

Clinical Features Associated With the Homozygous Trp⁶⁴Arg Mutation of the β 3-Adrenergic Receptor

No Evidence for Its Association With Obesity in Japanese

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Abstract—To characterize the clinical features associated with the Trp⁶⁴Arg mutation of the β 3-adrenergic receptor (β 3-AR), the effects of this mutation, in particular the homozygous state (Arg/Arg), on obesity, blood pressure, and plasma lipoproteins were investigated in 2 populations: subjects residing on a small isolated island (group 1; n=746) and patients residing in Tokyo who attend a clinic for metabolic diseases (group 2; n=371). The allelic frequency of the Trp⁶⁴Arg mutation was 23.4% in group 1 and 18.3% in group 2. No significant difference in the body mass index was observed between subjects with 3 different genotypes in each group. There was a trend that the Arg/Arg had higher systolic blood pressure than the Trp/Trp in both groups, but the differences were not statistically significant. The plasma LDL cholesterol levels were significantly lower in Arg/Arg than in Trp/Trp in men from the group 1 cohort (2.82 ± 0.84 versus 3.19 ± 0.7 mmol/L, $P < 0.05$). These results suggest that the homozygous Trp⁶⁴Arg mutation is not a major contributing factor for obesity, but potentially contributed to higher systolic blood pressure and low plasma levels of LDL cholesterol in Japanese men. (*Arterioscler Thromb Vasc Biol.* 1998;18:941-946.)

Key Words: β 3-adrenergic receptor ■ obesity ■ hypertension ■ diabetes ■ cholesterol

Obesity is associated with various diseases, such as hypertension, glucose intolerance, and hyperlipidemia, which collectively contribute to the development of ischemic heart disease.¹ Both genetic and environmental factors are involved in the development of human obesity.² The role of genetic factors has long been enigmatic, until recent discovery of molecules that govern the metabolism of adipocytes.³ β 3-AR¹ is one of these molecules.^{4,5} In conjunction with β 1-AR and β 2-AR, accumulating evidence supports an important role for the β 3-AR in mediating adrenergic regulation of the metabolism of adipose tissues. β 3-AR is expressed in brown adipose tissue of neonates and visceral adipocytes in adults,⁶ where it regulates thermogenesis and lipid mobilization. Furthermore, the β 3-AR has been shown to be widely expressed in other tissue, such as heart⁷ and gastrointestinal tract.⁸ Targeted disruption of this receptor gene in mice resulted in modest obesity, supporting its critical role in the development of obesity.⁹

In 1995, a missense mutation that replaces tryptophan with arginine (Trp⁶⁴Arg) of the human β 3-AR gene was reported to be associated with increased capacity to gain weight in the French population,¹⁰ the features of insulin resistance syndrome in Finns,¹¹ and the earlier onset of NIDDM in obese Pima Indians,¹² even in a heterozygous state. These observations were supported by subsequent reports in various ethnic

populations, including Japanese,¹³⁻¹⁸ Danes,¹⁹ and Austrians.²⁰ However, the role of this mutation in the pathogenesis of obesity is still controversial, and contradictory results have been reported with respect to the effects of the Trp⁶⁴Arg mutation on obesity.²¹⁻²⁵

Phenotypic expression of certain mutations can be more remarkable in a homozygous state than in a heterozygous state. In this regard, it is noteworthy that several studies have shown reduced resting metabolic rates,¹² hyperinsulinemia with obesity,¹³ and reduced insulin sensitivity⁹ in subjects homozygous for the Trp⁶⁴Arg mutation. But other studies have shown lack of association between the homozygous mutation and obesity.^{24,25} A limitation of these studies, however, is that relatively few individuals homozygous for the Trp⁶⁴Arg allele were studied.

In the current study, we attempted to more completely describe parameters associated with the Trp⁶⁴Arg mutation of the β 3-AR gene and also test the hypothesis that the mutation accounts for obesity. We investigated the association of this mutation with multiple clinical parameters, including blood pressure, plasma lipoproteins, and liver function, in an isolated population living on a small island in Japan and in patients residing in Tokyo who attend a clinic for metabolic diseases that collectively contained a total of 67 subjects homozygous for the Trp⁶⁴Arg mutation.

