Lack of Relationship Between β_3-Adrenergic Receptor Gene Polymorphism and Gestational Diabetes Mellitus in a Taiwanese Population

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The Trp64Arg polymorphism of β_3-adrenergic receptor (ADRB3) has been reported to be associated with insulin resistance and gestational diabetes mellitus (GDM). The objective of this study is to investigate whether the ADRB3 Arg variant confers susceptibility to GDM in a Taiwanese population. A total of 299 pregnant women (mean ± SD, 31.1 ± 4.2 years) was recruited. Two-hour, 75-g oral glucose tolerance tests (OGTT) were conducted at 24 to 31 weeks gestation (28.3 ± 1.6 weeks). Forty-one GDM subjects and 258 controls with normal glucose tolerance (NGT) level were genotyped for the ADRB3 Trp64Arg polymorphism. The genotype distribution and allele frequency of ADRB3 did not significantly differ between GDM and NGT subjects (9.8% *v* 14.5%). Body weight gain during pregnancy was not different between ADRB3 genotypes. However, the GDM subjects with the Arg64 variant had higher fasting ($P = .04$) and postload 120 minutes ($P = .03$) insulin levels as compared with the GDM subjects with the Trp64Trp allele. In all subjects, the women with the Arg64 allele (n = 76) had significantly higher level of insulin secretion (the ratio of $\Delta insulin_{60}/\Delta glucose_{60}$) during OGTT than those without (n = 223) ($P = .03$) despite similar plasma levels of glucose and insulin in both genotypes. Our results indicated that the ADRB3 Trp64Arg variant is not related to the development of GDM and has no effect on obesity during pregnancy in a Taiwanese population. However, ADRB3 polymorphism might be a possible determinant of insulin resistance.

GESTATIONAL DIABETES mellitus (GDM) is carbohydrate intolerance of varied severity that begins or is first recognized during pregnancy. Women with GDM history have a significantly increased risk of developing type 1 and type 2 diabetes.[1] Possible explanations for diabetogenicity during pregnancy include impaired β-cell function, increased insulin degradation, and decreased tissue sensitivity to insulin.[2] Identification of the risk factors for GDM is required for preventive strategies.

The β_3-adrenergic receptor (ADRB3) is a pivotal receptor mediating catecholamine-stimulated thermogenesis and lipolysis. A missense mutation in the ADRB3 gene, Trp64Arg, results in the substitution of a tryptophan by an arginine in the first intracellular loop of ADRB3.[3-5] Walston et al[3] reported the ADRB3 Trp64Arg variant was associated with early onset of type 2 diabetes mellitus and lower resting metabolic rate in Pima Indians. ADRB3 Trp64Arg polymorphism was also demonstrated to be associated with obesity[4-6] and increased tendency to gain weight.[5,6] However, the observations in the literature are not consistent. Other investigators reported that the polymorphism of ADRB3 has no effect on obesity, type 2 diabetes[7,8] or energy expenditure in obese postmenopausal women.[9]

There is evidence to suggest the association of obesity and insulin resistance with GDM. Higher body mass index (BMI) predicts increased GDM risk.[10] Pregnant women displayed insulin resistance, which was most pronounced in women with GDM.[11] Few studies to date have examined whether the ADRB3 Arg variant could be related to the development of GDM. In Austrian Caucasians, the Trp64Arg variant of the ADRB3 gene was associated with mild GDM.[12] However, a conflicting result has been reported in Greek women.[13] Thus, we investigated the association of Trp64Arg polymorphism of ADRB3 with GDM and body weight gain during pregnancy in a Taiwanese population.

SUBJECTS AND METHODS

Subjects

Two hundred and ninety-nine Taiwanese women aged 20 to 42 years (mean ± SD, 31.1 ± 4.2) at 24 to 31 weeks gestation (28.3 ± 1.6 weeks) were recruited from Taipei Municipal Women's and Children's Hospital. Two-hour, 75-g oral glucose tolerance tests (OGTT) were performed in the morning after an overnight fast by a standard protocol. GDM was diagnosed using established criteria (that is, 2 or more abnormal values during OGTT; in which normal glucose values were <5.3, <10.0, <8.6 mmol/L at 0, 1, and 2 hours, respectively[14]). Two hundred and fifty-eight subjects were classified as having normal glucose tolerance (NGT) and 41 subjects were identified as having GDM. This study was reviewed and approved by the Institutional Review Board of the hospital. The purpose of the study was fully explained to all subjects, and written informed consent was obtained before enrollment in the study.

