Association of β_3-Adrenergic Receptor Gene Polymorphism With Insulin Resistance in Japanese-American Men

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The Trp64Arg variant of the β_3-adrenergic receptor (β_3-AR) gene is relatively common in Japanese people. We hypothesized that this variant may be associated with obesity and insulin resistance when combined with a westernized lifestyle. To test this hypothesis, we investigated the relationships between the β_3-AR gene variant and obesity and insulin resistance in Japanese-American men, who are known to have a higher prevalence of type 2 diabetes mellitus (DM). The subjects were 152 Japanese-American men living in Hawaii, 83 with normal glucose tolerance (NGT), 40 with impaired glucose tolerance (IGT), and 29 with DM. The frequency of the Trp64Arg allele of the β_3-AR gene was 0.18, almost identical to that of the mainland Japanese. The prevalence of the Trp64Arg allele was 30.1% in NGT, 35.0% in IGT, and 41.4% in DM subjects (nonsignificant). The Trp64Arg variant of the β_3-AR gene showed no significant relationship with obesity or insulin resistance in NGT subjects. However, fasting and 2-hour insulin levels and insulin resistance as determined by homeostasis model assessment (HOMA) were significantly higher in IGT subjects with the Trp64Arg variant. Although indices of obesity were the same in IGT subjects with and without the Trp64Arg variant, differences in the body mass index (BMI) and percent body fat between NGT and IGT subjects were greater for individuals with the Trp64Arg variant. Thus, there is an association between the Trp64Arg variant of the β_3-AR gene and insulin resistance in Japanese-Americans with IGT.

THE β_3-adrenergic receptor (β_3-AR) is thought to be involved in thermogenesis and lipolysis in humans.[1] A high frequency of the Trp64Arg variant of the β_3-AR gene was found in Pima Indians, who are known to have the world's highest prevalence of type 2 diabetes mellitus (DM), and individuals with the Trp64Arg variant have an earlier onset of DM and a slightly lower metabolic rate.[1] An association of the Trp64Arg variant with an increasing waist to hip ratio (WHR), hypertension, insulin resistance, and hyperinsulinemia has been observed in Finns.[2] In severely obese French Caucasian subjects, weight gain from age 20 and over has been associated with the Trp64Arg variant of the β_3-AR gene.[3]

The association of the Trp64Arg variant of the β_3-AR gene and type 2 DM in Japanese subjects is not clear.[4,5] The prevalence of type 2 DM is higher in Japanese-Americans versus the Japanese living in Japan.[6-8] The frequency of the Trp64Arg allele of the β_3-AR gene is 0.18 to 0.22 in the Japanese, which is higher versus Caucasians[5,9-12] but lower versus Pima Indians. In a westernized environment, the Trp64Arg variant may be associated with increasing insulin resistance prior to the onset of DM. To investigate this hypothesis, we assessed the relationship between the Trp64Arg variant of the β_3-AR gene and obesity and insulin resistance in Japanese-American men.

SUBJECTS AND METHODS

Subjects

To investigate the association of β_3-AR gene polymorphism and insulin resistance in Japanese-American men, we studied Japanese men living on the island of Hawaii who emigrated from Hiroshima, Japan. The subjects were 152 Japanese-American men aged 66.9 ± 13.5 years (mean ± SD). The study was approved by the ethics committee of Hiroshima University School of Medicine, and all subjects provided informed consent for participation.

Analytical Methods

Physical measurements, a 75-g oral glucose tolerance test, and serum lipid determinations were performed after an overnight fast. Samples were immediately frozen and stored at −80°C. The presence of the Trp64Arg variant of the β_3-AR gene was then determined using a polymerase chain reaction–restriction fragment length polymorphism method[2,13] with DNA isolated from peripheral leukocytes and the restriction enzyme *Mva*I. The percent body fat was determined using bioelectric impedance analysis.[14-16] Immunoreactive insulin (IRI) was determined by radioimmunoassay. DM and impaired glucose tolerance (IGT) were diagnosed according to World Health Organization criteria.[17] Insulin resistance was evaluated with homeostasis model assessment (HOMA).[18,19]

Statistical Analysis

Data were analyzed using SAS Version 6.10 (SAS Institute, Cary, NC) and are shown as the mean ± SD. Mean values were compared by Student's unpaired *t* test or age-adjusted analysis of covariance (ANCOVA) as appropriate, and chi-square analysis was used to determine the significance of differences between groups.