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Selected Abbreviations and Acronyms

AR	=	adrenergic receptor
BMI	=	body mass index
DBP	=	diastolic blood pressure
GOT	=	glutamic oxaloacetic transaminase
GPT	=	glutamic pyruvic transaminase
γ -GTP	=	γ -glutamyltransferase
HDL-C	=	HDL-cholesterol
LDL-C	=	LDL-cholesterol
NIDDM	=	non-insulin-dependent diabetes mellitus
PCR	=	polymerase chain reaction
SBP	=	systolic blood pressure
TC	=	total cholesterol
TG	=	triglyceride
TP	=	total protein

Methods

Subjects

The first cohort (group 1) consisted of participants in a regular health check survey that live on a small isolated island with approximately 10 000 inhabitants located 290 km from the Japanese mainland.²⁶ A total of 746 subjects (224 men and 522 women, aged 30 to 89 years; mean age 61.5 \pm 11.33 years) were randomly recruited.

The second cohort (group 2) was recruited from patients attending a clinic for metabolic diseases at Tokyo University Hospital. The 371 subjects chosen (180 men and 191 women, aged 21 to 88 years; mean age 58.6 \pm 11.4 years) suffered from diabetes and/or hyperlipidemia. Of these 371, 107 (28.8%) were treated with oral hypoglycemic drugs, 230 (62%) were treated with hypolipemic drugs, and 91 (24.5%) were treated with antihypertensives.

The subjects' age, sex, accompanying diseases, and medications were collected, and height (m), weight (kg), and blood pressure were measured. BMI was calculated as kg/(m)². Severe and moderate obesity were defined as BMI>28 and BMI=24 to 28, respectively. Blood pressure (SBP/DBP) was determined based on the average of 2 or more readings obtained on 2 or more visits. ECGs were recorded and the ischemic changes were defined as follows: ECG evidence representing previous myocardial infarction, such as abnormal Q wave, or ischemic changes, such as depression of ST-T. Patients with liver cirrhosis were excluded from the present study.

Laboratory Tests

Blood samples were obtained from all subjects after a 12-hour fast. Serum concentrations of TC, TG, HDL-C, TP, GOT, GPT, γ -GTP, and choline esterase were measured using conventional methods.

LDL-C was calculated using the Friedewald equation. Urinalysis also was performed.

DNA Analysis

Genomic DNA was extracted from whole blood. PCR was used to amplify a genomic DNA fragment containing codon 64 of the β 3-AR in a volume of 25 μ L containing 50 ng of genomic DNA; 10 pmol each of the primers up (5'-CGCCCAATACCGCCAACAC-3') and down (5'-CCACCAGGAGTCCCATCACC-3'); 2.5 μ L of 10% DMSO, 10 \times PCR buffer (supplied by Perkin-Elmer Cetus), 10 \times BSA, 0.4 μ L of 2.5 mmol/L dNTP, and 0.625 U of *Taq* polymerase, essentially according to Widén et al.¹¹ The PCR reactions began with denaturation at 93°C for 2 minutes, annealing at 60°C for 2 minutes, and extension at 65°C for 5 minutes, followed by 39 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 65°C for 2 minutes, with a final extension at 72°C for 10 minutes.

The amplified PCR products were digested with 5 U of *Bst*NI and were separated by electrophoresis through 3% agarose gel. DNA was visualized by staining with ethidium bromide.

Statistical Analysis

All data are expressed as mean \pm SD. ANOVA, Student's *t*, Kruskal-Wallis, Mann-Whitney *U*, and χ^2 tests were performed to estimate the effects of each genotype on quantitative variables and qualitative variables, respectively (Statview 2 statistical package). Statistical significance was established at the *P*<0.05 level.

Results

Genotype Frequencies

Table 1 illustrates the number of subjects homozygous (Arg/Arg), heterozygous (Trp/Arg), and wild type (Trp/Trp) for the Trp⁶⁴Arg allele in groups 1 and 2. The 3 genotypes were distributed in both study groups in accordance with Hardy-Weinberg equilibrium. The calculated frequency of the Trp⁶⁴Arg allele was 23.4% for group 1 and 18.3% for group 2. No significant difference in the allelic frequencies was observed between the 2 study groups, indicating that these 2 populations represent the general Japanese population with respect to the β 3-AR locus.

