Relationship between the β_3 -Adrenoceptor Gene Variant and Body Fat in Japanese Children

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KUROKAWA, N., NAKAI, K., KAMEO, S., LIU, Z.-M. and SATOH, H. *Relationship between the β_3 -Adrenoceptor Gene Variant and Body Fat in Japanese Children.* Tohoku J. Exp. Med., 2003, **201** (4), 271-276 — In this study, we investigated whether the variant of the β_3 -adrenoceptor gene is associated with accumulation of body fat in Japanese junior high school students. A total of 87 junior high school students, 50 boys and 37 girls, participated in this survey. The β_3 -adrenoceptor gene variant was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Accumulation of body fat was assessed by skinfold thickness at the triceps and subscapular with a skinfold caliper. There was no difference between boys and girls in the frequency of the β_3 -adrenoceptor gene allele. There were no significant differences in body mass index and percent body fat between the Arg carriers (Trp/Arg or Arg/Arg) and the Arg noncarriers (Trp/Trp) for either sex. The Trp64Arg variant of the β_3 -adrenoceptor gene does not seem to have a strong effect on the accumulation of body fat in this group of Japanese junior high school students. ——— β_3 -adrenoceptor gene variant; Trp64Arg; body fat; children
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Obesity is both environmentally and genetically influenced, and is defined as a state of excessive adipose tissue mass, resulting from an imbalance of energy intake and expenditure. Recently molecular biological studies have identified specific genes influencing human obesity (Chagnon et al. 2003). Several energy expenditure-related gene mutation have been suggested to play a role in causing human obesity.

The β_3 -adrenoceptor (ADRB3) is mainly

expressed in brown and white adipose tissue. It has been shown that ADRB3 agonists stimulated lipolysis and thermogenesis through the activation of adenylyl cyclase followed by an increase in the intracellular concentrations of cyclic AMP. Therefore, ADRB3 plays a role in the regulation of metabolism (Krief et al. 1993; Lonnqvist et al. 1995).

In 1995, it was first shown in Pima Indians who have a high frequency of missense mutation (Trp64Arg) at the junction between the first

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transmembrane domain and the first intracellular loop of ADRB3 gene (Walston et al. 1995). Subjects with this variant tended to have a lower metabolic rate (Walston et al. 1995) and had an apparently increased capacity to gain weight (Clement et al. 1995). We have found that the mean body mass index (BMI) of the Arg carriers (Trp/Arg or Arg/Arg) was higher than that of the Arg noncarriers (Trp/Trp) in adulthood, based on a meta-analysis of the relationship between the ADRB3 gene variant and BMI (Kurokawa et al. 2001).

The relationship between the ADRB3 variant and age may partially explain the controversial results of some association studies. The age-dependent effect of the Trp64Arg variant was previously suggested by other investigators (Corbalan et al. 2003). In this regard, the Trp64Arg variant of ADRB3 seems to be a risk factor for obesity that is age dependent, being specifically relevant for young adults. In addition, there was an association between the Trp64Arg variant and an earlier onset of obesity in Finnish individuals who developed morbid obesity (Oksanen et al. 1996).

In Japanese children, two studies have assessed the influence of the Trp64Arg variant of the ADRB3 gene. Both studies found that the BMI was significantly higher in the Arg carriers than in the Arg noncarriers (Endo et al. 2000; Arashiro et al. 2003). Furthermore, the former concluded that the Trp64Arg variant of ADRB3 appears to be a genetic risk factor for obesity in Japanese children. However, we have assumed that the BMI does not correctly evaluate the accumulation of body fat in childhood. Especially, since standing height and body weight rapidly change in childhood, the BMI will also change greatly. Even if there was a statistical adjustment, it may be limited to judge the obesity using only the BMI and obesity index.

Therefore, in this study, we investigated the relationship between the ADRB3 gene variant and accumulation of body fat. For subjects, we

used Japanese junior high school students in Sendai, Japan.

METHODS

This study was approved by The Ethical Committee of Tohoku University School of Medicine.

Subjects

A total of 87 junior high school students (50 boys and 37 girls), 12–15 years old with a mean age ($\pm$ S.D.) of 13.25 ± 0.87 , participated after informed consent had been obtained from the subjects and their parents.

Anthropometric and body composition measurements

Height was measured to the nearest 0.1 cm, and subjects were weighed wearing light clothing (usually a T-shirt and shorts) and without shoes. Weight was measured to the nearest 0.1 kg. BMI was calculated as weight in kilograms divided by the square of the height in meters ($\text{weight}/\text{height}^2$). Waist and hip with light clothing were measured to the nearest 0.1 cm using a tape measure.