RESULTS

The frequency of the Trp64Arg allele of the β_3-AR gene was 0.18. The distribution of Trp/Trp, Trp/Arg, and Arg/Arg genotypes was 101 (66.4%), 47 (30.9%), and four (2.6%), respectively, with frequencies similar to those reported in other studies in the Japanese.[13,20,21] Trp64Arg heterozygotes (Trp/Arg) and Trp64Arg homozygotes (Arg/Arg) were defined as the β_3-AR gene variant. Subjects with the β_3-AR gene variant had a significantly higher 2-hour IRI level after the glucose load and a higher serum triglyceride concentration (Table 1). The frequency of the β_3-AR gene variant (Arg/Arg and Trp/Arg) was 30.1%, 35.0%, and 41.4% in subjects with normal glucose tolerance ([NGT] n = 83), IGT (n = 40), and DM (n = 29), respectively, but the differences were not statistically significant (Table 2).

Table 3 shows a stratified analysis of the three glucose

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Table 1. Characteristics of the Subjects (N = 152)

Characteristic	No Variant	Variant	P*
No. of subjects	101	51	
Age (yr)	66.4 ± 14.4	67.9 ± 11.7	.485
BMI (kg/m²)	24.5 ± 3.4	24.4 ± 3.5	.854
WHR	0.88 ± 0.05	0.89 ± 0.05	.248
Body fat (%)	22.7 ± 5.9	23.5 ± 5.6	.421
Fasting glucose (mmol/L)	5.3 ± 1.0	5.6 ± 1.6	.195
2-hour glucose (mmol/L)	8.3 ± 3.8	8.6 ± 4.3	.651
F-IRI (pmol/L)	54.3 ± 31.3	69.3 ± 54.6	.075
2-hour IRI (pmol/L)	394.1 ± 258.2	553.7 ± 400.9	.012
HOMA†	1.85 ± 1.27	2.44 ± 2.03	.062
Total cholesterol (mg/dL)	219.2 ± 38.3	228.2 ± 36.5	.164
Triglyceride (mg/dL)	158.5 ± 82.7	191.8 ± 88.2	.027
HDL cholesterol (mg/dL)	52.8 ± 18.7	48.8 ± 14.2	.147
Systolic blood pressure (mm Hg)	130.5 ± 18.2	131.7 ± 13.9	.635
Diastolic blood pressure (mm Hg)	74.8 ± 9.3	74.0 ± 8.3	.595

NOTE. Data are the mean ± SD.
Abbreviation: HDL, high-density lipoprotein.
*Unpaired *t* test.
†Insulin resistance evaluated by HOMA.

tolerance groups with respect to the presence or absence of the Trp64Arg variant. In the NGT group, the presence of the Trp64Arg variant was not associated with significant differences in fasting IRI (F-IRI), 2-hour IRI, or insulin resistance (HOMA). There also were no significant differences in the body mass index (BMI), WHR, and percent body fat between individuals with and without the Trp64Arg variant. IGT subjects had a significantly higher BMI than NGT subjects both with (26.0 ± 4.9 *v* 23.0 ± 2.5 kg/m², $P = .001$) and without (25.6 ± 3.3 *v* 24.1 ± 3.3 kg/m², $P = .033$) the Trp64Arg variant, according to age-adjusted ANCOVA. The percent body fat in IGT subjects with the Trp64Arg variant was also significantly higher than that in their NGT counterparts (24.8% ± 6.3% *v* 22.0% ± 5.4%, $P = .007$). Furthermore, in IGT subjects, F-IRI ($P = .029$), 2-hour IRI ($P = .007$), and insulin resistance (HOMA, $P = .021$) were significantly higher in individuals with the Trp64Arg variant versus those without, although no differences were observed for the BMI, WHR, or percent body fat between the two groups. In subjects with DM, there were no significant differences in the indices of obesity and insulin resistance between subjects with or without the Trp64Arg variant.

