Lack of Association Between Genetic Variation in the β3-Adrenergic Receptor Gene and Insulin Resistance in Patients With Coronary Heart Disease

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The β-adrenergic system plays a critical role in regulating lipolysis and thermogenesis. Recent studies have suggested that a missense Trp64Arg mutation in the β_3-adrenergic receptor gene is involved in visceral obesity and insulin resistance. We investigated the effect of this mutation on insulin resistance in patients with angiographically documented coronary heart disease ([CHD] n = 137) and normal subjects (n = 188). Plasma glucose and insulin responses to a 75-g oral glucose tolerance test and insulin resistance measured by the insulin suppression test, were determined in 58 (42%) patients with CHD and 121 (64%) controls. The genotype and allele frequency of the β_3-adrenergic receptor did not differ between patients with CHD and controls. The blood pressure, body mass index (BMI), waist to hip ratio, fasting plasma glucose, insulin, and lipid, and plasma glucose and insulin responses to the glucose load were relatively similar in subjects with and without the mutation in CHD and normal groups. The degree of insulin sensitivity, ie, the steady-state plasma glucose concentration, was not significantly different between subjects with and without the mutation in the CHD group (11.3 ± 1.2, n = 11 *v* 11.9 ± 0.6 mmol/L, n = 47, *P* = NS) and control group (8.4 ± 0.7, n = 30 *v* 8.2 ± 0.4 mmol/L, n = 91, *P* = NS). We conclude that Trp64Arg polymorphism of the β_3-adrenergic receptor gene does not likely play a major role in the development of CHD in the Chinese population. In addition, it appears to have no association with the insulin resistance syndrome in either CHD or non-CHD subjects.

OBESITY, particularly central obesity, glucose intolerance, hypertension, and dyslipidemia are all part of the insulin resistance syndrome and are implicated in the development of coronary heart disease (CHD).[1,2] Although both environmental and genetic factors contribute to this syndrome, several genes have been tested over the past few years for a potential linkage with these phenotypes. The β_3-adrenergic receptor mediates lipolysis and thermogenesis[3,4] and thus has a central role in regulation of the basal metabolic rate and lipid metabolism. Molecular abnormalities of the β_3-adrenergic receptor gene may lead to obesity and insulin resistance. Recently, a missense mutation replacing tryptophan with arginine at codon 64 of the β_3-adrenergic receptor gene was shown to be associated with features of the insulin resistance syndrome, an early onset of type 2 diabetes mellitus, and an increased capacity to gain weight.[5-7] However, subsequent reports found that this mutation was not related to insulin resistance in diabetic or nondiabetic subjects[8] or in patients with essential hypertension.[9]

It has been suggested that patients with CHD are characterized by the insulin resistance syndrome.[2] In fact, our group[10] and others[11,12] have previously shown that nondiabetic normotensive subjects with angiographically documented CHD are characterized by hyperinsulinemia and insulin resistance. Very recently, Higashi et al[13] have shown that the frequency of Arg64Trp mutation of the β_3-adrenergic receptor gene is significantly higher in patients with CHD. However, they failed to find differences in fasting glucose, insulin, and lipid concentrations between subjects with and without the mutation in the Japanese population. Since the fasting insulin level is only a surrogate of insulin resistance,[14,15] it is still unclear whether the degree of insulin resistance is distinct in different genotypes, particularly those patients with CHD. This study was initiated to examine the distribution of this mutation in Chinese CHD and non-CHD populations, and to investigate the relationship, if any, between this mutation and insulin resistance in patients with CHD.

SUBJECTS AND METHODS

Subjects

A total of 325 nondiabetic normotensive (systolic blood pressure [SBP] ≤ 40 mm Hg and diastolic blood pressure [DBP] ≤ 90 mm Hg) subjects were included in the study. Among them, 137 were confirmed as CHD patients with angiographic evidence of CHD. One hundred eighty-eight control subjects who were free of chest symptoms and had normal resting electrocardiograms were selected from individuals receiving an annual physical examination. CHD subjects with a history of recent acute myocardial infarction or unstable angina pectoris were excluded. Eighty-nine patients were receiving calcium-channel blockers (diltiazem or amlodipine), and 44 were treated with nitrate. None were taking lipid-lowering agents, β-blockers, or diuretics that might have adverse effects on carbohydrate and lipid metabolism.[16,17] Blood pressure was measured in the right arm after 30 minutes of rest with the subject seated. The waist circumference was measured with a soft measuring tape across the umbilicus; hip circumference was measured at the widest part of the gluteal region. The protocol was approved by the Human Subjects Committee of the National Defense Medical Center. Written informed consent was obtained from all subjects before enrollment in the study.

