic and cardiovascular complications⁽¹⁾. There are several putative candidates for this genetic factor. These include the *ob* gene and *db* gene which seem to control body weight^(2,3), neuropeptide Y which is thought to function as a central stimulator of feeding behavior⁽⁴⁾, uncoupling protein-2 which may induce hyperinsulinemia⁽⁵⁾, and the β_3 -adrenergic receptor (AR) gene⁽⁶⁾. The β_3 -AR, which is expressed in brown and white adipose tissues including visceral adipocytes, may contribute to the regulation of the resting metabolic rate, lipolysis, and thermogenesis. Therefore, an abnormality in the β_3 -AR could explain the linkage between body fat distribution and metabolic disturbances such as insulin resistance^(6,11).

We examined the potential role of this gene as a contributor to obesity in school-age Japanese children.

Subjects

Two hundred and twenty-five obese children were recruited from the community near University of Tsukuba, and from the outpatient clinic of Pediatrics, Kinu Medical Association Hospital and Tsukuba Metropolitan Hospital, Ibaraki prefecture. The subjects whose obesity index was greater than twenty percent constituted the obese group. Obesity index, $\{(\text{real body weight} - \text{standard body weight}) / \text{standard body weight}\} \times 100$, was calculated using the standard body weight for Japanese children selected according to gender, age, and height⁽¹²⁾. As the control group, two hundred and twenty-five healthy children based on school medical examination were matched with respected gender and age with each of the obese subjects. In addition, the body mass index, which is widely used for evaluation of physique in western countries, was compared with the Criteria for Overweight or obesity proposed by the Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Services⁽¹³⁾. BMI was calculated as body weight (in

kilograms) divided by height (in meters) squared. All of the subjects live in the same region of Ibaraki prefecture in Japan. All subjects and their parents gave informed consent.

Measurements

Venous blood samples were drawn after an overnight fast. Serum concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured with an auto-analyzer (Hitachi, Tokyo, Japan). Serum glucose was enzymatically measured and serum insulin concentration was measured by a radioimmunoassay kit (Eiken, Tokyo, Japan). Blood pressure was measured with a autosphygmomanometer on the right arm (Nippon Colin, Komaki, Japan).

Genetic Analysis

Genomic DNA was prepared from peripheral blood leukocytes. The Trp64Arg polymorphism of the β_3 -AR was detected using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis according to the established method^(7,9).

Data analyses

The results are presented as means \pm standard deviation. The difference in triglyceride levels between the two groups was assessed by the Mann-Whitney test. Systolic and diastolic blood pressure were adjusted for age and weight in each gender subsample. Student's t test, and chi-square test were used in statistical comparisons of other anthropometric and clinical measurements. All analyses were performed with the statistical software packages (StatView 5.1, Abacus Concepts Inc., Berkeley, USA and SPSS 6.1, SPSSInc., Chicago, USA).

Results

Table 1 demonstrates allele and genotype frequencies of the Trp64Arg mutation of the β_3 -AR gene in school-age Japanese children. Obese children were divided into the following subgroups: obesity index \geq +50%, morbidly obese; between +30% and +50%, moderately obese; between +20% and +30%, slightly obese. Each group was comprised in age of 121 boys and 104 girls, ranging from seven to fifteen (11.7 ± 1.7 year; mean \pm SD). There was no difference in genotype distribution by gender. The allele frequency of the mutation was 0.12 in non-obese control children, and 0.24 in obese children. There was a significantly higher frequency of the mutant allele in the obese group than in the control group ($p < 0.01$), although the frequencies of the mutant allele were similar among obese sub-groups. Allele frequencies were consistent with the Hardy-Weinberg equilibrium. In the control group, 0.9% were mutant homozygote, 23.1% were heterozygote, and 76.0% lacked mutation. Furthermore, the values observed in the obese group were, respectively, 4.9%, 39.1%, and 56.0%.