Biochemical and Genetic Analyses

Venous blood samples were drawn in the fasting state 60 and 120 minutes after glucose ingestion. Plasma and serum samples were prepared and stored at −20 °C until analysis. Plasma glucose, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglyceride concentrations were measured using commercially available kits (Olympus System Reagents; Olympus Diagnostica GmbH, Clare, Ireland.) by an autoanalyzer (Olympus AU400 System; Olympus, Tokyo, Japan.). Serum free fatty acids (FFA) were assayed by an enzymatic method with a NEFA-HR kit (Wako Pure Chemical, Osaka, Japan). Concentrations of insulin and C-peptide were measured by radioimmunoassay with a

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Submitted October 24, 2003; accepted April 20, 2004.

Supported by the National Science Council, Taiwan (NSC 91-2320-B-264-001) and the Department of Health, Taipei City Government, Taipei, Taiwan (Contract No. 89012).

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0026-0495/04/5309-0018$30.00/0

doi:10.1016/j.metabol.2004.04.006

Table 1. Genotype and Allele Frequency of the ADRB3 Gene Trp64Arg Variant in GDM and Control Subjects by the Glucose Tolerance Status

	NGT No. (%)	GDM No. (%)
No. of subjects	258	41
Genotype		
$Trp^{64}Trp$	189 (73.3)	34 (82.9)
$Trp^{64}Arg$	63 (24.4)	6 (14.6)
$Arg^{64}Arg$	6 (2.3)	1 (2.4)
	$\chi^2 = 1.91$, $df = 2$, $P = .384$	
Allele		
Trp	441 (85.5)	74 (90.2)
Arg	75 (14.5)	8 (9.8)
	$\chi^2 = 2.94$, $df = 1$, $P = .086$	

BioChem ImmunoSystems kit (BioChem Pharma, Rome, Italy) and RIA kit (Daiichi, Tokyo, Japan), respectively. The ratios of the 60-minute increase in insulin ($\Delta insulin_{60}$) relative to the 60-minute increase in glucose ($\Delta glucose_{60}$) during OGTT were calculated as measures of insulin secretion.[12]

Genomic DNA was extracted from peripheral blood lymphocytes using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Genotypes for ADRB3 codon 64 were determined as described previously.[5] The DNA fragment was amplified by polymerase chain reaction (PCR) in a volume of 10 μL containing 50 to100 ng genomic DNA, 10 pmol of each primer, 200 μmol/L of each deoxynucleotide triphosphate (dNTP), 1.5 mmol/L $MgCl_2$, 4% formamide, 0.8% bovine serum albumin (BSA), and 1 U Taq polymerase. The cycling parameters were 94°C for 2 minutes followed by 30 cycles of 94°C for 1 minute, 64°C for 20 seconds, and 72°C for 30 seconds with a final extension at 72°C for 10 minutes. PCR products were digested with BstN1 (New England BioLab, Beverly, MA) for 2 hours at 60°C. Fragments were analyzed on 3% agarose gel with ethidium bromide and visualized under ultraviolet light.

Statistical Analysis

All data were presented as mean ± SD. Statistical analysis was performed using the SPSS statistical package (SPSS, Chicago, IL). Chi-squared analysis was used to compare allele frequencies. The Mann-Whitney *U* test (or the *t* test for normally distributed values) was used to compare genotypes for the differences in biochemical variables. A *P* value of less than .05 was considered statistically significant.

RESULTS

Table 1 lists the genotype and allele frequency of ADRB3 in the NGT (n = 258) and GDM (n = 41) subjects. The allelic frequencies of the Arg 64 were 14.5% and 9.8% in the NGT and GDM groups, respectively. There were no significant differences between these 2 groups in ADRB3 genotype ($P = .39$) and allele frequency ($P = .09$).

Because only 1 GDM and 6 NGT subjects had homozygous Arg64Arg, the subjects carrying Arg64 allele were considered to be the variant group. The NGT subjects were further divided into 2 subgroups based on the presence or absence of Arg64 allele. The GDM subjects were also divided into 2 subgroups by ADRB3 variant. The clinical characteristics and metabolic parameters for subgroups in NGT and GDM subjects are shown in Tables 2 and 3. The clinical and metabolic parameters did not differ significantly between Arg64 variant and Trp64Trp subgroups in NGT subjects. Interestingly, the GDM subjects carrying Arg64 allele had higher fasting ($P = .04$) and postload 120-minute insulin concentrations ($P = .03$) than those GDM subjects with Trp64Trp allele. In addition, the GDM subjects with Arg64 allele had higher mean value of $\Delta insulin_{60}/\Delta glucose_{60}$ than those without, however, the difference was of borderline statistical significance ($P = .08$). Furthermore, fasting plasma lipid levels (total cholesterol, LDL-C, HDL-C, triglyceride, and FFA) did not significantly differ between Arg64 variant and nonvariant subjects either in the NGT or GDM groups (data not shown).

