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The Combined Effect of β2- and β3-Adrenergic Receptor Genotypes on Hyperglycemic Risk in the Korean Population

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Abstract

Adrenergic receptors play a major role in thermogenesis and lipolysis in brown and visceral adipose tissues, and have been implicated in the pathogenesis of obesity and metabolic disorders. The purpose of this study was to estimate the effects of β2-adrenergic receptor (β2AR) and β3-adrenergic receptor (β3AR) genotypes on hyperglycemia and obesity in the Korean population. A representative sample consisting of 530 Korean men and women were measured for height, weight, BMI, WHR, obesity index and body composition. The genotypes of β2AR polymorphism in codon 27 and β3AR polymorphism in codon 64 were analyzed by the PCR RFLP method. Serum concentrations of fasting glucose, total cholesterol, HDL cholesterol and triglyceride were determined. The frequencies of β2AR and β3AR genotype were: both wild type, 62.5%; only β2AR variant type, 12.8%; only β3AR variant type, 18.8%; and both variant type, 5.8%; the frequency of E and R alleles were 0.098 and 0.137, respectively. Among the physiological parameters, fasting glucose level was significantly higher in subjects with both variant type compared with the three other types ($p<0.05$). Subjects with both variant type had 12%, 12% and 9.3% increases in serum glucose levels compared with wild type, only β2AR variant type, and only β3AR variant type, respectively. When logistic regression analysis was conducted to estimate the risk for hyperglycemia, the subjects were selected for fasting blood glucose concentrations of more than 6.105 mmol/L (110 mg/dL), and the odds ratios were 1.215 (p=0.636) for only β2AR variant type, 1.659 (p=0.089) for only β3AR variant type, and 3.078 (p=0.011) for both variant type. These results suggest that the interaction of β2AR and β3AR variant genotypes has a strong association with increased glucose levels, and might be a significant risk factor for hyperglycemia among Korean subjects.

Key words: β2-adrenergic receptor, β3-adrenergic receptor, polymorphism, hyperglycemia, obesity

INTRODUCTION

Obesity and hyperglycemia are closely related to metabolic disorders with etiologies that involve the combined effects of genetic and environmental factors (1,2). Genes involved in the regulation of catecholamine function may be of particular importance for human obesity (3-5). The β2 and 3-adrenergic receptors (β2AR and β3AR), which are predominantly expressed in brown and visceral adipose tissues in humans, are involved in the regulation of lipolysis and thermogenesis (3-6). A high level of visceral fat deposition is a well known, important risk factor for insulin resistance and abnormal blood glucose levels (7,8).

Many studies have reported that β2AR and β3AR genetic polymorphisms are involved in obesity and related metabolic disorders (3,6,9-17). Several polymorphisms of β2AR gene have been found in the humans, including missense mutations resulting in the replacement of arginine by glycine at position 16 (Arg16Gly), glutamine by glutamate at position 27 (Gln27Glu), and threonine by isoleucine at position 164 (Thr164Ile) (17-19). Large et al. (20) reported that the Gln27Glu polymorphism was associated with obesity, and that subjects with Gln27Glu genotype had an average fat mass that was 20 kg higher than controls. The frequency of homozygocity for Glu27 was almost seven-times higher in obese females than in non-obese (24.4 vs 3.4%) (20). Recent studies in Pima Indians, Finns, and Caucasians have shown that a missense mutation in the β3AR gene, resulting in the replacement of tryptophan by arginine at position 64 (Trp64Arg), is associated with earlier onset of type 2 diabetes, and decreased resting metabolic rate, insulin resistance, and weight gain (9,10,21,22). However, there is still considerable debate on the association with obesity or hyperglycemia in the Korean population, and

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much research remains to be conducted, particularly considering ethnic differences and environmental factors. Therefore, the purpose of the present study was to estimate the interaction effect of β2AR and β3AR gene polymorphisms on the risk of hyperglycemia and obesity in the Korean population.