Table 1

 Allele and genotype frequencies of the Trp64Arg mutation of the β_3 -AR gene in school-age Japanese children

	obesity index (%)	number (M/F)	genotypes			allele frequency of Trp64Arg
			Arg/Arg n (%)	Arg/Trp n (%)	Trp/Trp n (%)	
control	(-20<and<+20)	225 (121/104)	2 (0.9)	52 (23.1)	171 (76.0)	0.12
obese	(+20 \leq)	225 (121/104)	11 (4.9)	88 (39.1)	126 (56.0)	0.24*
{ slight moderate morbid	(+20 \leq and<30)	67 (37/ 30)	1 (1.5)	29 (43.3)	37 (55.2)	0.23*
	(+30 \leq and<50)	99 (54/ 45)	4 (4.0)	40 (40.4)	55 (55.6)	0.24*
	(+50 \leq)	59(30/ 29)	6 (10.2)	19 (32.2)	34 (57.6)	0.26*

 *; $p < 0.01$, versus control

In Table 2, both the obesity index, a basis for defining obesity in Japanese children, and the Criteria for overweight or obesity proposed by the Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Service are shown. The association between overweight and the β_3 receptor mutation was analyzed by the chi-square test. The frequency of homozygosity or heterozygosity for this mutation was significantly higher in the overweight group than in the no-overweight group in these two methods of determining obesity.

Table 3 presents anthropometric and clinical characteristics in both the obese and the control groups. In both groups there were no significant differences in mean levels of serum lipids, glucose, and insulin. Mean levels of systolic or diastolic blood pressure in the control group did not show any difference between subjects with the mutation and those without mutation. However, significantly higher systolic and diastolic blood pressure was observed in obese subjects with the mutation than in obese subjects without the mutation

Table 2

 The association between overweight and the Trp64Arg mutation of the β_3 -AR gene in school age Japanese children

		Obesity Index*		At Risk of Overweight†		Overweight†	
		<20% (n=225)	20% \leq (n=225)	No (n=207)	Yes (n=207)	No (n=113)	Yes (n=113)
{ Arg/Arg Arg/Trp	n (%)	54 (24.0)	99 (44.0)	92 (44.4)	49 (23.7)	51 (45.1)	29 (25.7)
		171 (76.0)	126 (56.0)	115 (55.6)	158 (76.3)	62 (54.9)	84(74.3)
χ^2		18.129		18.972		8.533	
p value		0.0005		0.001		0.0056	

*; The standard body weight for Japanese children (see ref.12)

†; Guidelines for overweight in adolescent preventive services (see ref.13)

Table 3 Anthropometric and clinical characteristics in control and obese group in school-age Japanese children according to the Trp64Arg mutation of the β_3 -AR gene

haracteristics	control		obese	
	Arg/Arg (n=54)	Arg/Trp (n=171)	Arg/Arg (n=99)	Trp/Trp (n=126)
sex M/F	30/24	91/80	44/55	77/49
age (yr)	11.7± 1.7	11.6± 1.7	11.6± 1.7	11.7± 1.8
height (cm)	147.0±10.9	147.2±11.8	149.1±10.5	148.7±11.6
body weight (kg)	38.9± 9.6	39.4±10.3	57.8±17.9	57.4±14.4
obesity index (%)	- 1.0± 9.1	0.0± 8.7	42.7±17.9	42.0±17.2
BMI (kg/m ²)	17.7± 2.1	17.8± 2.3	25.6± 3.8	25.6± 3.7
serum TC (mg/dl)	174.0±25.1	174.4±25.2	181.4±32.9	182.9±30.2
serum HDL-C (mg/dl)	57.6±11.2	56.1±10.4	52.2±11.2	51.8±10.8
serum TG (mg/dl)	77.1±31.9	71.8±26.6	52.2±11.2	51.8±10.8
serum glucose(mg/dl)	NA	NA	92.2±12.6	92.6±14.8
serum insulin (μ g/ml)	NA	NA	19.5±15.2	16.8±10.4
systolic BP (mmHg)	108.7±12.2	105.4±11.5	112.3±11.7	108.2±11.8*
diastolic BP (mmHg)	59.9± 8.5	59.8± 7.9	63.2± 8.4	60.2± 8.3†

Data reported as mean±SD.

