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β_3-adrenergic-receptor polymorphism: a genetic marker for visceral fat obesity and the insulin resistance syndrome

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Summary We investigated whether the polymorphism of the β_3-adrenergic receptor (β_3-AR) gene, which is associated with insulin resistance in non-diabetic subjects and an earlier onset of non-insulin-dependent diabetes mellitus in Pima Indians, was associated with visceral fat obesity and features of the insulin resistance syndrome in Japanese premenopausal obese women. There was no difference between 131 obese women and 256 control subjects (0.23 vs 0.17, $p = 0.112$) in the frequency of the Arg^{64} allele. The visceral fat area measured by computerised tomography scan was greater in homozygous $Arg^{64}Arg$ (172 ± 17 cm^2, $n = 6$) and heterozygous $Trp^{64}Arg$ (178 ± 47 cm^2, $n = 48$) women than in women homozygous for the $Trp^{64}Trp$ (121 ± 46 cm^2, $n = 77$) genotype ($p < 0.01$). This was also reflected by increased total body fat but not by increased body mass index. The association between the Trp^{64} allele and visceral fat mass by multiple regression analysis, was independent of age, body mass index and total fat mass ($p < 0.004$). Moreover, homozygous carriers of the Arg^{64} allele had higher systolic blood pressure, higher fasting and post-load glucose and insulin concentrations, higher cholesterol, and triglyceride and lower HDL-cholesterol concentrations than homozygous carriers of the Trp^{64} allele. Some of these differences were also observed between heterozygous $Trp^{64}Arg$ and homozygous $Trp^{64}Trp$ genotypes (glucose tolerance, insulin and cholesterol concentration). We conclude that in obese women the β_3-AR polymorphism may be used as a genetic marker for visceral fat obesity and the insulin resistance syndrome. [Diabetologia (1997) 40: 200–204]

Keywords β_3-adrenergic-receptor gene, obesity, insulin resistance syndrome, genetics, polymorphism.

Obesity is a complex phenotype resulting from the combined effects of genes, behavioural and lifestyle factors, and their interactions [1]. The associations between obesity and increased risk of morbidity and mortality are well documented [2]; however, distribution of body fat is also of importance [3]. A syndrome with a clustering of multiple risk factors for coronary atherosclerosis has been described and defined by Reaven [4] as syndrome X; by Kaplan [5] as the deadly quartet; and by Matsuzawa et al. [6] as visceral fat obesity, which is more strongly associated with metabolic and cardiovascular diseases than subcutaneous fat obesity [7, 8]. From clinical and basic experiments, the imbalance of sex hormones [9], aging [10], excessive intake of sucrose [11] and lack of physical exercise [10] have been suggested to be major factors for visceral fat accumulation. However, it is not known whether a major gene exists which promotes visceral fat accumulation, although Bouchard et al. [12] demonstrated six times more variation between twin pairs than within twin pairs for an

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Abbreviations: β_3-AR, β_3-adrenergic receptor; NIDDM, non-insulin-dependent diabetes mellitus; WHR, waist-to-hip ratio; CT, computerised tomography; RMR, resting metabolic rate.

Table 1. Genotype frequency of the β_3-adrenergic receptor in obese women and control subjects

Genotype	Obese women	Control subjects
Arg/Arg	6 (4.6)	5 (2.0)
Trp/Arg	48 (36.6)	77 (30.0)
Trp/Trp	77 (58.8)	174 (68.0)

Date are *n* (%)

increase in visceral fat with long-term overfeeding. The β_3-adrenergic-receptor (β_3-AR) is mainly expressed in visceral adipose tissue [13] and is considered responsible for lipolysis and delivery of non-esterified fatty acid into the portal vein [14]. Recently, Pima Indians were found to have a high frequency of the Arg^{64} allele, and those with the mutation to have an early onset of non-insulin-dependent diabetes mellitus (NIDDM) and a tendency to have a low metabolic rate [15]. This mutation is also associated with an increased capacity to gain weight [16], and difficulty in losing weight [17]. Widén et al. [18] reported that this mutation is associated with abdominal obesity and resistance to insulin by waist-to-hip ratio (WHR), which is an indirect measurement of visceral fat. However, WHR does not accurately measure visceral fat mass [19]. The measurement of visceral fat mass by computerised tomographic (CT) scanning is the gold standard for quantitation of visceral obesity. Therefore, in this study, we investigated the association between β_3-AR polymorphism, visceral fat mass measured by CT scanning and features of the insulin resistance syndrome in obese women.

