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Effects of the β3-Adrenergic Receptor Genotype on Hyperglycemic Risk Among Korean Women

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The β3-adrenergic receptor (β3AR) plays a major role in thermogenesis and lipolysis in brown and visceral adipose tissue, and has been implicated in the pathogenesis of obesity and metabolic disorders. The purpose of this study was to estimate the effects of β3AR gene polymorphism on the risk of hyperglycemia in 980 Korean women who attended a weight loss program in a local clinic. Each subject's height, weight, BMI, WHR, obesity index and body composition were measured. The genotype of the β3AR gene in codon 64 was analyzed by the PCR RFLP method. Serum concentrations of fasting glucose, of total and HDL cholesterol, and of TG were determined. Genotype distributions were as follows : 67% WW type, 31% WR type, and 2% RR type. Among the many measured parameters, fasting glucose levels were significantly higher in the WR/RR type compared with the WW type ($p=0.011$). When the subjects were divided into two groups by a fasting blood glucose level higher or lower than 6.105mmol/L (110mg/dl), the frequency of hyperglycemia showed a significant difference in relation to β3AR genotype as measured by x^2-analysis ($p=0.014$); the frequency of hyperglycemia was significantly higher (at 24.8%) in WR/RR type subjects, compared to 18.2% in WW type subjects. When all of the measured parameters were included in stepwise logistic regression analyses to find the risk factors for hyperglycemia, the odds ratios for hyperglycemia were 1.573 ($p=0.011$) for the WR/RR type of the β3AR gene, 1.053 ($p=0.001$) for TG, 1.044 ($p=0.037$) for BMI, and 1.026 for age ($p=0.031$). These data suggest that the WR/RR genotype of the β3AR has a very strong association with increased blood glucose level and might be a significant risk factor for hyperglycemia among Korean women.

Key words : β3-adrenergic receptor, polymorphism, hyperglycemia, fasting blood glucose, BMI, Korean women

INTRODUCTION

Obesity and related metabolic disorders have been shown to be determined by the combined effects of genetic and environmental factors.[1),2)] A high level of visceral fat deposition is a well known high risk factor for insulin resistance and abnormal blood glucose levels.[3),4)] The β3-adrenergic receptor (β3AR), which plays a key role in regulating the energy balance through the stimulation of thermogenesis and lipolysis, is predominantly expressed in brown and white adipose tissue in rodents, and in visceral fat tissue in humans.[5)] The β3AR is a G-protein-coupled transmembrane receptor and activates adenylate cyclase, leading to an increase in cellular cAMP level. Activated cAMP-dependent protein kinase induces the action of terminal cellular components, such as lipolysis in white adipocytes and thermogenesis in brown adipocytes. Many studies have reported that β3AR genetic polymorphisms are involved in obesity, insulin resistance and related metabolic disorders.[6)-17)] In 1995, a missense mutation in the β3AR gene, resulting in the replacement of tryptophan by arginine at position 64 (Trp64Arg), was reported to be associated with the earlier onset of type 2 diabetes and a decreased resting metabolic rate in Pima Indians,[5)] and induced insulin resistance and abdominal fat gain in the Finns,[18)] and in Caucasians.[7)] Other researchers reported that Japanese with the variant type of the β3AR gene had a higher BMI and a tendency for an earlier age of onset of type 2 diabetes.[9),19)] However, there is still considerable debate on the nature of the association of the β3AR genotype with obesity and blood glucose levels, and much research remains to be conducted, particularly considering ethnical differences and environmental factors. The purpose of the present study was to investigate the effect of the β3AR gene polymorphism on the risk of hyperglycemia in Korean women.

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MATERIALS AND METHODS

1. Subjects

All subjects were recruited from new participants in the weight loss program in Kirin Oriental Medical Hospital (Seoul, Korea), prior to starting their program. A total of 980 Korean women subjects were recruited. The physical and biochemical characteristics of the subjects are shown in Table 1. Genomic DNA was obtained with informed consent. Study subjects were divided into 2 groups by genotypes of 3AR; the WW type (wild type) group, and the WR and RR types group (WR/RR) (variant type). Subjects were classified as hyperglycemic if their fasting blood glucose levels were in excess of 6.105mmol/L (110mg/dL), according to the American Diabetes Association, 1997 criteria.[20]

Table 1. The clinical characteristics of the total subjects.