In all subjects, the women with Arg64 alleles were considered to be carriers (n = 76) and those with Trp64Trp were considered to be noncarriers (n = 223). There were no significant differences between carriers and noncarriers in the clinical characteristics (age, week of pregnancy, prepregnancy BMI, and body weight gain). No significant differences were found between carriers and noncarriers with respect to the metabolic parameters (plasma lipid levels, fasting concentrations of glucose, C-peptide, and insulin, and postload plasma concentrations of glucose, C-peptide, and insulin during OGTT) (data not shown). Interestingly, carriers had significantly higher insulin secretion ($\Delta insulin_{60}/\Delta glucose_{60}$) than noncarriers (193.8 ± 84.4 *v* 174.0 ± 98.8 pmol/mmol, $P = .03$).

Furthermore, we also examined whether the Arg64 allele of ADRB3 was related to obesity. A total of 299 subjects were divided into 2 groups according to prepregnancy BMI: non-obese group (BMI < 26.4 kg/m^2), and obese group (BMI ≥ 26.4 kg/m^2). The allelic frequencies of the Arg64 were 14.3% in the non-obese group (n = 273) and 9.6% in the obese group (n = 26), revealing no significant difference between these 2 groups ($P = .32$).

Table 2. Clinical Characteristics of NGT and GDM Subjects According to ADRB3 Genotype

	NGT		GDM	
Characteristics	Trp64Trp	Arg64	Trp64Trp	Arg64
No. of subjects	189	69	34	7
Age (yr)	30.8 ± 4.2	30.7 ± 4.1	32.9 ± 4.3	31.0 ± 2.0
Week of pregnancy	28.3 ± 1.7	28.3 ± 1.4	27.9 ± 1.7	28.3 ± 1.1
BMI (kg/m^2, initial)	21.4 ± 2.9	21.1 ± 2.9	22.9 ± 3.4	23.7 ± 2.6
BMI (kg/m^2, actual)	25.3 ± 2.9	25.2 ± 3.0	27.2 ± 3.4	27.5 ± 3.1
Body weight gain (kg)	9.9 ± 3.3	10.2 ± 3.5	10.4 ± 3.7	9.8 ± 3.0

NOTE. Data are the mean ± SD.
Abbreviation: BMI, body mass index.

Table 3. Plasma Concentrations of Glucose, Insulin, and C-Peptide Responses on 75-g Oral Glucose Tolerance Tests in NGT and GDM Subjects by ADRB3 Genotype

Characteristics	NGT		GDM	
	Trp64Trp	Arg64	Trp64Trp	Arg64
Fasting				
Glucose (mmol/L)	4.5 ± 0.4	4.6 ± 0.3	5.1 ± 0.7	5.1 ± 0.5
Insulin (pmol/L)	87.7 ± 34.9	93.0 ± 45.2	113.2 ± 41.8[a]	145.6 ± 23.9[a]
C-peptide (ng/mL)	1.2 ± 0.8	1.3 ± 0.9	1.6 ± 0.8	1.7 ± 0.4
60 min				
Glucose (mmol/L)	8.1 ± 1.3	8.2 ± 1.2	11.0 ± 1.3	10.1 ± 2.4
Insulin (pmol/L)	732.1 ± 463.9	771.9 ± 351.4	824.6 ± 537.6	999.4 ± 529.4
C-peptide (ng/mL)	5.9 ± 2.7	5.9 ± 2.4	6.8 ± 3.0	6.4 ± 2.3
120 min				
Glucose (mmol/L)	6.8 ± 1.1	6.9 ± 1.0	9.2 ± 1.3	9.3 ± 1.7
Insulin (pmol/L)	603.8 ± 384.2	611.7 ± 351.5	858.1 ± 411.1[b]	1,185.9 ± 397.9[b]
C-peptide (ng/mL)	5.6 ± 2.8	5.4 ± 2.5	7.8 ± 3.4	7.9 ± 1.3
$\Delta insulin_{60}/\Delta glucose_{60}$ (pmol/mmol)	182.9 ± 109.2	195.6 ± 96.3	124.6.9 ± 93.6	176.5 ± 76.2

NOTE. Data are the mean ± SD.

Values with the same letter superscripts were significantly different: [a,b]$P < .05$ by Mann-Whitney U test.