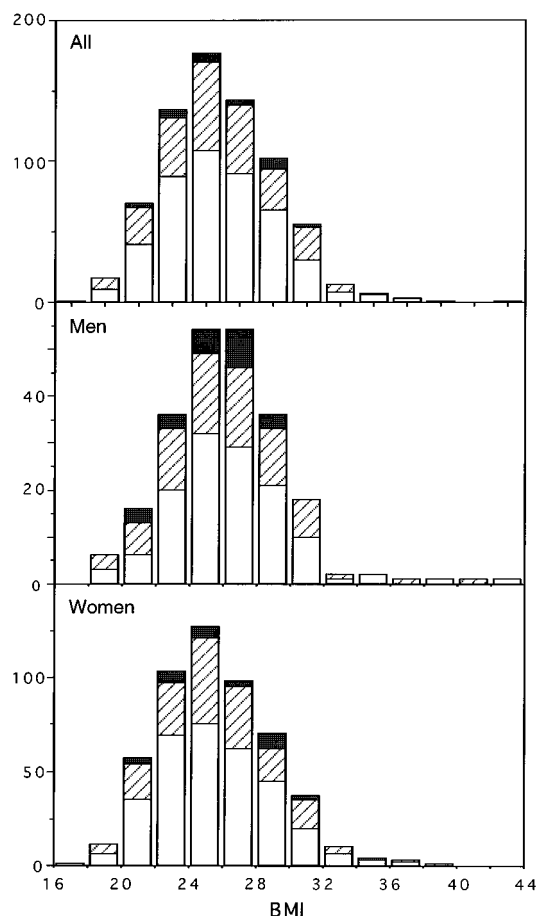
Body Mass Index

Univariate analyses in each study group did not reveal any difference in age and BMI between Arg/Arg, Trp/Arg, and Trp/Trp subjects in both men and women (Table 1). Allelic

TABLE 1. Clinical Characteristics of Subjects According to Trp⁶⁴Arg Mutation of β 3-AR Gene

	Men				Women			
	Arg/Arg	Trp/Arg	Trp/Trp	<i>P</i>	Arg/Arg	Trp/Arg	Trp/Trp	<i>P</i>
Group 1, n	22	77	125		29	169	324	
Age, y	63.6 \pm 12.6	63.2 \pm 12.2	60.0 \pm 11.3	0.11	60.8 \pm 11.8	61.5 \pm 10.6	61.4 \pm 11.4	0.96
BMI, kg/(m) ²	23.4 \pm 2.7	23.8 \pm 3.2	24.4 \pm 3.4	0.28	24.1 \pm 3.4	23.7 \pm 3.5	23.6 \pm 3.4	0.75
SBP, mm Hg	149 \pm 23	142 \pm 19	141 \pm 19	0.16	137 \pm 18	139 \pm 20	139 \pm 20	0.91
DBP, mm Hg	83 \pm 12	82 \pm 11	83 \pm 11	0.99	78 \pm 9	80 \pm 11	80 \pm 11	0.49
Group 2, n	8	49	121		8	54	127	
Age, y	58.5 \pm 11.1	55.5 \pm 12.5	56.0 \pm 12.3	0.82	63.1 \pm 7.6	60.9 \pm 10.5	61.1 \pm 9.2	0.82
BMI, kg/(m) ²	23.0 \pm 2.5	23.7 \pm 3.8	23.1 \pm 2.7	0.63	23.0 \pm 3.5	23.0 \pm 3.1	22.6 \pm 3.0	0.96
SBP, mm Hg	131 \pm 22	123 \pm 17	122 \pm 14	0.31	126 \pm 16	128 \pm 19	124 \pm 16	0.24
DBP, mm Hg	83 \pm 4	76 \pm 13	76 \pm 9	0.17	77 \pm 7	79 \pm 10	75 \pm 7	0.05

Data are mean \pm SD, analyzed by ANOVA.



Distribution of allelic frequencies for Trp⁶⁴Arg mutation of β 3-AR gene according to BMI in total (All), men, and women. Homozygotes and heterozygotes for the Trp⁶⁴Arg mutation are indicated as solid and hatched bars, respectively.

frequency in subjects with severe obesity (BMI>28) was not significantly different from that in moderately obese (BMI 24 to 28) or nonobese people (BMI<24). The Figure compares the distribution of BMI among the 3 genotypes in the total (top) men (middle), and women (bottom) from group 1. Male Arg/Arg were found more frequently in subjects with BMI between 26 and 28 than in subjects in any other BMI range, although this difference was not statistically significant. Furthermore, no significant difference in allelic frequency was detected between any BMI quartile either in men, women, or the total (data not shown). Notably, the BMI of all 51 Arg/Arg was <35. In group 2, no significant correlation was detected between BMI and the Trp⁶⁴Arg mutation (Table 1). Furthermore, analysis of the combined populations of groups 1 and 2 revealed no significant correlation between BMI and the Trp⁶⁴Arg mutation (Table 2).