		Total subject (n=980)
Physical Characteristics		
Age	(year)	26.97 ± 0.23[1]
Weight	(Kg)	66.27 ± 0.37
BMI	(Kg/m^2)	25.74 ± 0.14
Obesity index	(%)	119.43 ± 0.63
WHR		0.87 ± 0.01
SBP	(mmHg)	116.02 ± 0.44
DBP	(mmHg)	71.74 ± 0.37
Body Composition		
Water	(Kg)	29.77 ± 0.12
Fat mass	(Kg)	22.87 ± 0.25
Lean body mass	(Kg)	43.47 ± 0.18
% body fat	(%)	33.81 ± 0.20
Serum Biochemical Characteristics		
Blood glucose	(mmol/L)	5.62 ± 0.04
TC	(mmol/L)	4.63 ± 0.03
LDL	(mmol/L)	2.78 ± 0.02
HDL	(mmol/L)	1.34 ± 0.01
TG	(mmol/L)	1.16 ± 0.02
T-bilirubin	(μmol/L)	11.75 ± 0.50
GOT	(IU/L)	20.78 ± 0.56
GPT	(IU/L)	24.70 ± 0.81

[1] Mean ± SE
BMI; body mass index, WHR; Waist Hip ratio, SBP; systolic blood pressure, DBP; diastolic blood pressure, % body fat; percent body fat mass, TC; total cholesterol, LDL; LDL cholesterol, HDL; HDL cholesterol, TG; triglyceride, T-bilirubin; Total bilirubin, GOT; glutamic oxaloacetic transaminase, GPT; glutamic pyruvic transaminase

All clinical data were obtained from subjects before starting their weight loss program. Blood pressure, height, weight, and waist and hip circumference, were measured. BMI was calculated as weight (Kg) divided by squared height (m), and WHR was calculated as waist circumference divided by hip circumference. The obesity index was calculated as follows : [obesity index = actual weight / ideal weight × 100], where ideal weight (kg) = (height (cm) - 100) × 0.9. Body composition was measured by bio-impedance analysis using a commercial device (Inbody 2.0, Biospace Co., Seoul, Korea).

2. Determination of the β3-adrenergic receptor (β3AR) genotype

Genomic DNA was extracted from whole blood using a Qiagen mini kit. PCR was used to amplify a genomic DNA fragment containing codon 64 of β3AR genomic DNA. Upstream primer (5'CCAGTGGGCTGCCAGGGG 3'), downstream primer (5'GCCAGTGGCGCCCACGG 3'), 3μl of dNTP mix (1mM), 0.2μl of Taq DNA polymerase (1 unit), and 3μl of PCR buffer (10×), were combined and adjusted with distilled water to make a total volume of 30μl. The amplification protocol consisted of 35 cycles of denaturation at 96°C for 40 seconds, annealing at 65°C for 30 seconds, and with an extension at 72°C for 30 seconds. The amplified PCR product was checked for correct size (248bp) by electrophoresis in a 3% agarose gel. The PCR product was subsequently digested with the enzyme BstNI for 1hour at 60°C, and was then subjected to electrophoresis in a 4% agarose gel. The resulting band patterns were 97, 64, 61, 15 and 11 bp for the Trp64 (WW) genotype; 158, 97, 64, 61, 15 and 11 bp for the Trp64Arg (WR) genotype; and 158, 61, 15 and 11 bp for the Arg64 (RR) genotype.

3. Biochemical analysis

Blood samples were obtained after subjects had fasted overnight for more than 12 hours. Blood samples were centrifuged at 2,000 rpm for 30 minutes, and the serum was stored at -70°C until analyzed. Sample concentrations of fasting glucose, total- and HDL cholesterol, triglycerides, GOT, GPT and total bilirubin were determined by auto-biochemical analyzer (SPOTCHEM, ARKRAY, Koto, Japan). LDL cholesterol levels were calculated using the Friedewald equation [LDL = TC-HDL-TG/5].[21]

4. Statistical analysis

All values are presented as mean ± SE. Age adjusted covariate analysis of variance was performed using the GLM (general linear model) procedure to examine the independent effect of the β3AR genotype on dependent variables. The Chi-square (x^2) test was used to compare the frequencies of β3AR genotypes between groups. Risk factors for hyperglycemia were analyzed by logistic regression analysis. Statistical significance was established at the level of $P<0.05$. All analyses were performed using SPSS 10.0 software.