Subjects and methods

We studied 131 premenopausal obese women (mean age: 38.5 ± 11.8 years; mean duration of obesity: 4.6 ± 1.7 years; mean body weight: 80.6 ± 14.6 kg; mean body mass index 33.3 ± 3.7 kg/m^2) who came to the out-patient clinic of our university hospital for treatment of their condition. We also examined 256 age-matched non-obese women (mean age: 35.8 ± 4.4 years; mean body weight 50.5 ± 5.3 kg; mean body mass index 22.3 ± 2.8 kg/m^2). All protocols were approved by the University Review Board of Kyoto Prefectural University of Medicine. Written informed consent was obtained from all subjects before enrollment in the study. In obese subjects the proportion of body fat (% body fat) was estimated from skin fold measurements, according to the method of Durnin and Womersley [20] and the body fat distribution was determined by CT scanning according to the procedure of Tokunaga et al. [21]. In obese and non-obese control subjects blood samples were drawn after an overnight fast. Blood glucose was measured by the glucose oxidase method, and serum total cholesterol and triglyceride levels were determined by enzymatic methods. HDL-cholesterol was measured by the heparin-calcium precipitation method. In obese subjects, the 75 g oral glucose tolerance test was also performed, and blood samples were collected at 0, 30, 60, 90, and 120 min to determine glucose and insulin levels. Serum insulin was assayed by double antibody radioimmunoassay. The blood glucose or serum insulin area was determined by the trapezoidal rules [18]. In 98 obese women (not 131) the resting metabolic rate (RMR) was measured by a closed-circuit indirect calorimeter (Sanborn-Wedge type spirometer: metabograph, Model SS-80; Fukuda Medical Laboratory Co., Tokyo, Japan) with a mouth piece, in a temperature-controlled room (22–24 °C) after overnight fast [22]. The RMR was obtained as the mean of a 30-min measurement period, and was adjusted [23] by age and lean body mass, which was calculated by subtracting body fat from body weight.

Table 2. β_3-adrenergic receptor genotypes and body composition, fat distribution, blood pressure, blood glucose, serum insulin and lipids in obese women

Genotype	Homozygous		Heterozygous		
	Arg/Arg	*p* value	Trp/Arg	*p* value	Trp/Trp
n	6		48		77
Body mass index (kg/m^2)	34.1 ± 1.7	0.055	33.2 ± 5.9	0.612	32.3 ± 4.5
Body fat (%)	44.8 ± 2.8	0.024	42.9 ± 3.7	0.012	41.8 ± 2.8
CT fat area (cm^2)					
Total	547 ± 66	0.444	580 ± 154	0.039	519 ± 146
Visceral	172 ± 17	0.006	178 ± 47	< 0.001	121 ± 46
Blood pressure (mm Hg)					
Systolic	165 ± 32	0.011	141 ± 27	0.100	133 ± 26
Diastolic	95 ± 17	0.209	83 ± 15	0.626	84 ± 18
Blood glucose					
Fasting (mmol/l)	6.9 ± 0.4	0.002	6.3 ± 1.9	0.617	6.0 ± 1.4
AUC[a] (mmol · min/l)	1283 ± 32	0.005	1344 ± 635	< 0.001	926 ± 407
Serum insulin					
Fasting (pmol/l)	79 ± 22	0.003	72 ± 29	< 0.001	43 ± 29
Log (AUC)[b] (pmol · min/l)	3.90 ± 0.06	0.031	3.89 ± 0.15	< 0.001	3.75 ± 0.22
Serum lipids (mmol/l)					
Total cholesterol	5.9 ± 0.9	0.008	5.4 ± 1.2	0.021	4.9 ± 0.7
Triglycerides	2.4 ± 0.8	0.002	1.3 ± 0.5	0.784	1.4 ± 0.8
HDL cholesterol	1.1 ± 0.1	0.010	1.3 ± 0.3	0.423	1.4 ± 0.3

