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The Gln27Glu β_2-Adrenergic Receptor Variant Is Associated with Obesity Due to Subcutaneous Fat Accumulation in Japanese Men

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The Trp64Arg β3-adrenergic receptor (AR) variant is associated with visceral obesity probably due to decreased lipolysis in visceral fat (H. Kim-Motoyama et al., *Diabetologia* 40, 469–472, 1997). Functional alteration of β2AR may also change fat distribution. We investigated the influence of the Gln27Glu β2AR variant upon obesity and fat distribution. We screened 278 unrelated Japanese men and detected 249 wild-type Gln27 homozygotes, 28 Gln27/Glu27 heterozygotes, and one mutant Glu27 homozygote. The frequency of mutant Glu27 allele was significantly higher in obese subjects than in nonobese/intermediate subjects (0.11 vs 0.04, $P = 0.004$). The Gln27/Glu27 heterozygotes had a significantly higher mean age-adjusted body-mass index (BMI) and mean age-adjusted subcutaneous fat area assessed by CT scan than the wild-type homozygotes but not the mean age-adjusted visceral fat areas. In summary, we have found that in Japanese men the Gln27Glu β2AR variant is associated with obesity due to subcutaneous fat accumulation.

Obesity is the result of excess energy storage due to increased fat accumulation and/or decreased lipolysis. Lipolysis in adipose tissue is regulated by catecholamines via β1–3 adrenergic receptors (βARs). The β1 and β2 isoforms play a major role in lipolysis (1–3) and the β3 isoform, which is expressed more highly in visceral fat than in subcutaneous fat, has also lipolytic activity in adipose tissue (4). We and several investigators have previously shown that a Trp64Arg β3AR variant is associated with obesity (5–7), especially with visceral obesity (8, 9), probably due to decreased lipolysis in visceral fat (10). Recently, of the three known variants of β2AR gene that alter receptor function (Arg16Gly, Gln27Glu, and Thr164Ile) (11, 12), the Gln27Glu β2AR variant was shown to be most strongly associated with obesity (13). Functional alteration of β2AR, as well as β3AR, may also change fat distribution. We investigated the influence of the Gln27Glu β2AR variant upon obesity and fat distribution.

SUBJECT AND METHODS

We performed association studies on the Gln27Glu β2AR variant in 278 unrelated Japanese men aged 21–65 years (51.3 ± 0.5 years, mean ± SE), body-mass index (BMI) ranged 17.1-41.6 kg/m^2 (mean 24.5 ± 0.2 kg/m^2), who underwent a medical checkup in a company-based clinic. Informed consent was obtained from all the subjects studied. Presence or absence of obesity was diagnosed according to the criteria of the Japan Society for the Study of Obesity: BMI $<$ 22 kg/m^2 was considered to be nonobese, 22 kg/m^2 $\leq$ BMI $<$ 26.4 kg/m^2 to be intermediate, and BMI $\geq$ 26.4 kg/m^2 (not less than 120% of BMI 22) to be obese in Japan (14). Following a 12-h fast, plasma glucose and insulin levels during a 75-g oral glucose load, serum total cholesterol, and triglycerides were measured. The V/S ratio was defined as the ratio of visceral fat area (V) to subcutaneous fat area (S) by CT scan at the level of the umbilicus. Visceral fat area was measured over the same density area as the subcutaneous fat layer (8). Genomic DNA was obtained from peripheral blood using standard methods, and the Gln27Glu β2AR variant was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. This PCR-RFLP analysis was performed as previously described (13), except that we used LA-Taq with GC buffer (Takara Corporation, Tokyo, Japan) for optimal amplification of GC-rich fragments. A C → G substitution at nucleotide 79 of β2AR gene destroys one of the three *Ita*I sites. Genotyping was repeated for all the subjects. Because only one Glu27 homozygote was detected, we compared Gln27 homozygotes and Gln27/Glu27 heterozygotes for all

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TABLE 1

Allelic Frequency of the Gln27Glu Variant According to BMI

	Glu27 allelic frequency
Nonobese[a]/intermediate[b] subjects (BMI < 26.4)	
Gln/Gln n = 201	
Gln/Glu n = 15	0.04
Glu/Glu n = 1	
Obese[c] subjects (BMI ≥ 26.4)	
Gln/Gln n = 48	
Gln/Glu n = 13	0.11*
Glu/Glu n = 0	

Note. Nonobese[a] (BMI < 22), intermediate[b] (22 ≤ BMI < 26.4), and obese[c] (26.4 ≤ BMI) according to the criteria of Japan Society for the Study of Obesity (Ref. 14).