DISCUSSION

Previously, we reported that the prevalence of DM among Japanese-Americans in Hawaii is 18.9%, three times higher than the prevalence of 6.2% in Japanese individuals living in Japan.[6,7] Insulin levels were significantly higher in Japanese-Americans before and after a glucose load versus mainland Japanese, even after adjustment for the BMI and blood glucose level.[7] Thus, a westernization of the lifestyle has increased the insulin resistance in Japanese-Americans, resulting in a higher prevalence of DM in this group.

Table 2. Genotype Frequency (%) of the β_3-AR in NGT, IGT, and DM Groups

	NGT		IGT		DM	
Genotype	No.	%	No.	%	No.	%
Arg/Arg + Trp/Arg	2/23	30.1*	1/13	35.0*	1/11	41.4*
Trp/Trp	58	69.9*	26	65.0*	17	58.6*

*χ^2 analysis was performed in 6 groups: $\chi^2 = 1.265$, $P = .531$.

Recently, Kim-Motoyama et al[21] and Kadowaki et al[22] found that nondiabetic Japanese subjects with Trp64Arg homozygosity have a significantly higher BMI and F-IRI and 2-hour IRI post–glucose load than subjects without the Trp64Arg variant. Furthermore, Sakane et al[13,20] reported that the Trp64Arg variant of the β_3-AR gene might be predictive of difficulty in losing body weight, reducing the WHR, and improving the glycemic control and insulin resistance in obese women with type 2 DM. However, the significance of the Trp64Arg variant in the development of type 2 DM in Japanese subjects has not been clarified. Fujisawa et al[4] reported that the mutated Arg allele and Arg/Arg genotype tended to be higher in type 2 diabetics.[4] On the other hand, Ueda et al[5] found no association between the Trp64Arg variant of the β_3-AR gene, obesity, and type 2 DM.

We found that both serum insulin and insulin resistance are significantly higher in IGT subjects with the Trp64Arg variant, even though almost all subjects were heterozygotes. These results are consistent with those of Silver et al,[23] who showed elevated 2-hour insulin levels during a 75-g glucose tolerance test in nondiabetic Mexican Americans. On the other hand, we found no significant differences for the indices of obesity in IGT subjects with and without the Trp64Arg variant, suggesting that the increase in insulin is independent of obesity. However, obesity may be necessary for the Trp64Arg variant to induce insulin resistance, because we found higher F-IRI, 2-hour IRI, and HOMA levels in obese (BMI ≥ 24.1) but not in non-obese (BMI < 24.1) nondiabetic subjects (data not shown). Garcia-Rubi et al[24] also reported that obese (BMI = 36 kg/m²) postmenopausal women who are heterozygous for the Trp64Arg variant of the β_3-AR gene showed greater insulin resistance as evaluated by the clamp technique than their obese counterparts with normal genes.

The mechanism by which the Trp64Arg variant alters insulin sensitivity is not fully elucidated. The β_3-AR gene regulates lipolysis in brown and white adipose tissue in the visceral region, and may affect insulin sensitivity by regulating serum free fatty acid levels.[25] A recent investigation reported an association between the β_3-AR gene variant, increased visceral fat mass, and increased insulin resistance.[22]

Subjects with IGT had a higher BMI, percent body fat, and WHR than those with NGT, and this tendency is more apparent for subjects with the Trp64Arg variant. Thus, visceral obesity may be one of the factors that increased insulin resistance in IGT subjects with the Trp64Arg variant of the β_3-AR gene.