Blood Pressure

The mean SBP of group 2 was significantly lower than that of group 1 in any given genotype and sex (Table 1). Since the difference remained even after eliminating patients treated with antihypertensive drugs, the lower SBP may be ascribed to either adherence to low-salt diets or the difference in other genetic and/or environmental factors. There was a trend for

TABLE 2. Body Mass Index and Blood Pressure Analyses of Combined Populations (Groups 1 and 2)

	Arg/Arg	Trp/Arg	Trp/Trp	P
N				
Men	30	127	247	
Women	37	222	247	
Total	67	349	702	
BMI, kg/(m) ²				
Men	23.5±2.7	23.8±3.4	23.8±3.1	0.87
Women	23.9±3.4	23.6±3.4	23.3±3.5	0.45
Total	23.7±3.1	23.6±3.4	23.5±3.4	0.71
SBP, mm Hg				
Men	144.3±18.2*	134.8±20.5	131.7±19.3	0.0026
Women	134.7±18.2	136.2±20.3	134.2±20.1	0.48
Total	138.8±21.5	135.7±20.3	133.1±19.8	0.025
DBP, mm Hg				
Men	82.9±10.4	79.8±12	79.4±10.3	0.28
Women	77.4±8.1	79.4±10.6	78.8±10.3	0.50
Total	80.4±10.6	79.5±11.1	79.0±10.3	0.49

Statistical analyses were performed on the combined population consisting of groups 1 and 2. Data are mean±SD, analyzed by ANOVA.

*Statistically significant difference at $P<0.05$ vs Trp/Trp genotype by Fisher test.

the Arg/Arg to have higher SBP than the Trp/Trp in each population, but the difference did not reach statistical significance by univariate analyses (Table 1). In group 2, there was also a trend for the Arg/Arg to have higher SBP than the Trp/Trp in men, although the difference was not statistically significant (Table 1). The number of patients under treatment for hypertension was 5 (31.2%) in the Arg/Arg, 24 (23.2%) in the Trp/Arg, and 64 (24.7%) in the Trp/Trp, respectively, but the difference was not statistically significant.

Next, the 2 populations were analyzed in combination, thus yielding 30 Arg/Arg men. The Arg/Arg had significantly higher SBP than the Trp/Arg and Trp/Trp (Table 2). No differences were observed in women. Because blood pressure is influenced by several factors, multivariate analyses using age, sex, BMI, group, and the genotype of β 3-AR as explanatory factors were performed to determine whether the effects of the β 3-AR mutation on elevated SBP is independent of other confounding factors. As expected, age, BMI, and group were strongly associated with elevated SBP ($P<0.0001$). The effects of the β 3-AR genotype were no longer significant ($P=0.18$). When only men were analyzed, a weak association between the β 3-AR mutation and SBP was found ($P=0.067$).

Incidence of NIDDM and Abnormal ECG

No difference in the incidence of NIDDM was observed between the Arg/Arg (3.9%), Trp/Arg (6.9%), and Trp/Trp subjects (4.9%) in group 1. The allelic frequency (18.6%) in the diabetic patients in the group 1 cohort was surprisingly similar to the value in group 2 (18.1%), of which 216 (58%) were diabetic.

TABLE 3. Biochemical Characteristics of Subjects According to Trp⁶⁴Arg Mutation of β 3-AR Gene