RESULTS

The frequencies of β3AR genotypes among all subjects were : 67% WW type, 31%, WR type; and 2% RR type (Table 2). The frequency of WR and RR variant types (WR/RR) of β3AR found in this study is similar to that of the Japanese population, is higher than Caucasian populations, and is lower than the Pima Indians.

Table 2. The relative genotype frequencies of the β3-adrenergic receptor of this study comparing the others.

Populations	Sample (n)	WW, Trp64	WR, Trp64Arg	RR, Arg64	R allele
Korean[1]	**1781**	**70**	**27**	**3**	**0.167**
1. H.H.Oh[2]	980	67	31	2	0.175
2. K.J.Ahn[3]	500	70	27	3	0.165
3. H.J.Lee[4)0]	114	68	28	4	0.180
4. J.Y.Oh[5]	53	70	26	4	0.170
5. S.W.Park[6]	134	74	23	3	0.145
Japanese[7]	**1033**	**66**	**30**	**4**	**0.189**
7. T.Nagase[8]	186	61	36	3	0.210
8. Y.Shima[9]	261	67	29	4	0.185
9. N.Sakane[10]	387	63	33	3	0.195
10. K.Yanagisawa[11]	199	72	23	5	0.165
Caucasians; German[12]	1259	88	11	1	0.065
Caucasians[13]	909	88	11	3	0.085
Caucasians; French-canadian[14]	468	87	13	9	0.155
Finnish[15]	170	53	42	5	0.260
Pima Indians[16]	642	46	45	9	0.315

[1] Calculated by study of 1-5 as total Korean populations (n=1781)
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[16] Walston et al, Time of onset of non insulin diabetes mellitus and genetic variation in the β3-adrenergic receptor gene, N Engl J Med 333 : 343-347, 1995

Table 3 compares physical, body composition, and biochemical characteristics of the subjects classed by β 3AR genotype. Subjects' weights, BMI, obesity index and WHR were higher in the WR/RR type compared with the WW type, but these differences were not statistically significant. Fasting glucose level was significantly higher in the WR/RR types compared with the WW type (p=0.011); subjects with variant (WR/RR) types of β3AR gene had 3.6% higher fasting glucose levels compared with the wild (WW) type. Subjects' blood lipid profiles were similar in all genotype classes. These results suggest that the β3AR genotype has a significant effect on blood glucose levels.

Table 3. The comparison of physical, body composition and blood biochemical characteristics by genotype of the β3-adrenergic receptor

Genotypes		WW type (n=668)	WR/RR type (n=318)	p-value
Physical Characteristics				
Weight	(Kg)	66.18±0.45[1]	66.46±0.65	0.697
BMI	(Kg/m²)	25.67±0.16	25.88±0.24	0.445
Obesity index	(%)	119.14±0.75	120.03±1.13	0.480
WHR		0.87±0.01	0.88±0.01	0.267
SBP	(mmHg)	115.79±0.52	116.48±0.77	0.415
DBP	(mmHg)	71.68±0.43	71.86±0.65	0.750
Body Composition				
Water	(Kg)	29.79±0.15	29.73±0.21	0.841
Fat mass	(Kg)	22.72±0.30	23.18±0.43	0.379
Lean body mass	(Kg)	43.54±0.22	43.33±0.30	0.611
% body fat	(%)	33.65±0.24	34.16±0.33	0.223
Blood Biochemical Characteristics				
Blood glucose	(mmol/L)	5.55±0.04	5.75±0.07	0.011**
TC	(mmol/L)	4.60±0.03	4.68±0.05	0.132
LDL	(mmol/L)	2.76±0.03	2.82±0.04	0.200
HDL	(mmol/L)	1.34±0.01	1.34±0.02	0.991
TG	(mmol/L)	1.16±0.02	1.17±0.03	0.549
T-bilirubin	(μmol/L)	12.14±0.68	10.95±0.51	0.259
GOT	(IU/L)	20.94±0.66	20.45±0.92	0.690
GPT	(IU/L)	25.38±0.96	23.21±0.89	0.210