* P = 0.004 vs. nonobese/intermediate subjects.

analysis. The chi-squared test was used to compare allelic frequencies. Analysis of covariance (ANCOVA) was used to estimate the differences in variables between the Gln27 homozygotes and the Gln27/Glu27 heterozygotes, with results presented as least-squares means ± SE. SAS software version 6.12 (SAS Institute Inc., Cary, NC) was used.

RESULTS

Allelic frequency of the Gln27Glu β2AR variant according to BMI in Japanese men. We detected 249 Gln27 homozygotes, 28 Gln27/Glu27 heterozygotes, and one Glu27 homozygote in the studied subjects. The allelic frequency of the Gln27Glu β2AR variant was 30/556 = 0.05. The frequency of the mutant Glu27 allele was significantly higher in obese subjects (26.4 ≤ BMI) than in nonobese/intermediate subjects (BMI < 26.4); 0.11 vs. 0.04, respectively (P = 0.004) (Table 1).

The Gln27Glu β2AR variant and obesity and fat distribution. The Gln27/Glu27 heterozygotes had a significantly higher mean BMI than the wild-type Gln27 homozygotes (mean BMI 26.5 ± 1.0 kg/m^2 vs. 24.3 ± 0.2 kg/m^2; P = 0.001) (Table 2). Furthermore, the Gln27/Glu27 heterozygotes had a significantly higher mean subcutaneous fat area (S) than the Gln27 homozygotes (mean S 17,636 ± 2,686 mm^2 vs. 12,807 ± 410 mm^2; P = 0.002). There was no difference between the means of visceral fat area (V) of those with the two genotypes. Thus the Gln27/Glu27 heterozygotes had a lower mean V/S ratio than the Gln27 homozygotes (mean V/S ratio 0.60 ± 0.05 vs. 0.73 ± 0.02; P = 0.023) (Table 2).

The Gln27 homozygotes were aged 51.8 ± 0.5 years and the Gln27/Glu27 heterozygotes were 46.7 ± 2.0 years (P = 0.004) (Table 2). To adjust for the age difference between the two groups, we further compared age-adjusted BMI, S, and V and there were still significant differences between age-adjusted BMI and S (Table 3). The age-adjusted BMIs of Gln27 homozygotes and Gln27/Glu27 heterozygotes were 24.4 ± 0.2 kg/m^2 and 25.8 ± 0.6 kg/m^2 (P = 0.02) and the age-adjusted S were 12,995 ± 437 mm^2 and 15,936 ± 1331 mm^2, respectively (P = 0.04). The age-adjusted V and V/S ratio were not different (Table 3). In the obese subgroup (BMI ≥ 26.4), mean age-adjusted S, V, and V/S ratio of the Gln27 homozygotes (n = 48) and the Gln27/Glu27 heterozygotes (n = 13) were 20,745 ± 1,382 mm^2, 26,206 ± 2,702 mm^2 (P = 0.08); 12,112 ± 627 mm^2, 12,624 ± 1,228 mm^2 (P = 0.71); and 0.63 ± 0.03, 0.54 ± 0.06 (P = 0.15), respectively.