Trp ⁶⁴ Arg	Men				Women			
	Arg/Arg	Trp/Arg	Trp/Trp	<i>P</i>	Arg/Arg	Trp/Arg	Trp/Trp	<i>P</i>
Group 1, n	22	77	125		29	169	324	
TC, mmol/L	4.70±1.03*	5.05±0.83	5.20±0.79	0.03	5.33±0.95	5.27±0.83	5.26±0.82	0.91
TG, mmol/L	1.21±0.62	1.43±0.88	1.56±1.26	0.35	1.38±0.57	1.19±0.56	1.24±0.72	0.29
HDL-C, mmol/L	1.33±0.32	1.40±0.37	1.30±0.37	0.18	1.29±0.33	1.39±0.31	1.40±0.31	0.22
LDL-C, mmol/L	2.82±0.84*	2.99±0.78	3.19±0.70	0.04	3.40±0.93	3.34±0.76	3.29±0.72	0.67
TP, g/L	77±4*	74±4	75±4	0.01	76±5	75±5	75±5	0.29
GOT, U	25 (21–38)	23 (20–28)	25 (21.8–30)	0.16	21 (19–24.3)	20 (18–25)	21 (18–25)	0.45
GPT, U	22 (16–34)	21 (17–32.3)	21 (15–28)	0.76	15 (12–21.3)	14 (11–20)	15 (12–21)	0.31
γ -GTP, U	37 (20–68)	28 (18–42)	30 (20–53)	0.31	12 (9–15)	12 (9–18.5)	14 (9–22.3)	0.15
Group 2, n	8	49	121		8	54	127	
TC, mmol/L	5.25±1.0	5.34±1.1	5.5±1.2	0.61	5.73±0.81	6.07±0.96	5.85±0.83	0.25
TG, mmol/L	1.60±0.94	1.80±1.18	1.99±1.79	0.67	1.48±0.66	1.60±0.82	1.37±0.89	0.25
HDL-C, mmol/L	1.39±0.47	1.4±0.39	1.47±0.47	0.59	1.6±0.23	1.75±0.51	1.69±0.54	0.38
LDL-C, mmol/L	3.14±1.1	3.16±1.03	3.07±1.16	0.89	3.39±0.68	3.7±0.94	3.51±0.89	0.38
TP, g/L	74±7	75±5	76±5	0.18	76±4	76±5	76±4	0.94
GOT, U	23 (21–30)	21.5 (18–26.5)	22 (18–27)	0.46	23.5 (19.5–26)	20 (17.5–26.5)	19 (16–24)	0.10
GPT, U	23 (14–35.5)	19 (12–29.5)	20 (14–29.5)	0.63	20 (12.5–29.5)	17.5 (12–24)	14 (11–18)	0.02
γ -GTP, U	47.5 (34–87)	39 (26–61)	41 (25.3–71)	0.71	36.5 (24–101)	24.5 (17–37.5)	23 (17–32)	0.05

The results are represented by mean±SD for nonskewed data (TC, TG, HDL-C, LDL-C, and TP) and median (25th to 75th percentile) for skewed data (GOT, GPT, and γ -GTP). ANOVA and Kruskal-Wallis were performed for the nonskewed and skewed data, respectively.

*Statistically significant difference at $P<0.05$ vs Trp/Trp genotype by Fisher test.

The prevalence of abnormal ECG changes indicative of ischemic heart disease was similar among the 3 genotypes in group 1.

Biochemical Data

Plasma levels of TC and LDL-C were significantly lower in the Arg/Arg than those in the Trp/Trp in men, but not in women, from group 1 cohort by ANOVA (Table 3). To examine whether other factors confounded the results, we eliminated the subjects treated with hypolipemic drugs, yielding 21 Arg/Arg, 64 Trp/Arg, and 110 Trp/Trp men available for the analyses. In these subjects not treated with hypolipemic drugs, the TC and LDL-C levels of the Arg/Arg men were still significantly lower than those of Trp/Trp men. To determine whether the effects of the β 3-AR genotype are independent of other potential confounding factors, multivariate analyses were performed using age, BMI, and the genotype of β 3-AR as explanatory factors. BMI, but not age, was strongly associated with plasma LDL-C levels ($P<0.003$). The effects of the β 3-AR mutation remained significant ($P<0.05$). In the group 2 cohort, no significant differences were observed in the plasma levels of either TC, TG, HDL-C, or LDL-C between different genotypes, probably because the effects of the β 3-AR mutation were severely confounded by associating hyperlipidemia and hypolipemic medication, which were prevalent in this particular population.

In the group 1 cohort, the plasma level of TP was significantly higher in the Arg/Arg than in the Trp/Arg and Trp/Trp in men, but not in women, by ANOVA (Table 3).

Since the results of liver function tests exhibited highly skewed distribution, nonparametric tests were employed for statistical analyses. We found a statistically significant association between the plasma levels of γ -GTP and the Trp⁶⁴Arg mutation in women from the group 2 cohort (Table 3).

Discussion

In both group 1 and 2 populations, the frequency of the Trp⁶⁴Arg mutation is similar to that reported in other Japanese populations.^{13–17,24,25} In addition, although condensation of the His¹⁰¹Gln mutation in the α -tocopherol transfer protein gene was found in the same population, most likely due to a founder effect,²⁶ these results indicate that Japanese appear to be relatively homogeneous in terms of the mutation in the β 3-AR gene.