MATERIALS AND METHODS

Subjects

All subjects were recruited among patients at a weight loss program at Kirin Oriental Medical Hospital (Seoul, Korea). A total of 530 Korean subjects, 38 men and 492 women were recruited. The physical and biochemical characteristics of the subjects are shown in Table 1. Genomic DNA was obtained with informed consent. The study subjects were divided into 4 groups by genotypes at β2AR and β3AR gene loci; wild type (QQ+WW), only β2AR variant (QE/EE+WW), only β3AR variant (QQ+WR/RR) and both variant type (QE/EE+WR/RR). Obesity was defined as a BMI greater than 27, and hyperglycemia as fasting serum glucose greater than 6.105 mmol/L (110 mg/dL) (23). All clinical data were obtained before starting a weight loss program. Blood pressure, height, weight, waist and hip circumference were measured. BMI was calculated as weight (kg) divided by squared height (m), and WHR was calculated waist circumference divided by hip circumference. The obesity index was calculated as [obesity index = real weight/ideal weight × 100, ideal weight = (height-100) × 0.9]. Body compositions were measured by bio-impedance analysis (Inbody 2.0, Biospace Co., Seoul, Korea).

Genotyping of the β2AR and β3AR variants

Genomic DNA was extracted from whole blood and subjected to PCR-RFLP to amplify a genomic DNA fragment containing codon 16 of β2AR and codon 64 of β3AR genomic DNA. Upstream primer of β2AR genomic DNA (5′GGCCCATGACCAGATCAGCA3′), and downstream primer (5′GAATGAGGCTTCCAGGCGTG3′) was used for amplification with 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The amplified PCR product was checked for correct size of 353bp by electrophoresis in a 2.5% agarose gel. The PCR product was subsequently digested with the enzyme Fnu4H for 2 h at 37°C. The resulting band patterns were 174, 97, 55, 27bp for Gln27 (QQ), 229, 174, 97, 55, 27bp for Gln27Glu (QE), 229, 97, 27bp for Glu27 the (EE) genotype. Upstream primer of β3AR genomic DNA (5′CCAGTGGGCTGCCAGGGG3′), downstream primer (5′GCCAGTGGCGCCCACGG3′), the amplification protocol consisted of 35 cycles of denaturation at 96°C for 40 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 30 seconds. The amplified PCR product was checked for correct size of 248bp by electrophoresis in a 3% agarose gel. The PCR product was subsequently digested with the enzyme BstNI for 1 h at 60°C. The resulting band patterns were 97, 64, 61, 15, 11bp for Trp64 (WW), 158, 97, 64, 61, 15, 11bp for Trp64Arg (WR), 158, 61, 15, 11bp for Arg64 (RR) genotype.

Table 1. Clinical characteristics of the subjects (n=530)

	Total subject
Sex	M 38, F 492
Age (years)	26.55±0.31[1)]
Weight (kg)	67.92±0.65
BMI (kg/m^2)	26.05±0.21
SBP (mmHg)	116.07±0.64
DBP (mmHg)	71.70±0.51
GOT (IU/L)	22.70±0.83
GPT (IU/L)	28.41±1.32

[1)]Mean±SE.

Serum biochemical analysis

Blood samples were obtained after fasting overnight for more than 12 h and centrifuged at 2,000 rpm for 30 min. Concentrations of fasting glucose, total and HDL cholesterol, triglyceride, GOT, GPT and total bilirubin were determined on an auto-biochemical analyzer (SPOTCHEM, ARKRAY, Koto, Japan). LDL cholesterol was calculated using the Friedewald equation [LDL = TC- HDL-TG/5].

Statistical analysis

All values are expressed as mean±SE. Age and sex adjusted univariate analysis of variance was performed using GLM (general linear model) procedure to examine the independent effect of the β2AR or β3AR genotype on dependent variables. The Fishers exact (x^2) test was used to compare frequencies of β2AR or β3AR genotype between groups. Risk factors of hyperglycemia were analyzed by logistic regression analysis. Statistical significance was established at the level of $p<0.05$. All analyses were performed using SPSS (ver. 10.0).

RESULTS AND DISSCUTION

Frequencies of β2AR and β3AR genotype

The frequencies of β2AR genotype were: QQ type, 81.3%; QE type, 17.9%; and EE type, 0.8%; the frequency of E allele was 0.098. The frequencies of β3AR genotypes were: WW type, 75.3%; WR type, 22.1%; and RR type, 2.6%; the frequency of R allele was 0.137. The combined frequencies of β2-, β3AR genotypes among all subjects were wild type, 62.5%; only 2AR variant type, 12.8%; only β3AR variant type, 18.9%; and both variant type, 5.8% (Table 2).

Table 2. Distribution of genotypes defined by the polymorphisms of β2- and β3-adrenergic receptor genes in the Korean population

		Genotype of β3AR		Total
		WW type	WR/RR type	
Genotype of β2AR	QQ type	331[1)] (62.5%)	100 (18.9%)	431 (81.3%)
	QE/EE type	68 (12.8%)	31 (5.8%)	99 (18.7%)
	Total	399 (75.3%)	131 (24.7%)	530 (100.0%)

[1)] *n*, Number of subjects.