TABLE 2

Characteristics of Subjects by the Gln27Glu β2AR Genotype

	Gln27 homozygote	Gln27/Glu27 heterozygote	P values
n	249	28	
Age (years)	51.8 ± 0.5	46.7 ± 2.0	0.004
BMI (kg/m^2)	24.3 ± 0.2	26.5 ± 1.0	0.001
S (mm^2)	12807 ± 410	17636 ± 2686	0.002
V (mm^2)	8862 ± 266	9068 ± 1023	0.81
V/S ratio	0.73 ± 0.02	0.60 ± 0.05	0.023
Fasting blood glucose (mmol/l)	5.9 ± 0.04	5.9 ± 0.2	0.81
2 hr blood glucose (mmol/l)	7.5 ± 0.2	8.5 ± 0.6	0.08
Fasting insulin (pmol/l)	41.6 ± 1.1	62.9 ± 15.7	0.13
2 hr insulin (pmol/l)	204.8 ± 8.9	254.8 ± 39.1	0.22
Total cholesterol (mmol/l)	5.5 ± 0.1	5.5 ± 0.2	0.94
Triglycerides (mmol/l)	1.5 ± 0.06	2.0 ± 0.4	0.20
Systolic blood pressure (mmHg)	125.5 ± 1.1	124.3 ± 3.7	0.72
Diastolic blood pressure (mmHg)	81.2 ± 0.7	79.5 ± 2.0	0.47

Note. Data are means ± SE.

The Gln27Glu β2AR variant and blood glucose, plasma insulin, lipid levels, and blood pressures. There were no significant differences between the Gln27 homozygotes and the Gln27/Glu27 heterozygotes in the levels of fasting blood glucose, blood glucose 2 hours after oral glucose load, fasting plasma insulin, plasma insulin 2 hours after oral glucose load, total cholesterol, triglycerides, and systolic or diastolic blood pressure (Table 2).

TABLE 3

Age-Adjusted BMI, S, V, and V/S Ratio According to βAR Gln27Glu Genotype

	Gln27 homozygote	Gln27/Glu27 heterozygote	P values
n	249	28	
BMI (kg/m^2)	24.4 ± 0.2	25.8 ± 0.6	0.02
S (mm^2)	12995 ± 437	15936 ± 1331	0.04
V (mm^2)	8884 ± 274	8873 ± 836	0.99
V/S ratio	0.72 ± 0.02	0.64 ± 0.05	0.13

Note. Data are means ± SE.

DISCUSSION

We screened for the β2AR Gln27Glu variant in Japanese men and found that the frequency of the mutant Glu27 allele was significantly higher in obese subjects than in nonobese/intermediate subjects (Table 1). Furthermore, the Gln27/Glu27 heterozygotes had a higher BMI and subcutaneous fat area than wild-type homozygotes (Tables 2 and 3). In the obese subgroup, there was a trend toward higher mean subcutaneous fat area of the Gln27/Glu27 heterozygotes than that of the Gln27 homozygotes.

We did not investigate the effect of Glu27 homozygosity because we detected only one Glu27 homozygote. He was 53 years old, had a BMI of 22.1 kg/m^2, an S of 16,231 mm^2, a V of 7,652 mm^2, a V/S ratio of 0.47, and was normoglycemic and normolipidemic. Large and coworkers demonstrated that in Swedish women Glu27 homozygotes had a higher BMI than Gln27 homozygotes (13). They also showed that the difference in BMI between Gln27/Glu27 heterozygotes and wild-type homozygotes was not significant (13). They studied female subjects and our study investigated only male subjects. There may be a gender or ethnic difference in the effect of the Gln27/Glu27 heterozygous variant.

We did not investigate young subjects. Eckwald and co-workers demonstrated that in early-onset obese Danish men, who were around the age of 20 years, the Gln27Glu β2AR variant was not associated with higher BMI (15). They investigated only BMI, although we directly assessed the association of the Gln27Glu variant with fat distribution by CT scan, in addition to BMI.

Our findings suggest that the Gln27Glu variant contributes to the genetic susceptibility to fat accumulation in subcutaneous adipose tissue and obesity in Japanese men. These results raise the possibility that the Gln27Glu β2AR variant and/or another variant in linkage disequilibrium may affect receptor function and decreased lipolysis especially in subcutaneous fat tissue, where β2AR plays a major role in lipolysis (1–3). In contrast to the Trp64Arg β3AR variant which associates with visceral fat increase (8, 9), the Gln27Glu β2AR variant associates with subcutaneous fat accumulation. In visceral fat tissue, where β3AR plays a pivotal role in lypolysis (16), the effect of the Gln27Glu variant of β2AR may have been obscure.