Skinfold thickness at the triceps and subscapular on the right side of the body was measured three times with a skinfold caliper. The measurements were averaged. Body fat was calculated by the formula proposed by Brozek et al. (1963) based on body density estimated by the method of Watanabe et al. (1993).

In addition, we estimated %body fat and fat content from total body resistance. Total body resistance was measured with a bioelectrical impedance analyzer (BIA).

DNA analysis

The buccal mucosa was swabbed with a sterile cotton swap to obtain genomic DNA. Genomic DNA was isolated enzymatically as follows. 1) Buccal mucosa cells were incubated in lysis solution of 500 μ l containing 5% Chelex-

100 (Bio-Rad, Hercules, CA, USA) and 20 μ l containing proteinase K solution (10 mg/ml) at 55°C for 30 minutes. 2) The mixture was boiled for 8 minutes. 3) Lipids were extracted with phenol/chloroform and the aqueous phase containing DNA was transferred to a clean tube. Then DNA was precipitated with ethanol, and finally dissolved in 100 μ l of TE (Nippongene, Tokyo).

Exon 1 of the ADRB3 gene was amplified by PCR according to the method described by Clement et al. (1995). Since the Trp64Arg variant of the ADRB3 gene eliminates a restriction site recognized by the Mva I (BstN1) restriction enzyme, this variant can be detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The digested samples were electrophoresed in a 4.0% agarose gel and the gel was stained with ethidium bromide. The presence of a fragment of 161 bp was considered to be an indication of ADRB3 gene variant.

Statistical analysis

All data are shown as means $\pm$ S.D. Allele frequencies were estimated by the gene counting

method. Data were analyzed by Student's *t*-test. A *p*-values less than 0.05 were defined as significant.

RESULTS

Of the 87 students, 55 subjects had the Trp/Trp genotype of the ADRB3 gene, 27 had the Trp/Arg genotype, and 5 had the Arg/Arg genotype. The frequency of the Trp64Arg allele was 0.21. The overall genotype distributions did not deviate significantly from the Hardy - Weinberg equilibrium. The allele frequency was not significantly different between the sexes.

The physical characteristics of the subjects with the Trp64Arg or Arg64Arg genotype (Arg carriers) or those with the Trp64Trp genotype are summarized in Table 1 for boys and Table 2 for girls. No significant differences were observed in any of the physical characteristics between the Arg carriers and the Arg noncarriers.

Of the 87 students, 23 subjects showed BMI values of higher than 20 kg/m², and 14 subjects showed %body fat calculated by Brozek's formula of higher than 20%. There were no sig-

TABLE 1. *Physical characteristics and genotypes of boys*

Boys (<i>n</i> =50)	The Arg carriers (<i>n</i> =18)	The Arg noncarriers (<i>n</i> =32)	<i>p</i> -value
	Mean S.D.	Mean S.D.	
Age (years)	13.28 $\pm$ 0.90	13.22 $\pm$ 0.91	0.912
Height (cm)	161.36 $\pm$ 7.98	161.93 $\pm$ 8.70	0.840
Weight (kg)	48.73 $\pm$ 9.60	51.94 $\pm$ 10.48	0.364
BMI (kg/m ²)	18.56 $\pm$ 2.34	19.71 $\pm$ 3.06	0.353
Waist (cm)	66.3 $\pm$ 7.03	69.88 $\pm$ 9.08	0.189
Hip (cm)	83.73 $\pm$ 6.56	85.84 $\pm$ 6.63	0.396
Waist/Hip ratio	0.79 $\pm$ 0.04	0.82 $\pm$ 0.06	0.130
Percent body fat (%: BIA)	14.08 $\pm$ 4.74	15.93 $\pm$ 4.87	0.169
Triceps (mm)	8.38 $\pm$ 3.08	10.09 $\pm$ 4.89	0.228
Subscapular (mm)	10.14 $\pm$ 1.07	11.4 $\pm$ 6.21	0.770
Triceps+Subscapular (mm)	18.52 $\pm$ 7.47	21.49 $\pm$ 10.88	0.524
Body density (g/ml)	1.069 $\pm$ 0.01	1.066 $\pm$ 0.01	0.524
Percent body fat (%: Brozek)	13.33 $\pm$ 3.04	14.55 $\pm$ 4.45	0.524

*Data are mean $\pm$ S.D. Triceps, triceps skinfold thickness; Subscapular, subscapular skinfold thickness.