Data are means ± SD. *p* values compared subjects carrying Arg/Arg or Trp/Arg genotype with subjects carrying Trp/Trp genotype.
[a] AUC, Area under curve; [b] transformed by the natural logarithm

Table 3. β_3-adrenergic receptor genotypes and body mass index, blood pressure and serum glucose and lipids in control subjects

Genotype	Homozygous		Heterozygous		
	Arg/Arg	*p* value	Trp/Arg	*p* value	Trp/Trp
n	5		77		174
Body mass index (kg/m^2)	23.0 ± 1.9	0.627	22.4 ± 2.7	0.991	22.3 ± 2.8
Blood pressure (mm Hg)					
Systolic	120 ± 11	0.092	110 ± 11	0.757	133 ± 26
Diastolic	70 ± 7	0.609	68 ± 7	0.374	68 ± 7
Blood glucose					
Fasting (mmol/l)	5.3 ± 0.6	0.572	5.1 ± 0.7	0.957	5.1 ± 0.7
Serum lipids (mmol/l)					
Total cholesterol	5.1 ± 0.8	0.465	5.0 ± 0.9	0.089	4.8 ± 0.9
Triglycerides	0.8 ± 0.3	0.633	0.9 ± 0.4	0.799	0.9 ± 0.3
HDL cholesterol	1.6 ± 0.2	0.331	1.8 ± 0.3	0.106	1.7 ± 0.3

Data are means ± SD. *p* values compared subjects carrying Arg/Arg or Trp/Arg genotype with subjects carrying Trp/Trp genotype

Table 4. Multiple regression analysis with visceral fat area as the response variable (transformed by the square root) in obese subjects

Explanatory variable	Regression cofficient (95 % CI)	*p* value
Age	0.024 (0.002–0.046)	0.032
Body mass index	0.118 (0.052–0.185)	0.001
Percent body fat	0.124 (0.022–0.226)	0.018
Fasting blood glucose	0.008 (0.002–0.019)	0.133
Fasting serum insulin	0.099 (0.011–0.186)	0.028
Trp^{64} Arg allele	0.936 (0.312–1.560)	0.004

PCR-RFLP screening for the Trp^{64}Arg polymorphism. As the Trp^{64}Arg polymorphism in the β_3-AR gene eliminates a Mva I restriction site, this polymorphism can be detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR primers were 5′-CCAATACCGCCAACACCAGT-3′ (upstream) and 5′AGGAGTCCCATCACCAGGTC-3′ (downstream), which flank the whole exon 1 of the β_3-AR gene. Genomic DNA (100 ng) in a total volume of 20 μl was used for PCR. PCR was performed by initial denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 67 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. We then incubated 5 μl of the PCR product for 1 h with 10 U of MvaI at 37 °C in a final volume of 10 μl without further purification. The samples were then run on a 3.0 % agarose gel, stained with ethidium bromide and analysed under ultraviolet light. In the presence of the polymorphism, the restriction site for MvaI is lost; therefore, the allele of this polymorphism corresponds to the 158 base pair (bp) undigested band.

Statistical analysis

All data are shown as mean ± SD. Differences in continuous variables between obese women and control subjects carrying the Arg/Arg, Trp/Arg or Trp/Trp genotype were tested by Mann-Whitney U-test. When necessary, Bonferroni *t*-test was performed after justification by one-way ANOVA. In obese subjects, a multiple regression analysis controlled for age, body mass index, percentage of body fat, fasting blood glucose and fasting serum insulin (explanatory variables) was performed to evaluate the impact of polymorphism of the β_3-AR gene (explanatory variable) on visceral fat mass (response variable). The statistical package of Social Science for Windows, version 6.01, was used for statistical analysis.