Among the 270 obese subjects with fasting glucose concentrations 6.105 mmol/L, the frequencies of QE/EE type were 20.0% in the obese subjects and 0.4% in the normal weight subjects ($p = 0.274$ by x^2-analysis, data not shown). Among the 104 hyperglycemic subjects with fasting glucose concentrations 6.105 mmol/L, the frequencies of WR/RR type were 34.6% in the hyperglycemic subjects and 3.1% in the normoglycemic subjects ($p = 0.007$ by x^2-analysis, data not shown). Fig. 1 shows the distribution of the β2AR and β3AR genotypes among hyperglycemic (n = 104) and normoglycemic (n = 386) subjects. The frequency distributions were significant different by x^2-analysis ($p = 0.007$). The frequency of both variant type was 10.6% in hyperglycemic subjects, and 4.9% in normoglycemic subjects, 116.3% higher in the hyperglycemic compared with normoglycemic subjects.

In a study by Hong et al. (24), the frequencies of β2AR genotypes were: 84.3% QQ type, and 15.7% QE type among Korean women. Although the QE allele was carried by 20.9% of the normal weight subjects and 23.9% in the obese subjects, the QE, EE alleles were carried by 14.5% and 0% of the normal weight subjects with diabetes and 22.7% and 2.3% of the obese subjects with diabetes among Korean population (25). Mori et al. (17) reported that QE, EE alleles occurred in 6.9%, 0.5% of normal weight subjects and 21.3%, 0% of obese subjects among 278 Japanese male subjects. The frequency of the R allele of β3AR found in this study was similar to the 0.185 or 0.195 in the Japanese population (26,27), higher than the 0.065 or 0.085 frequency in the Caucasian population (28,29) and lower than the 0.315 frequency in Pima Indians (3). Ahn et al. (30) reported that the frequency of subjects with the variant types is significantly higher in obese subjects with type 2 diabetes patients than in non-obese type 2 diabetes patients, and suggested that the β3AR genotype might be associated with obesity related type 2 diabetes.

Therefore, variant types of β2AR are more frequent in subjects with metabolic disorders such as obesity and diabetes than in normal subjects in East-Asian populations; variant types of β2AR occurred less frequently in this study than in other Korean population studies of obese subjects. In this study, variant types of β3AR occurred more frequently among hyperglycemic subjects than among normoglycenic subjects.

Interaction effects of β2AR and β3AR genotypes on physical and biochemical characteristics

Table 3 shows the physical and biochemical characteristics of the subjects according to the β2AR and β3AR genotypes. Weight and obesity index were significantly higher only β2AR variant type compared with wild type ($p < 0.05$). WHR and body fat mass were significantly higher only β2AR variant type, or both variant type compared with wild type ($p < 0.05$). Fasting glucose concentrations were significantly higher subjects with both variant type compared with the three other types ($p < 0.05$). Subjects with variant alleles in both β2AR and β3AR genes had 12%, 12% and 9.3% increased serum glucose levels compared to those with wild type or only β2AR or β3AR variant types, respectively. LDL cholesterol was significantly higher in only β3AR variant type compared with three other groups, and HDL cholesterol was significantly lower in only β2AR variant type compared with wild type.

BMI, body fat mass and WHR were significantly higher in subjects who were homozygous for the variant type of the β2AR gene than those with the wild type (18-20). Mori et al. (17) also suggested that the β2AR

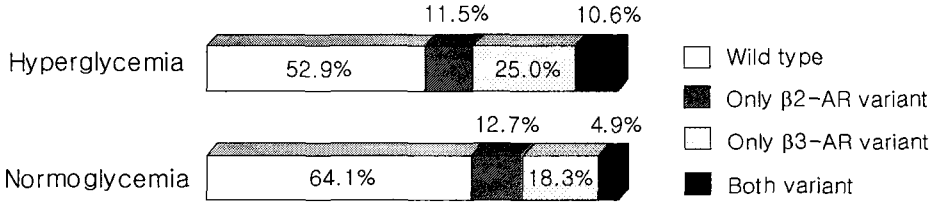

Fig. 1. Distribution of combined genotypes of the β2- and β3-adrenergic receptor genes among subjects with hyperglycemia (n=104) and normoglycemia (n=386). Hyperglycemia was defined as blood glucose concentration higher than 6.105 mmol/L.