In summary, we performed an association study in Japanese men and found that the Gln27Glu β2AR variant is significantly more frequent in the obese subgroup than in the nonobese subgroup and is associated with a higher BMI and subcutaneous fat area. These results suggest the possibility that the β2AR locus is one of the genetic factors that influence susceptibility to obesity, especially due to accumulation of subcutaneous fat tissue in humans.

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REFERENCES

1. Arner, P. (1995) *Ann. Med.* **27,** 435–438.
2. Enocksson, S., Shimizu, M., Lönnqvist, F., Nordenström, J., and Arner, P. (1995) *J. Clin. Invest.* **95,** 2239–2245.
3. Barbe, P., Millet, L., Galitzky, J., Lafontan, M., and Berlan, M. (1996) *Br. J. Pharmacol.* **117,** 907–913.
4. Krief, S., Lönnqvist, F., Raimbault, S., Baude, B., Spronsen, A. V., Arner, P., Strosberg, A. D., Ricquier, D., and Emorine, L. J. (1993) *J. Clin. Invest.* **91,** 344–349.
5. Walston, J., Silver, K., Bogardus, C., Knowler, W. C., Celi, F. S., Austin, S., Manning, B., Strosberg, A. D., Stern, M. P., Raben, N., Sorkin, J. D., Roth, J., and Shuldiner, A. R. (1995) *N. Engl. J. Med.* **333,** 343–347.
6. Kadowaki, H., Yasuda, K., Iwamoto, K., Otabe, S., Shimokawa, K., Silver, K., Walston, J., Yoshinaga, H., Kosaka, K., Yamada, N., Saito, Y., Hagura, R., Akanuma, Y., Shuldiner, A. R., Yazaki, Y., and Kadowaki, T. (1995) *Biochem. Biophys. Res. Commun.* **215,** 555–560.
7. Yoshida, T., Sakane, N., Umekawa, T., Sakai, M., Takahashi, T., and Kondo, M. (1995) *Lancet* **346,** 1433–1434.
8. Kim-Motoyama, H., Yasuda, K., Yamaguchi, T., Yamada, N., Katakura, T., Shuldiner, A. R., Akanuma, Y., Ohashi, Y., Yazaki, Y., and Kadowaki, T. (1997) *Diabetologia* **40,** 469–472.
9. Sakane, N., Yoshida, T., Umekawa, T., Kogure, A., Takakura, Y., and Kondo, M. (1997) *Diabetes Care* **20,** 1887–1890.
10. Umekawa, T., Yoshida, T., Sakane, N., Kogure, A., Kondo, M., and Honjyo, H. (1999) *Diabetes* **48,** 117–120.
11. Green, S. A., Turki, J. M., Innis, M., and Liggett, S. B. (1994) *Biochemistry* **33,** 9414–9419.
12. Green, S. A., Cole, G., Jacinto, M., Innis, M., and Liggett, S. B. (1993) *J. Biol. Chem.* **268,** 23116–23121.
13. Large, V., Hellström, L., Reynisdottir, S., Lönnqvist, F., Eriksson, P., Lannfelt, L., and Arner, P. (1997) *J. Clin. Invest.* **100,** 3005–3013.
14. Guideline for diagnosis, treatment and patient education of obesity edited by Japan Society for the Study of Obesity (1993) Ishiyaku-Shuppan, Japan (in Japanese)
15. Eckwald, S. M., Sørensen, T. I. A., Tybjærg-Hansen, A., Andersen, T., and Pederson, O. (1998) *Diabetes* **47,** 1657–1658.
16. Lönnqvist, F., Thörne, A., Nilsell, K., Hoffstedt, J., and Arner, P. (1995) *J. Clin. Invest.* **95,** 1109–1116.