We previously found that the consumption of animal fat and simple carbohydrates is 1.5 times higher in Japanese-Americans versus the Japanese in Japan, although total energy intake is almost identical between the two groups.[8] In addition to the higher influx of dietary fat and carbohydrate, the impaired lipolysis observed in adipose tissue in subjects with the β_3-AR variant may contribute to the development of obesity and insulin resistance in IGT Japanese-American men with this

Table 3. Clinical and Biochemical Characteristics of NGT, IGT, and DM Groups According to the Presence or Absence of the Trp64Arg Variant of the β_3-AR Gene

Characteristic	NGT (n = 83)			IGT (n = 40)			DM (n = 29)		
	No Variant	Variant	*P*	No Variant	Variant	*P*	No Variant	Variant	*P*
No. of subjects	58	25		26	14		17	12	
Age (yr)	63.1 ± 15.9	64.4 ± 14.3		70.8 ± 10.4	73.0 ± 6.0		70.8 ± 11.4	69.3 ± 8.5	
BMI (kg/m^2)	24.1 ± 3.3	23.0 ± 2.5	.168	25.6 ± 3.3	26.0 ± 4.9	.554	24.4 ± 3.6	25.5 ± 2.4	.388
WHR	0.87 ± 0.05	0.88 ± 0.05	.734	0.89 ± 0.04	0.90 ± 0.06	.552	0.89 ± 0.04	0.91 ± 0.05	.452
Body fat (%)	22.4 ± 6.4	22.0 ± 5.4	.914	23.3 ± 4.5	24.8 ± 6.3	.215	22.6 ± 6.4	24.9 ± 5.0	.355
Fasting glucose (mmol/L)	5.0 ± 0.5	5.0 ± 0.4	.805	5.2 ± 0.8	5.4 ± 0.7	.542	6.5 ± 1.5	7.3 ± 2.5	.347
2-hour glucose (mmol/L)	5.9 ± 1.2	5.7 ± 1.0	.432	9.0 ± 1.0	9.0 ± 1.0	.821	15.3 ± 3.1	14.3 ± 5.0	.507
F-IRI (pmol/L)	48.8 ± 25.8	51.7 ± 33.0	.602	61.0 ± 37.3	95.4 ± 69.6	.029	64.6 ± 35.9	76.1 ± 61.7	.609
2-hour IRI (pmol/L)	324.3 ± 207.4	415.4 ± 285.6	.109	490.8 ± 266.9	812.2 ± 487.2	.007	484.3 ± 332.9	540.3 ± 379.6	.698
HOMA*	1.52 ± 0.88	1.57 ± 1.01	.766	1.99 ± 1.23	3.31 ± 2.56	.021	2.77 ± 1.90	3.23 ± 2.35	.647

NOTE. Data are the mean ± SD. *P* values are from ANCOVA adjusted for age.
*Insulin resistance evaluated by HOMA.

variant compared with Japanese men. Thus, westernized eating patterns, such as an increase in fat intake, may augment insulin resistance in individuals with the Trp64Arg variant of the β_3-AR gene prior to the onset of DM.

In diabetics, F-IRI and 2-hour IRI levels tended to be lower in subjects with the Trp64Arg variant of the β_3-AR gene compared with their IGT counterparts, whereas in those without this variant, insulin levels were almost identical. Thus, the increase in insulin resistance in subjects with the β_3-AR gene variant among the IGT group does not translate as a factor in DM. This suggests the importance of an impairment of the insulin secretory capacity, one of the characteristics of the pathogenesis of DM in the Japanese.[26]

In this investigation, we analyzed the data according to stratification by glucose tolerance. However, multiple comparisons present several problems because of differences in clinical characteristics in each category of glucose tolerance and the reduced number of subjects in each group. The inconsistency of the results for the study of the association of the β_3-AR gene variant and insulin resistance may be partially due to such stratification.

In conclusion, Japanese-American men with IGT showed increased insulin resistance if they had the Trp64Arg variant of the β_3-AR gene. Further studies are needed for the comparison of IGT and DM in Japanese subjects in Japan and Japanese Americans in Hawaii, with the same category of glucose tolerance segregating heterozygotes and homozygotes for the β_3-AR gene variant to clarify if a westernized lifestyle may exaggerate the genetic susceptibility to DM in Japanese subjects.

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