Table 3. Comparison of physical and serum-biochemical characteristics by combined genotypes of β2- and β3-adrenergic receptor genes in the Korean population

	Combined genotype of the β2AR and β3AR gene			
	Wild type	Only β2AR variant	Only β3AR variant	Both variant
No (M / F)	331 (24/307)	68 (7/61)	100 (6/94)	31 (1/30)
Age (years)	26.7±0.4	25.4±0.9	27.4±0.8	24.9±0.9
Physical characteristics				
Weight (kg)	66.96±0.73[1)a2)]	71.92±2.25[b]	67.43±1.41[ab]	70.97±3.98[b]
BMI (kg/m^2)	25.70±0.25[a]	27.33±0.63[b]	26.21±0.44[ab]	26.40±1.28[ab]
Obesity index (%)	122.69±1.08[a]	129.75±2.72[b]	126.55±2.16[ab]	128.00±5.20[ab]
WHR	0.87±0.01[a]	0.90±0.01[b]	0.89±0.01[ab]	0.90±0.02[b]
Body fat mass (kg)	22.91±0.43[a]	25.60±1.17[b]	23.86±0.82[ab]	25.97±2.19[b]
Serum-biochemical characteristics				
Glucose (mmol/L)	5.58±0.05[a]	5.55±0.17[a]	5.71±0.07[a]	6.24±0.36[b]
TC (mmol/L)	4.63±0.04	4.40±0.10	4.77±0.09	4.51±0.13
LDL (mmol/L)	2.74±0.04[a]	2.68±0.09[a]	2.95±0.07[b]	2.65±0.11[a]
HDL (mmol/L)	1.32±0.02[b]	1.18±0.04[a]	1.26±0.04[ab]	1.27±0.07[ab]
TG (mmol/L)	1.25±0.03	1.21±0.09	1.30±0.07	1.30±0.11

[1)]Mean±SE.
[2)]Values were for comparisons of 4 groups by GLM (covariance) analysis adjusted for age and sex at the significance level of $p<0.05$.

variant genotype contributes to genetic susceptibility to fat accumulation in subcutaneous adipose tissue and obesity in Japanese men. On the other hand, fasting blood glucose concentrations, serum lipid profiles and visceral fat area by CT scan showed no difference by β2AR genotype. The β2AR variant genotype was associated with subcutaneous fat accumulation, in contrast to the β3AR variant genotype which was associated with visceral fat increase (31-33). Non-diabetic Japanese subjects with β3AR variant genotype have significantly higher BMI, fasting insulin levels, and 2 hr postprandial insulin levels compared with those with the wild type of the β3AR gene; and the β3AR variant genotype is associated with visceral obesity and low triglyceride, suggesting a suppressive effect on triglyceride levels via decreased lipolysis and free fatty acid-stimulated hepatic triglyceride production (31,33). A study in 152 Japanese-American men indicated that triglyceride and 2 hr postprandial insulin concentrations were increased in the β3AR variant genotype (34). Non-diabetic Mexican Americans with the β3AR variant genotype also had elevated 2 hr insulin concentrations during a 75 g glucose tolerance test. Many researchers have reported that fasting glucose, 2-hour glucose, fasting insulin and 2-hour insulin levels were significantly higher only β3AR variant type compared with wild type (33-35), indicating that variant types of the β3AR gene may contribute to a worsening insulin resistance and thereby hyperglycemia. The Trp64Arg polymorphism of the β3AR gene, associated with lower lipolytic activities, has been studied in relation to obesity, insulin resistance and hyperglycemia in many populations such as Pima Indians, Caucasians and Japanese; the results of these studies suggest that the Trp64Arg polymorphism might play a role in the pathogenesis of metabolic disorders by impairing lipolysis, thermogenesis and insulin sensitivity. Although significant associations between β3AR genotype and metabolic disorders have been reported in several studies, many controversies persist. No effects of the β3AR gene mutation on BMI, WHR and body fat mass were found in the Thai population (36) or in the Pima Indians (37). Another study conducted among Finns also found no differences in BMI among subjects with different β3AR genotypes (21). The association between insulin resistance and β3AR polymorphisms has been controversial as well. Neither fasting glucose nor glucose × fasting insulin levels were affected by β3AR genotype in Japanese subjects, and a study in Finns also found no difference in basal or post-glucose insulin concentrations (21,36). In addition, there are other studies where the Trp64Arg polymorphism of the β3AR gene does not appear to be associated with obesity (38,39). These data suggest the possibility that the effects of β3AR polymorphism on the obesity involve complex interactions with other risk factors such as age, gender, or ethnicity, rather than being an independent risk factor for differences in weight, BMI, WHR and body fat. In other words, obesity and related metabolic disorders may be determined by environmental and life style factors as well as genetic factors in which many candidate genes and their interactions may be involved.