[1] Mean ± SE
**Values for comparisons of two groups are significantly different at p<0.01 by GLM (covariance) analysis, adjusted for age
BMI; body mass index, WHR; Waist Hip ratio, SBP; systolic blood pressure, DBP; diastolic blood pressure, % body fat; percent body fat mass, TC; total cholesterol, LDL; LDL cholesterol, HDL; HDL cholesterol, TG; triglyceride, T-bilirubin; Total bilirubin, GOT; glutamic oxaloacetic transaminase, GPT; glutamic pyruvic transaminase

The frequency of hyperglycemia in relation to β3AR genotype is shown in Table 4. When the subjects were divided into two groups by the cut off point of fasting blood glucose level at 6.105 mmol/L, the frequency distributions showed a statistically significant difference as measured by x^2-analysis (p=0.014). The frequency of hyperglycemia was 18.2% in WW type subjects, and was 24.8% in the WR/RR type subjects, giving an increased frequency of hyperglycemia of 36.3% in the WR/RR types compared with the WW type.

To identify the main risk factors for hyperglycemia, all factors which showed significant differences between hyperglycemia and normoglycemia were introduced into a stepwise logistic regression analysis; four main factors were identified : β3AR genotype, TG, BMI and age (Table 5). The odds ratios for hyperglycemia were 1.573 (p=0.011) for the WR/RR types of the β3AR gene, 1.053 (p=0.001) for TG, 1.044 (p=0.037) for BMI and 1.026 (p=0.031) for age, where the following variables were included in the analysis : type of β3AR gene, age, weight, BMI, fat mass, WHR, TC, LDL, HDL and TG. This result suggests that the WR/RR types of the β3AR gene can be a significant risk factor for hyperglycemia among Korean women.

Table 4. The genotype frequency of the β3-adrenergic receptor according to fating blood glucose level

	Hyperglycemia[1)]	Normoglycemia	Total	p-value[4)]
WW[2)]	110[3)] (18.2)	496 (81.8)	606 (100)	
WR/RR[2)]	73 (24.7)	222 (75.3)	295 (100)	
Total	183 (20.3)	718 (79.7)	901 (100)	0.014

1) Hyperglycemia was defined as a blood glucose level of more than 6.105mmol/L (110mg/dL)
2) WW; wild type of β3AR gene, WR/RR; variant type of β3AR gene
3) *n*, Number of subjects
4) Values were compared by x^2-analysis

Table 5. Final logistic regression model of hyperglycemia (n=980)

Variable[1)]	β	SE	p-value	odds ratio	95% CI	
Triglyceride	0.052	0.015	0.001	1.053 / 0.2 mmol/L	1.023	1.084
WR/RR type[2)]	0.453	0.177	0.011	1.573	1.111	2.226
Age	0.026	0.012	0.031	1.026 / year	1.002	1.050
BMI	0.043	0.021	0.037	1.044 / Kg/m^2	1.003	1.088

Hyperglycemia defined as a blood glucose level of more 6.105mmol/L (110ml/dL)
1) Including the variable were a variant type of β3AR gene, age, weight, BMI, fat mass, WHR, TC, LDL, HDL and TG.
2) Variation of β3-adrenergic receptor gene

DISCUSSION

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 β 3AR genotype and metabolic disorders have been reported in several studies, many controversies persist.