NA; not applicable

*; p<0.01, versus obese subjects with mutation after correction by gender, age, and body weight

†; p<0.001, versus obese subjects with mutation after correction by gender, age, and body weight

(systolic; 112.3±11.7mmHg versus 108.2±11.8mmHg, p<0.01 and diastolic; 63.2±8.4mmHg versus 60.2±8.3mmHg, p<0.001).

Discussion

Several studies reported the association of Trp64Arg mutation of the β_3 -AR gene with obesity in adults. Walston et al. was the first to report that obese Pima Indians with this mutation demonstrated early onset of noninsulin-dependent diabetes mellitus (NIDDM) and tended to have a low metabolic rate⁽⁷⁾. Widén et al. studied Finns and found that this mutation was associated with the clinical features of the insulin resistance syndrome including increased blood pressure and high concentrations of insulin, glucose, and lipids⁽⁸⁾. Clément et al. found this mutation had a significant relationship with an increased capacity to gain weight in morbidly obese French subjects⁽⁹⁾. In Japan, Kadowaki et al. found that in Japanese without NIDDM, the frequency of the mutant allele among obese subjects was significantly higher than that among non-obese subjects⁽¹¹⁾. Fujisawa et al. observed a higher frequency of the mutant allele and noted significantly increased BMI in the homozygotes only⁽¹⁴⁾. However, in all the studies only analyzed, so it is not clear whether this

mutation predisposes young subjects to obesity and its complications.

There are various methods of determining obesity using height and weight. However, since the subjects in this study were Japanese children, those with an obesity index $\geq 20\%$ were classified as the obese group using the standard body weight for Japanese children determined according to gender, age, and height⁽¹²⁾. We confirmed by comparison in each child a good agreement between this cut-off value and “at risk of overweight” according to the criteria for overweight or obesity based on the BMI proposed by the Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Services⁽¹³⁾. Therefore, the screening level is considered to be similar between the two methods of determining overweight associated with health sequelae.

Our matched-pair study using the obesity index, the total allele frequency of this mutation was similar to that reported for Japanese adults (table 1). There was also a similar tendency towards a higher frequency of this mutant allele among obese children (table 2). Our result supports that this variant has the potential to contribute to obesity even during the early years of life.

On the contrary, several investigators reported different results in some ethnic groups^(15,18). Gagnon et al. reported that there was no significant association between this mutation and obesity in two different cohorts, a Quebec Family Study and obese Swedish subjects⁽¹⁶⁾. Elbein et al. found that carriers of this mutation did not have earlier NIDDM onset, higher BMI, or higher waist-hip ratio than Northern European subjects without mutation in forty-two families ascertained to have two or more NIDDM siblings⁽¹⁷⁾. Silver et al. scanned the complete β_3 -adrenergic receptor gene in obese and diabetic Nauruans and reported that neither this mutation or any other mutation in the β_3 -AR gene was a major contributor to genetic susceptibility to NIDDM and obesity in Nauruans despite the very high prevalence of NIDDM in this group⁽¹⁸⁾.