Results

There was no difference between the obese women and the non-obese control subjects (0.23 vs 0.17, $p = 0.112$) in the frequency of the Trp^{64}Arg allele (Table 1). In univariate analysis of obese subjects as shown in Table 2, those with the mutation had an increased percentage of body fat ($p < 0.024$, Arg/Arg; $p < 0.012$, Trp/Arg), higher total fat area by CT scanning ($p < 0.039$, Trp/Arg), higher visceral fat mass ($p < 0.006$, Arg/Arg; $p < 0.001$, Trp/Arg), higher systolic blood pressure ($p < 0.011$, Arg/Arg), higher fasting blood glucose ($p < 0.002$, Arg/Arg), increased blood glucose area under the curve ($p < 0.005$, Arg/Arg; $p < 0.001$, Trp/Arg), higher fasting serum insulin level ($p < 0.003$, Arg/Arg; $p < 0.001$, Trp/Arg), increased insulin area under the curve ($p < 0.031$, Arg/Arg; $p < 0.001$, Trp/Arg), higher total cholesterol ($p < 0.008$, Arg/Arg; $p < 0.021$, Trp/Arg), higher triglyceride ($p < 0.002$, Arg/Arg) and lower HDL cholesterol ($p < 0.010$, Arg/Arg), compared to those without the mutation, although there was no difference in body mass index or diastolic blood pressure. Furthermore, the adjusted RMR, that was measured in 98 obese women (not 131), was lower in those with the mutation ($p < 0.042$, Arg/Arg [$n = 4$; 20 ± 104 kcal/day]; $p < 0.022$, Trp/Arg [$n = 36$; 118 ± 131 kcal/day]) than in those without the mutation ($n = 58$; 227 ± 227 kcal/day). However, there was no difference in body mass index, blood pressure, blood glucose or serum lipids in control subjects (Table 3) with or without the mutation of the β_3-AR gene by both Mann-Whitney U-test and Bonferroni *t*-test.

When adjustments were made for age, body mass index, percentage of body fat, fasting blood glucose and fasting serum insulin in a multiple regression analysis, a significantly positive association was found

between $Trp^{64}Arg$ allele of the β_3-AR gene and visceral fat mass ($p < 0.004$).

Discussion

Mutation of the β_3-AR gene was present with an allelic frequency of 0.23 in Japanese obese women and 0.17 in non-obese control subjects. These values were similar to those reported previously [17, 24, 25], which was higher than that in black and white Americans [15], French [16], Finns [18], Swedish [26] and Danes [27] but not Pima Indians [15]. These findings suggest that this mutation may play an important role in the Pima Indians and Japanese.