Since the original discovery of the Trp⁶⁴Arg mutation of the β 3-AR gene, numerous studies have addressed the problem concerning the association between this variant and obesity, NIDDM, and/or insulin resistance.^{10–25} Overall, the effects of this mutation in heterozygous form on adiposity and/or insulin resistance, if present, appear to be small. Several studies have reported the effects of this mutation in the homozygous state; 3 Arg/Arg subjects exhibited progressive weight gain in young white Danish.¹⁹ However, the number of Arg/Arg subjects employed seems too small to perform meaningful statistical analyses. In contrast, our study subjects contained a total of 67 Arg/Arg from 2 different populations, rendering our comparison meaningful. No significant difference was found in mean BMI among the subjects with 3 different genotypes in each population ana-

lyzed separately or in combination, even in the Arg/Arg homozygotes. In accordance with our results, several groups have recently published results failing to support the hypothesis that the Trp⁶⁴Arg mutation of the β 3-AR gene is associated with obesity.^{21–25}

Gagnon et al²² argued that the Trp⁶⁴Arg mutation of the β 3-AR gene may be functionally silent based on previous findings that the third intracellular domain (i3), but not i1, which contains the Trp⁶⁴ codon, is involved in both ligand-binding interactions and in receptor coupling to G proteins. They proposed that this notion is supported by the fact that cows, rats, and mice have arginine instead of tryptophan at the same position. In fact, the Trp⁶⁴Arg mutant receptor was shown to be pharmacologically and functionally indistinguishable from the wild-type β 3-AR when expressed in Chinese hamster ovary cells.²⁷ We are not in complete agreement with these arguments, because the β 3-AR gene mutation was found to be associated with other clinical factors, such as SBP and some of the laboratory data. Because obesity is a complex phenotype regulated by numerous factors, the effects of functional defects of the β 3-AR may easily be mitigated by other confounding factors, thus resulting in apparently contradictory findings.

In contrast to previous reports showing association between the β 3-AR gene mutation and early onset¹² and high prevalence of NIDDM,¹⁵ we did not find an increase in the prevalence of NIDDM either in homozygotes or in heterozygotes in either study population. One limitation of the current study is that we did not measure insulin sensitivity or fat distribution, both of which were reported to be abnormal in patients carrying the Trp⁶⁴Arg mutation.^{17,19}

There was a trend for the Arg/Arg men to have higher SBP than the Trp/Trp men (Table 1), but the difference obtained from separate analyses on whole subjects from a single cohort did not reach the level of statistical significance. Although combined analyses of group 1 and 2 revealed elevation of SBP in the Arg/Arg men (Table 2), multivariate analyses failed to demonstrate independent effects of the genotype of β 3-AR on SBP. Since the group 2 cohort had significantly lower SBP than group 1, probably due to medical intervention (Table 1), the difference in the combined analysis may result from the effects of stratification.

Several previous studies have reported associations between hypertension and the β 3-AR gene mutation in both heterozygous^{11,20} and homozygous forms.¹⁶ Functional β 3-AR was demonstrated to be present in cardiomyocytes isolated from human hearts,⁷ and β 3-AR agonists were shown to have both negative inotropic and positive chronotropic activities.²⁸ Thus, impaired action of the β 3-AR of the heart may contribute to elevation of blood pressure. Alternatively, several reports have shown that patients with the Trp⁶⁴Arg mutation of the β 3-AR gene are hyperinsulinemic. Elevation of SBP may manifest as a result of insulin resistance, as postulated by Widén et al.¹¹ Apparently, both sex and age are important in the phenotypic expression of elevated SBP. Further studies are required to clarify the precise mechanisms at work.

We found a decrease in the plasma LDL-C levels in the Arg/Arg men from group 1. These associations were ob-

served only in men, suggesting sex-specific expression of the phenotype of the mutation. The reduced lipolysis due to defective β 3-AR function limits the supply of free fatty acids to the liver, thereby decreasing the production of VLDL, which in turn reduces the LDL-C levels. The fact that men have more visceral fat, the metabolism of which is under the regulation of the β 3-AR, than women, in whom subcutaneous fat is predominant, may explain the sex difference observed. Similar association between the decreases in the plasma TG levels and the Trp⁶⁴Arg mutation has recently been reported by Kim-Motoyama et al.¹⁷

Higher plasma levels of TP and γ -GTP in the Arg/Arg subjects were also novel findings. This result may represent a real effect or a spurious chance statistical finding.

In conclusion, our current results did not support the original hypothesis that the Trp⁶⁴Arg variant of the β 3-AR is a determinant of adiposity in Japanese, even in the Arg/Arg homozygotes.

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