TABLE 2. *Physical characteristics and genotypes of girls*

Girls (<i>n</i> =37)	The Arg carriers (<i>n</i> =14)	The Arg noncarriers (<i>n</i> =23)	<i>p</i> -value
	Mean S.D.	Mean S.D.	
Age (years)	13.07±0.92	13.39±0.78	0.280
Height (cm)	155.62±4.97	153.77±7.32	0.273
Weight (kg)	44.26±7.78	45.77±7.26	0.364
BMI (kg/m ²)	18.19±2.47	19.28±2.21	0.103
Waist (cm)	63.55±4.39	63.95±4.81	0.700
Hip (cm)	84.44±6.40	86.23±5.73	0.287
Waist/Hip ratio	0.75±0.04	0.74±0.04	0.437
Percent body fat (%: BIA)	18.85±4.74	21.18±4.33	0.100
Triceps (mm)	10.52±4.36	11.8±3.82	0.228
Subscapular (mm)	13.66±3.94	13.91±3.78	0.827
Triceps+Subscapular (mm)	24.18±7.88	25.71±7.10	0.348
Body density (g/ml)	1.055±0.01	1.054±0.01	0.348
Percent body fat (%: Brozek)	19.12±2.29	19.56±2.05	0.348

*Data are mean±s.d. Triceps, triceps skinfold thickness; Subscapular, subscapular skinfold thickness.

nificant differences in the frequency of Arg carriers and Arg noncarriers in those subjects.

In addition, there were no noticeable differences in BMI, %body fat calculated by Brozek's formula or BIA values between Arg/Arg carriers and Trp/Trp carriers.

DISCUSSION

We found that there were no significant associations between the Trp64Arg variant of ADRB3 gene and the accumulation of body fat in Japanese junior high school students in Sendai.

In the present study, the BMI did not differ between subjects with and without the Trp64Arg variant of the ADRB3 gene. Moreover, neither skinfold thickness measured with a caliper nor %body fat measured with a BIA, both of which are considered to be more useful obesity-related indexes than the BMI, had any significant relationship to the Trp64Arg variant of the ADRB3 gene.

The association of the Trp64Arg variant with BMI in Japanese was first examined by Kadowaki et al. (1995). Thereafter, there have been several reports on the relationship between

the Trp64Arg variant of ADRB3 gene and obesity in Japanese adults. However, some of them suggest that the Trp64Arg variant of ADRB3 gene may have weak effects on the development of obesity in Japanese. We revealed that the relationships between this variant and BMI using meta-analysis method in adulthood (Kurokawa et al. 2001).

This study was conducted to confirm whether the Trp64Arg variant of ADRB3 gene affects the accumulation of body fat using an obesity-related index in children.

The association of the Trp64Arg variant with BMI in children was first examined by Hinney et al. (1997). They reported that there was no significant relationship between heterozygous Trp64Arg variant of ADRB3 gene and BMI in children, adolescents and young adults in Germany. Their report suggested that the Trp64Arg variant of ADRB3 is not of major importance in regulation of body weight.

On the other hand, two studies of Japanese children found that the mean BMI in the Arg carriers was significantly higher than those of the Arg noncarriers (Endo et al. 2000; Arashiro et al. 2003). However, it was pointed out that

the obesity-related index calculated only by height and weight as the BMI and obesity index are not an equivalent measure of adiposity for both genders, and it is suggested that the use of the obesity-related indices are a limitation for assessing the true degree of adiposity of an individual child (Ellis et al. 1999). In addition, since standing height and body weight rapidly change in childhood, the BMI will also change greatly. Even if there was a statistical adjustment, it may be limited to judge the obesity using only the BMI and obesity index. The BMI is likely to overestimate the body fat percentage in obese subjects (Deurenberg et al. 1991). This may indicate that the principal factor of accumulation of body fat in childhood is not the Trp64Arg variant of the ADRB3 gene but other factors. Especially, it was reported that there are children accumulating visceral fat which is not distinguished by the BMI and obesity index (Matsuzawa et al. 1995). Accordingly, although we estimated body fat using skinfold thickness and BIA, there were no significant differences in any of the physical characteristics between the Arg carriers and the Arg noncarriers.

In conclusion, our data suggest that the Trp64Arg variant of the ADRB3 gene would not have much of an effect on the accumulation of body fat in this group of Japanese junior high school students. However, since we investigated only a small number of school children, further studies will be needed to clarify the relevance of the Trp64Arg variant of ADRB3 gene to the accumulation of body fat in childhood.

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