The β2AR genotype has significant effects on obesity phenotypes, but, β3AR genotype has no effect; and no combined effects of β2AR and β3AR genotypes were

observed. β2AR and β3AR genotypes have no effects on blood glucose; however, a significant additive effect of β2AR and β3AR genotypes was observed. Therefore, the β2AR genotype had a very strong association with obesity, and the β3AR genotype was associated with increased TG and LDL cholesterol. The combination of β2AR and β3AR variant genotypes was associated with increased serum glucose concentrations.

Interaction effects of β2AR and, β3AR genotypes on obese and hyperglycemic subjects

Among the 178 obese subjects in the study, those with variant alleles of both β2AR and β3AR had higher weight, BMI, WHR and body fat mass compared with those with the wild types and just only β3AR variant type ($p<0.05$) (Fig. 2 upper panel). Among the hyperglycemic subjects, weight, BMI, WHR and body fat mass were significantly higher both variant type compared with wild type ($p<0.05$) (Fig. 2 lower panel). These results clearly demonstrate that obesity is a common phenotype among Koreans with variant types of both β2AR and β3AR genes, especially among hyperglycemic subjects.

Obese subjects with only β3AR variant type have been shown to have higher WHR and visceral fat mass, but decreased plasma free fatty acid and TG levels, than wild type (33). The authors suggested that the β3AR genotype was more associated with fat distribution than

