Anthropometric measurements

The subjects' body height and weight were measured with a portable standiometer and a digital scale to the nearest 1 mm and 0.1kg, respectively. Relative weight was calculated based on the table published in 1990 by the Ministry of Education of the Japanese Government[8]. The ponderal index was calculated as body weight in kilograms divided by the cubic of the height in meters. Percent fat was measured by the biological impedance method using a body composition analyzer, RJL Spectrum (RJL Systems, Detroit, MI, USA). Right triceps and subscapular skinfold thickness were measured using Harpenden calipers, and the sum was calculated. Both systolic and diastolic blood pressure were measured using automated recorder (Dinamap Model 8104). The abdominal wall fat index was measured by ultrasonography using ultrasound equipment (Toshiba Model SSA-250A) and a linear-array probe(7.5 MH_z)[9].

Assays

Blood samples were obtained after fasting in this study. Blood glucose and insulin were assayed using the glucose oxidase method[10] and double-antibody radioimmunoassay[11], respectively. The serum cholesterol and triglyceride levels were measured using an enzyme assay[12,13]. High-density lipoprotein cholesterol was assayed using the precipitation method[14]. Apolipoproteins were assayed using the turbidimetric immunoassay method[15]. Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were assayed using colorimetric methods[16].

Detection of the Trp64Arg polymorphism

Polymerase chain reaction (PCR) was carried out in a volume of 20 μl containing 100 ng of genomic DNA extracted from leukocytes, 20 pmol each of the following primers: (5'CGCCCAATACCGCCAACAC3' and 5'CCACCAGGAGTCCCATCACC3'), 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, 200 μM each dNTP, and 0.5 units of Taq polymerase (Takara Shuzo Co., Ltd, Kyoto, Japan)[3]. The PCR reactions (PTC-100, MJ Reserch, Inc., Mass., USA) were performed with an initial denaturation at 94℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 30 sec, annealing at 65℃ for 30 seconds, extension at 72℃ for 30 seconds, with a final extension at 72℃ for 10 min.

A 15 μl aliquot of the amplified PCR products was incubated at 60℃ for 16 h with the addition of 5 μl of a mixture containing 10 mM Tris-HCl (pH7.9), 10mM MgC12, 50mM NaCl, 100 μg/ml BSA, and 10 units of BSTNI, a restriction enzyme specific for the sequence CC(A/T)GG(New England Biolabs, Inc., Mass., USA).

A 12μl portion of the digest samples was separated by electrophoresis at 50 V for one and half hours in a 3% agarose gel (Sigma chemical, Co.) and visualized by staining with ethidium bromide.

Statistical analysis

Mean values were compared between subjects with and without the mutation in the β3AR gene using Student's t-test and the Mann-Whitney U test.

RESULTS

Genotypes and allele frequencies of the Trp64Arg polymorphism

The sizes of the BstNI-digested fragments of the 210 bp PCR product were 99, 62, 30, 12, and 7 bp for the Trp64 homozygotes, 161, 99, 62, 30, 12, and 7 bp for the Trp64/Arg64 heterozygotes, and 161, 30, 12, and 7 bp for the Arg64 homozygotes. The smaller fragments of 30, 12, and 7 bp could not be visualized on the gel.

Of 259 subjects, 151 (58.3%), 101 (39.0%), and 7 (2.7%) demonstrated Trp64 homozygotes, Trp64/Arg64 heterozygotes, and Arg64 homozygptes, respectively. The allele frequencies for Trp64 and Arg64 in all subjects were 0.778 and 0.222, respectively. The genotypes and allele frequencies in 7, 10, and 13-years-old subjects are shown in Table 1.

Trp64Arg polymorphisim in relation to the anthropometric and biochemical data

The mean values of all anthropometric and biochemical data of the boys did not differ from these of the girls in each age group. Data of both sexes were analyzed together.

Seven-year-old subjects with the mutation (Trp64/Arg64 heterozygotes and Arg64 homozygotes) had a larger relative weight, higher ponderal index, and highter levels of triglyceride and insulin than those without the mutation (Trp64 homozygptes) ($p < 0.05$) (Table 2). There were no significant differences in the other variables.

In either 10 or 13-year-old, no significant differences were observed in the anthropometric and biochemical data between subjects with or without the mutation.

Table 1. Genotype and allele frequencies of the Trp64Arg polymorphism in Japanese obese children

	7-year-old subjects	10 year-old subjects	13 year-old subjects
Genotypes			
Trp64 homozygotes	45(62.5)	83(59.7)	23(47.9)
Trp64/Arg64 heterozygotes	26(36.1)	52(37.4)	23(47.9)
Arg64 homozygotes	1(1.4)	4(2.9)	2(4.2)
Alleles			
Trp64	116(80.6)	218(78.4)	69(71.9)
Arg64	28(19.4)	60(21.6)	27(28.1)

Number (%) of subjects.