In the studies of Kim-Motoyama *et al.*[22)] and Sakane *et al.*,[23)] the β3AR variant genotype was reported to be associated with increased visceral fat mass. It was reported that obese subjects with variants of the β3AR genotype had higher WHR and visceral fat mass, but decreased plasma free fatty acid and TG levels, than subjects with the wild genotype. The authors suggested that the β3AR genotype was more associated with fat distribution than with weight gain. The variant type may increase the visceral fat mass, because fat in individuals carrying the β3AR variant types was distributed in visceral fat rather than in subcutaneous fat. Impaired lipolysis associated with visceral obesity may lead to insulin resistance and hyperinsulinemia. The visceral fat mass was correlated with blood glucose level, insulin resistance, and free fatty acid or triglyceride levels. Kadowaki *et al.*[19)] found that non-diabetic Japanese subjects with the β3AR variant genotype had significantly higher BMI values than subjects with the wild type of the β3AR gene; this study showed that the β3AR variant genotype was associated with visceral obesity and low triglyceride levels, via decreased lipolysis and via free fatty acid stimulated hepatic triglyceride production. In Germany, Hinny *et al.*[24)] showed that the Trp64Arg polymorphism of the β3AR gene resulted in a genetic predisposition to the development of obesity in children, adolescents and young adults. Ahn *et al.*[25)] reported that the frequency of patients with the variant allele was significantly higher in obese NIDDM patients than in non-obese NIDDM patients, suggesting that the β3AR genotype might be associated with obesity related NIDDM. Non-diabetic Japanese subjects with the β3AR variant genotype were found to have significantly higher fasting insulin levels, and 2-hour insulin levels, compared to subjects having the wild type of the β3AR gene.[19)] A study of 152 Japanese-American men indicated that triglyceride and 2-hour insulin levels were higher in the β3AR variant genotype.[26)] Silver *et al.*[14)] also demonstrated elevated 2 hour insulin levels in subjects carrying the β3AR variant genotypes, in a 75g glucose tolerance test of non diabetic Mexican Americans. McFarlane-Anderson *et al.*[27)] reported that fasting glucose and 2-hour glucose levels were significantly higher in the variant type of the β3AR gene compared with the wild type.

Some researchers reported differences between the WR and RR variants of the β3AR gene. In a study of

380 healthy Danes, the WR type of the β3AR gene was not associated with an altered insulin sensitivity index or changes in other features of the insulin resistance syndrome, but the RR type had a significantly higher BMI, and higher fasting serum C-peptide, triglyceride and LDL cholesterol levels, when compared with the WW type.[15] The RR type also had a significantly lower insulin sensitivity index than the WW type, indicating that the RR type of the β3AR gene may contribute to a worsening insulin resistance and thereby hyperglycemia. In studies by Buttner *et al*,[28] fasting insulin levels in type 2 diabetes mellitus subjects were not elevated in the case of the WR type of the β3AR gene, whereas the RR type subjects had elevated insulin levels after 3 and 4 hours of fasting; the HbA1c levels were also elevated in the diabetic RR types compared with the diabetic WW and WR types of the β3AR gene.

On the other hand, no effects of variant β3AR genes on BMI, WHR and body fat mass were found among 211 Thai subjects; this study also found that fasting glucose and glucose × fasting insulin levels were not different by β3AR genotype.[29] No effects of β3AR genotype on weight, BMI, WHR, body fat mass, and fasting plasma insulin, glucose and glycerol levels, were found in a another study of Pima Indians.[30] A study conducted among Finns also showed no difference in BMI, and in basal or post glucose insulin concentration, among subjects with different β3AR genotypes.[18] In addition, there are other studies where Trp64Arg polymorphism of the β3AR gene does not appear to be associated with obesity.[31)-33)]

In the present study, the effects of β3AR genotype were evaluated among 980 Korean women. The frequencies of the Trp64Arg genotypes, WW, WR, and RR, were 67%, 31%, and 2%, respectively (Table 2). The frequency of the variant types (WR/RR) of β3AR is similar to that found in the Japanese population, higher than in Caucasian populations, and lower than among Pima Indians. For obesity markers such as weight, BMI, obesity index and WHR, this study found no differences among the β3AR genotypes, although fasting glucose levels were significantly higher in the WR/RR types compared with the WW type (Table 3). The frequency of the WR/RR types, as well as BMI, WHR, body fat mass and TG levels, were significantly higher in hyperglycemic than in normoglycemic subjects (Table 4 and 5). When stepwise logistic regression analysis was conducted to create a model for predictors of hyperglycemia risk, one of the significant risk factors was found to be the WR/RR type of the β3AR gene which showed an odds ratio of 1.573 (p=0.011).

In conclusion, the variant genotypes of the β3AR gene (WR/RR) had no significant association with obesity markers such as weight, BMI, WHR, body fat mass and lipid profile; however, a strong association was found between the β3AR genotype and both fasting blood glucose level and frequency of hyperglycemia. These results suggest that the variant types of the β3AR gene could be an important risk factor for hyperglycemia in Korean women.

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