DISCUSSION

The Trp64Arg polymorphism of ADRB3 has been reported in various ethnic groups including Pima Indians,[3] Finns,[4] French Caucasians,[5] Chinese,[15] Taiwanese,[16] and Japanese.[6,8,17] The Arg64 allelic frequency of ADRB3 found in this Taiwanese population is 13.9%. This frequency is similar to those previously reported in nonpregnant Taiwanese,[16] Chinese,[15] and Caucasian[4,5] populations, which is lower than the rate reported in Pima Indians[3] and Japanese,[6,8] but is higher than those reported in Austrian pregnant women[12] and Greek pregnant women.[13]. Festa et al[12] showed that the presence of Trp64Arg substitution in ADRB3 tended to cause the development of GDM. Conversely, Alevizaki et al[13] reported that the frequency of Trp64Arg heterozygotes was similar in the GDM and control groups (6.7% *v* 6.9%). In the present study, the frequency of the Arg64 allele was not significantly different between the GDM and control groups (9.8% *v* 14.5%). In fact, we observed that the Arg allele tended to be less frequent in the GDM group. Festa et al[12] defined GDM using the criteria based on a relatively low cutoff point. The association between ADRB3 variant and GDM was still absent when we analyzed our data using these lower criteria. Therefore, the lack of relationship between ADRB3 polymorphism and GDM in the Taiwanese population is not due to the different diagnostic criteria. Herein, we provide further evidence that the ADRB3 Arg variant itself is not a major determinant of GDM.

ADRB3 is one of the factors responsible for lipolysis and delivery of FFA into the portal vein. The Trp64Arg polymorphism of ADRB3 was associated with impaired catecholamine-induced lipolysis,[18] increased fasting FFA concentration,[19] and decreased serum triglyceride levels.[20] Because FFA has been suggested to play a role in inducing insulin resistance in pregnancy,[2] we also examined whether the ADRB3 Arg variant might influence plasma FFA level. There was no significant effect of Trp64Arg variant on fasting plasma concentrations of triglyceride and FFA in the Taiwanese subjects during pregnancy.

The Trp64Arg polymorphism of ADRB3 was related to body weight gain[5] and an increased risk for obesity.[21,22] Moreover, ADRB3 Arg variant was associated with increased weight gain during pregnancy[12] or obesity in the GDM women.[13] However, our results showed no significant difference in Arg64 allele frequency in non-obese women compared with the obese group, indicating little influence of the ADRB3 Arg variant on obesity during pregnancy in the Taiwanese population.

García-Rubi et al[23] reported that the obese postmenopausal women with heterozygous Trp64Arg had higher insulin resistance than a matched group of women with wild-type homozygotes, using the hyperinsulinemic-euglycemic glucose clamp technique. In the present study, plasma concentrations of fasting and postload 120-minute insulin in GDM subjects carrying the ADRB3 Arg variant were significantly higher than those in the nonvariant GDM subjects. In contrast to the insulin levels, plasma fasting and postload glucose levels were similar in both groups, implicating that the elevated insulin levels might not improve glycemic control in GDM subjects carrying the ADRB3 Arg variant. Although the ratio of $\Delta insulin_{60}/\Delta glucose_{60}$ is not a standard insulinogenic index, it may provide some information with regard to insulin secretion. In all subjects, the women with Arg 64 allele of ADRB3 had significantly higher insulin secretion ($\Delta insulin_{60}/\Delta glucose_{60}$) than those without, despite the fact that similar plasma glucose concentrations in these 2 groups are seen. Therefore, a potential effect of ADRB3 Arg variant on insulin resistance could not absolutely be excluded entirely in this study. Further studies, such as using the hyperinsulinemic-euglycemic glucose clamp technique, are required to clarify the effect of ADRB3 Arg variant on insulin resistance during pregnancy.

In summary, the ADRB3 Trp64Arg variant may not contribute to the development of GDM in a Taiwanese population. The ADRB3 Arg variant itself has no effect on obesity and plasma FFA level during pregnancy.

ACKNOWLEDGMENT

We thank all of the pregnant women who participated in this study, all of the nursing staff at the out-patient clinic of Obstetrics and Gynecology, and the Taipei Municipal Women's and Children's Hospital for their assistance. We gratefully acknowledge Professor Shau-Ku Huang and Beverly Plunkett, the Department of Medicine, School of Medicine, Johns Hopkins University and Dr Wei-Shiung Yang, the Department of Internal Medicine, National Taiwan University Hospital for carefully reviewing the manuscript and offering constructive advice.

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