Univariate analysis of the obese women revealed an increase of visceral fat mass, and the characteristics of the insulin resistance syndrome in those with this mutation, i.e. an increased systolic blood pressure, higher fasting blood glucose, increased blood glucose area under the curve, higher fasting serum insulin, increased insulin area under the curve, higher serum total cholesterol, higher serum triglyceride, and decreased serum HDL cholesterol, compared with those without the mutation; although, in non-obese control subjects with and without the mutation of the β_3-AR gene we could not find any difference in clinical and laboratory data. Kadowaki et al.[24] and Fujisawa et al.[25] both reported higher body mass index among Japanese subjects homozygous for the $Trp^{64}Arg$ mutation than those heterozygous and those without the mutation. In this study there was no significant difference between body mass index values and the mutation in either obese or non-obese subjects, although obese women with the mutation had an increased percentage of body fat compared to those without the mutation. The reason for the difference between the present findings and those reported previously is unclear, the gender difference (present, female; Kadowaki [24], male; Fujisawa [25], not shown) and the difference in degree of obesity in the subjects (present, obese body mass index (mean) 33.3 kg/m^2, control 22.3; Kadowaki [24], 24.7–22.1; Fujisawa [25], 22.8) may explain this discrepancy. The present result showing the higher visceral fat mass (Arg/Arg, $p < 0.006$; Trp/Arg $p < 0.001$) in obese subjects with the β_3-AR mutation is consistent with our previous finding (Trp/Arg, $p < 0.01$) with 88 obese women in another study [17] and is in agreement with the result in Finns [18] showing that women with a mutation had a higher WHR ($p < 0.008$). A larger visceral fat mass is a factor usually accompanied by the insulin resistance syndrome [10]; therefore, it is not surprising that in the present study obese subjects with the mutation have the features of the insulin resistance syndrome. Indeed, the findings showing an increased systolic blood pressure in obese subjects homozygous for the $Trp^{64}Arg$ mutation is in analogy with the weak trend toward higher systolic blood pressure in subjects with the mutant allele reported by Kadowaki et al. [24]. The higher fasting blood glucose (Arg/Arg) and the increased glucose area under the curve (Arg/Arg, Trp/Arg) in obese subjects found in the present study, have previously been found to be more common among Finns [18], Pima Indians [15] and Japanese with NIDDM [25]. Kadowaki et al. [24] also reported an increased fasting serum insulin (Arg/Arg; Trp/Arg) and area under the curve (Arg/Arg; Trp/Arg) in obese subjects homozygous for the mutation as compared to those heterozygous and those without the mutation. The increased total cholesterol (Arg/Arg; Trp/Arg), increased fasting triglyceride (Arg/Arg) and decreased HDL cholesterol (Arg/Arg) in obese subjects with the mutation are in contrast with the findings of Kadowaki et al. [24] who did not find any differences in the prevalence of dyslipidaemia between the different alleles, but are in analogy with those reported in young Danes [27]. The discrepancy between our findings and the data of Kadowaki et al. [24] may again be explained by the difference in the degree of obesity in each study. Concerning the RMR, in the present study, we could measure it in only 98 obese women of the total 131. The finding showed that those with the mutation (Arg/Arg; Trp/Arg) had a lower adjusted RMR, as compared to those without the mutation, being consistent with our previous finding in 88 obese women in another study [17] and is in agreement with the findings in Pima Indians [15].

Multivariate analysis showed that visceral fat mass among the $Trp^{64}Arg$ allele of β_3-AR gene was independent of body mass index and percentage of body fat. Thus, the polymorphism in its homozygous and heterozygous forms may itself increase the visceral fat mass, because there is more β_3-AR in visceral fat than in subcutaneous fat [13]. The underlying mechanisms may involve impaired lipolysis [14], with an associated enlargement of the adipose cells and thereby relative visceral obesity, which may lead to insulin resistance and hyperinsulinism. The latter may cause down-regulation of β_3-AR, which will promote further progression of obesity and establish a vicious circle, although there are reports showing that when a heterologous system of this mutation was expressed in CHO cells, its mutant receptor was pharmacologically and functionally indistinguishable from the wild type β_3-AR [28] and that there was no difference in β_3-AR function between $Trp^{64}Arg$ heterozygotes and Trp^{64} homozygotes [29]. Although the physiological relevance of β_3-AR in humans remains to be clarified, pharmacological studies on β_3-AR agonists in obese subjects have revealed a decrease in their insulin resistance [29].

The effect of β_3-AR polymorphism seems to be smaller than was initially expected. However, judging

from our present findings and those obtained in the Pima Indians, ethnic differences in the importance of the mutation must be considered. Essentially, this mutation may represent a real "thrifty gene" [30] in the Pima Indians and Japanese.

In conclusion, the $Trp^{64}Arg$ allele is associated with visceral fat obesity and features of the insulin resistance syndrome. Therefore, our findings suggest that the β_3-AR polymorphism may be used as a genetic marker for visceral fat obesity and the insulin resistance syndrome in obese subjects. Since only six homozygotes were identified among 131 obese women, the results must be interpreted with caution. Further studies using larger population samples will be required.

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