Table 2. Clinical characteristics of subjects according to the presence of the Arg64 mutation

Characteristic	7-year-old subjects		10 year-old subjects		13 year-old subjects	
	Mutation	No mutation	Mutation	No mutation	Mutation	No mutation
Sex(boys/girls)	18/9	32/13	39/17	62/21	10/15	15/8
Body hight (cm)	120.0±5.5	128.3±5.3	142.9±8.5	142.0±7.4	155.8±7.1	153.9±8.0
Body weight (kg)	40.8±5.2	38.9±4.6	53.6±9.6	52.9±9.3	70.8±11.9	69.9±12.7
Relative weight (%)	154.8±10.6	149.3±8.9*	152.4±9.0	152.9±10.7	151.4±12.8	155.2±13.4
Ponderal index (kg/m^3)	19.0±1.3	18.3±1.1*	17.8±2.3	18.3±1.2	18.6±1.2	19.0±1.2
Percent fat (%)	31.8±5.5	30.4±3.2	33.6±7.1	32.2±5.1	32.3±3.8	32.6±4.3
Skinfold thickness	51.5±8.2	49.2±9.8	55.0±7.5	54.2±9.3	56.3±8.5	57.6±9.0
Systolic blood pressure (mmHg)	109.3±8.7	111.7±11.2	117.8±12.1	116.4±11.6	121.5±9.7	117.2±12.2
Diastolic blood pressure (mmHg)	54.1±9.9	55.0±7.5	60.0±11.8	57.9±9.9	55.7±6.4	55.1±9.9
Abdominal wall fat index	0.56±0.19	0.59±0.20	0.66±0.26	0.63±0.19	0.76±0.30	0.68±0.27
Blood glucose (mg/dl)	87.6±5.9	86.1±5.8	86.8±6.6	88.0±6.1	87.8±6.4	89.3±7.3
Serum insulin (mU/ml)	9.3±3.0	7.6±2.5†	14.4±15.0	12.1±5.8	16.1±7.9	14.4±9.3
Serum lipids (mg/dl)						
Total cholesterol	186.4±30.0	181.9±29.2	186.0±26.6	185.9±27.8	175.0±28.2	180.2±19.2
HDL-cholesterol	57.1±9.7	58.6±12.1	54.9±11.1	55.2±10.8	52.1±8.9	52.3±9.6
Triglyceride	95.6±46.0	74.8±31.4*	105.8±63.8	101.3±48.6	95.7±43.5	103.0±61.6
Serum apolipoproteins (mg/dl)						
Apo A-1	136.6±19.3	137.1±20.9	130.7±18.4	132.0±17.3	122.1±17.7	130.0±21.4
Apo B	84.4±22.1	80.6±20.6	82.1±19.3	84.4±19.3	76.5±17.9	83.9±17.1
Apo E	5.8±1.4	5.8±1.9	5.9±1.3	6.0±1.5	5.5±1.4	6.1±1.7
Transaminase (IU/l)						
GOT	28.8±10.3	28.1±7.4	27.7±10.0	30.4±16.3	31.9±34.9	32.3±18.8
GPT	27.7±23.1	23.3±15.7	28.4±19.8	36.4±37.5	39.4±63.5	45.4±53.5

The values are expressed as mean±SD.

*$p<0.05$ for comparison between two group using student *t*-test

†$p<0.05$ for comparison between two group using the Mann-Whitney U-test.

DISCUSSION

A pathogenic role for the mutation of Trp64Arg in the β3AR gene has been reported in several ethnic groups. A cumulative change in weitht from 20 to 45 years of age was larger in French obese patients with the mutation than in those without the mutation[4]. In Finland, the mutation was associated with a larger ratio of waist to hip circumference and higher levels of blood glucose and serum insulin in 50-year-old nondiabetic subjects[3]. Fifty-year-old Pimas homozygous for the mutation had an earlier onset of non-insulin dependent diabetes mellitus and tended to have a lower resting metabolic rate[5]. In adults, the mutation has deleterious effects on the progression of obesity, resulting in insulin resistance by altering the balance of energy metabolism. In children, Endo et. al. have reported that the mutaion is associated with relative weight and ponderal index in Japanese schoolchildren with obesity. They did not, however, discuss insulin resistance or hyperinsulinemia[17].

This study provides evidence that the mutation of Trp64Arg is associated with anthropometric data and biochemical data — especially insulin — in Japanese younger obese children. In 7-year-old subjects, the mutation had a significant effect on an excess of body weight, expressed as a larger relative weight and higher ponderal index. The percent fat and skinfold thickness measured using the biological impedance method and Harpenden caliper, respectively, were also larger in the subjects with mutation than in those without the mutation. These differences, however, did not reach a statistical significance. These findings suggest that the mutation plays a modest role in determining the amount of body fat, but rather acts to accelerate the course of childhood obesity. In addition to these anthropometric data, the mutation was associated with higher levels of triglyceride and insulin. Obese adults tend to have insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension[18]. These metabolic abnormalities and the associated dysregulation of blood pressure are known as syndrome X. Although this study did not rigorously evaluate glucose intolerance, the mutation of Trp64Arg may play a role in altering glucose and insulin levels and affecting lipoprotein metabolism in Japanese children with obesity.

A question arising from the results of this study is why the differences in the anthropometric and biochemical data were not observed in either 10 or 13-year-old subjects. The contribution of the mutation of Trp64Arg to obesity may be masked by the differences in other genes and lifestyles including eating habits and the change of physical constitution during preadolescence and adolescence. As reported previously[4], the difference in the body weight between French obese patients with and without the mutation of Trp64Arg was obtained not at 20, but at 50 years of age[4]. From these findings, we can infer that the strength of the effect of the mutation varies with age.

In conclusion, the results of this study suggest that a polymorphism in the β3AR gene contributes to an increased capacity for weight gain and is associated with hyperinsulinemia and hypertriglyceridemia in younger obese children.

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