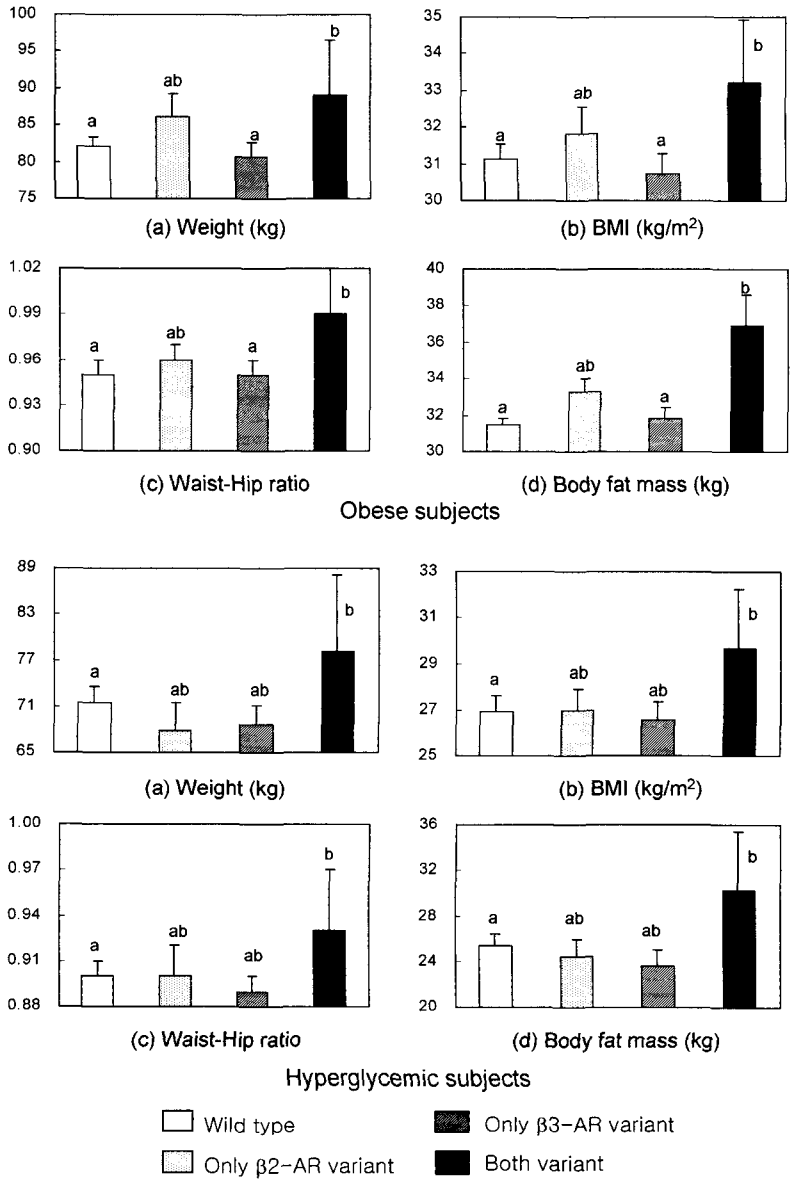

Fig. 2. Comparison of physical characteristics by combined genotype of the β2- and β3-adrenergic receptor gene in obese subjects (n=178) and in hyperglycemic subjects (n=104).
[a,b]Values were for comparisons of 4 groups by GLM (covariance) analysis adjusted for age and sex at the significance level of $p<0.05$. Mean ± SE.

with weight gain. The visceral fat mass was correlated with blood glucose concentrations, insulin resistance, and free fatty acid or triglyceride levels. Non-diabetic Japanese subjects with the β3AR variant genotype had significantly higher BMI values than subjects with the wild type of the β3AR gene; that study showed that the β3AR variant genotype is associated with visceral obesity and low triglyceride levels, via decreased lipolysis combined with free fatty acid stimulated hepatic triglyceride production (31). In a study of obese Pima Indians, there was no statistically significant effect of the β3AR genotype on the rate of free fatty acid appearance in plasma or the rate of fasting lipid oxidation (33,40).

Combined variant type of β2AR and β3AR by genetic risk factors on the hyperglycemia

Logistic regression analysis was used to estimate the genetic risk factors for hyperglycemia in Korean subjects (Table 4). The odds ratios for hyperglycemia were 1.215 (p=0.636) for only β2AR variant type, 1.659 (p=0.089) for only β3AR variant type, and 3.078 (p=0.011) for both variant type. This result suggests that with variant types of both β2AR and β3AR genes can be a significant risk factor for hyperglycemia in Koreans.

In this study, significant interactions were observed between β2AR and β3AR genotypes in relation to hyperglycemia and obesity among Korean subjects. Among the total of 530 subjects, serum glucose level was significantly higher both variant types compared with wild type or single variant types (Table 3). For obesity markers such as weight, BMI, obesity index and WHR, the β2AR variant was an independent risk factor, but the β3AR variant was not (Table 3). However, in 178 obese subjects or in 104 hyperglycemic subjects, the effect of with both variants was significant (Fig. 2). Dionne et al. (28) also reported that fat mass was significantly higher both variant types compared with only β3AR variant type, suggesting an interaction effect of β2AR and β3AR types on body fatness. When we tried a forward stepwise regression analysis to create a model for predictors of genetic risk factor for hyperglycemic change in the Korean subjects, the odds ratios for hyperglycemic change were significantly higher 3.078 for with both variant types compared with only β2AR or only β3AR variant type (Table 4). The lipolytic effects of catecholamine in adipose tissue are mediated by members of adrenergic receptors, so variant type of β2AR and β3AR gene can regulate energy metabolism and lipolysis by oxidation of endogenous triglyceride in the smooth muscle and visceral and brown adipose tissue (2-6). Therefore, variant types of β2AR and β3AR genes can interfere with lipolysis in adipose tissue and in the Korean population, who has higher frequency of R allele than frequency of E allele, lead to pathogenesis of obesity and related metabolic disorders, including hyperglycemia.

Table 4. Logistic regression of hyperglycemia by variants of the β2- and β3-adrenergic receptor genes

Variable	β	p-value	Odds ratio
Variant of β2AR gene	0.195	0.636	1.215
Variant of β3AR gene	0.506	0.089	1.659
Variant of both genes	1.124	0.011	3.078

Hyperglycemia was defined by fasting blood glucose level more than 6.105 mmol/L (110 mg/dL).

In conclusion, with the variant type (QE/EE) β2AR allele was significantly associated with obesity markers such as weight, WHR and body fat mass. The variant type (WR/RR) β3AR allele was significantly associated with serum lipid disruptions such as increased LDL and triglyceride. However, each variant type of β2AR and β3AR was significantly associated with obesity markers in the obese and hyperglycemia of Korean subjects. Also, a strong association was found between the interaction of the β2AR and β3AR genotypes and both fasting blood glucose level, frequency of hyperglycemia. Therefore, combined effect of with the β2AR and β3AR genotypes can be a genetic risk factor for hyperglycemia in the Korean population.

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