However, Susulic et al. demonstrated a knock-out gene study which showed that β_3 -AR-deficient mice had moderately increased fat stores⁽¹⁰⁾. The study also indicated that β_3 -ARs plays an important role in regulating energy balance. This finding in mice was supported by observations in a human study by Yoshida et al.⁽¹⁹⁾. They treated obese Japanese women with a combined low-calorie diet and exercise regimen. Three months later, subjects with this mutation had more difficulty losing weight than those without the mutation. Some workers take note of the interaction between β_3 -ARs and Gs proteins^(20,21). The study by Pietri-Rouxel et al. on stable expression of the genes encoding the wild-type or this mutation in two different cell types: hamster CHO K-1 and human HEK 293 provides interesting functional analysis of this mutation⁽²²⁾. They reported that the binding and activation constants of the receptor for catecholamines did not differ between the wild type and mutation type, but the maximal adenylyl cyclase activity of cells expressing mutated receptors is lower than for wild-type cells for the various agonists, suggesting that the mutated receptors are coupled less efficiently to the Gs protein than the wild type. In the future, more investigations into the stereochemistry of these complex forms will be reported through the use of newer techniques of molecular biology and chemistry become available.

Widén et al. supposed that the increased diastolic blood pressure in non-diabetic heterozygous subjects

was secondary to increased visceral adiposity and associated insulin resistance⁽⁶⁾. In this study, systolic and diastolic blood pressure was higher in obese subjects with mutation after correction by gender, age, and body weight, although there was no significant evidence for insulin resistance. More interestingly, Kurabayashi et al. investigated elderly Australians and reported that female heterozygotes had significantly higher BMI, higher diastolic blood pressure, longer reproductive life, with an earlier menarche, a higher gravidity and parity⁽²³⁾. They considered that this mutation was a candidate gene for hypertension, independent of factors controlling weight, and for evolutionary reasons it has remained in the population. Their suggestion about this mutation is seen to be helpful when considering the reason the allele frequency of this mutation is higher in Japanese than in some other populations, where much larger average BMIs were reported.

There is at least an association between the Trp64Arg mutation of the β_3 -AR gene and obesity and/or metabolic disturbances among some ethnic groups including Japanese. In this regard, there seems to be a possibility that other factors, such as dietary behavior and the other aspects of life style, may mask the influence of β_3 -AR mutation in some populations. What needs to be emphasized in our study is that this mutation predisposes individuals to obesity early in life (the mean age of all subjects in this study was 11.7 ± 1.7 yr). Given that this mutation is associated with obesity in a young population, it appears that adult obesity and the future development of metabolic disturbances are connected to reduced thermogenesis, basal energy expenditure, and lipolysis in adipose tissue.

In conclusion, the presence of the Trp64Arg mutation of the β_3 -AR gene was found to predispose Japanese children to obesity.

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The β_3 -adrenergic system plays an important role in regulating the energy balance through thermogenesis and lipolysis in white and brown adipose tissues. Recently, a possible pathogenic mutation in human β_3 -AR genes (codon64 TGGTrp→CGGArg; Trp64Arg) has been reported to be associated with obesity and insulin resistance among several population. We investigated the Trp64Arg mutation of the β_3 -AR gene in 450 school-age Japanese children. One group of 225 children (121 boys and 104 girls) with a mean age of 11.7 and an obesity index greater more than twenty percent constituted the obese group. Two hundred and twenty-five non-obese children were matched to each obese subject for gender and for age to the control group.

The allele frequency of this mutation in the obese group was significantly higher than that in the control group (0.24 versus 0.12, $p < 0.01$). The prevalence of this mutation was also significantly more increased in the obese group than in the control group (44.0% versus 24.0%, $\chi^2 = 11.3$, $p < 0.001$). Significantly higher blood pressure was observed in obese subjects with this mutation than in obese subjects without mutation after correction for gender, age, and body weight (systolic; 112.3 ± 11.7 mmHg versus 108.2 ± 11.8 mmHg, $p < 0.01$ and diastolic; 63.2 ± 8.4 mmHg versus 60.2 ± 8.3 mmHg, $p < 0.001$). In each group, there was no significant differences in body weight, obesity index, body mass index, and serum lipid concentrations between β_3 -AR genotypes.

Our results suggest that the Trp64Arg mutation of the β_3 -AR gene is associated with obesity and even some physiological systems, such as blood pressure, in school-age Japanese children.

Key Word: β_3 -adrenergic receptor gene, obesity, school-children, blood pressure