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Coronary angiography was performed according to the method of Sones and Shirey[18] or Judkins.[19] An arteriogram was considered positive for CHD if there was a postnitroglycerin stenosis of the principal vessels greater than 50% of the luminal diameter, or a greater than 70% reduction in the luminal diameter of a first-order branch (or second-order branch if the first-order branch was larger than the major vessel distal to its junction with the major vessel).

Blood was drawn after an overnight fast for measurement of plasma glucose,[20] insulin,[21] triglyceride,[22] and cholesterol[23] concentrations. In addition, high-density lipoprotein (HDL) cholesterol was determined in the supernatant of the plasma after magnesium chloride–phosphotungstic precipitation of apolipoprotein B–containing lipoproteins.[24] The low-density lipoprotein (LDL) cholesterol concentration was estimated by the formula of Friedewald et al. [25]

Fifty-eight (42%) CHD patients and 121 (64%) controls received additional metabolic evaluations. After the fasting blood samples, all subjects underwent a 75-g oral glucose challenge, and blood samples were taken 30, 60, 120, and 180 minutes later for determination of plasma glucose and insulin. The total area under the plasma glucose and insulin concentration curves was calculated and designated as the glucose (millimoles per liter per hour) and insulin (picomoles per liter per hour) response, respectively. A modified insulin suppression test was performed as previously described.[26] Briefly, intravenous catheters were placed in both arms after an overnight fast. Blood was sampled from one arm for determination of plasma glucose and insulin, and the contralateral arm was used for administration of the test substance. Somatostatin was infused at 350 μg/h to suppress endogenous insulin secretion while insulin (25 mU/m^2/min) and glucose (240 mg/m^2/min) were administered simultaneously. Blood was sampled hourly until 2 hours into the study, and then every 10 minutes at 150, 160, 170, and 180 minutes. The insulin concentration typically reaches a plateau after 30 minutes, whereas the glucose concentration reaches a plateau after 120 minutes. The four values for individual glucose and insulin concentrations obtained from 150 to 180 minutes were averaged, and represent the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations. Because SSPI concentrations were similar in all subjects, SSPG provided a measurement of insulin-mediated glucose disposal, ie, the higher the SSPG, the more insulin-resistant the person.

Extraction and Amplification of Genomic DNA

Genomic DNAs were extracted from peripheral blood lymphocytes by DNAZOL Reagent (GIBCO BRL Life Technologies, Gaithersburg, MD). Arg64Trp polymorphism of the β_3-adrenergic receptor gene was detected by a mismatch–polymerase chain reaction amplification of genomic DNA followed by restriction endonuclease digestion as described by Widen et al.[6] The allele containing the *Bst*N I restriction site in the presence of thymine was designated as "without Arg mutation," and the allele lacking the restriction site was designated as "with Arg mutation."

Statistical Analysis

Results are expressed as the mean ± SEM. Differences in distribution of the β_3-adrenergic receptor genotype and allele were analyzed by χ^2 tests. An unpaired *t* test was used to compare differences in biochemical variables between genotypes. Since only two CHD patients and four controls were found to have homozygous Arg64Arg, their data were combined with those for Arg64Trp and designated as with mutation. Logistic multiple regression analysis was performed to compare several variables after adjustment for age and body mass index (BMI). All statistical analyses were conducted using a Macintosh (Apple Computer, Cupertino, CA) computer with the Statview 4.0 Statistical Package (Abacus Concepts, Berkeley, CA).

RESULTS

The genotype and allele frequency of the β_3-adrenergic receptor in patients with CHD and controls are shown in Table 1. The genotype distribution did not deviate from Hardy-Weinberg equilibrium. Only two CHD patients and four controls were found to have homozygous Arg64Arg. There were no differences in genotype and allele frequency between the two groups. For a subgroup of CHD patients aged less than 60 or separated by sex, there were also no statistical differences in genotype (data not shown). In addition, the distribution of allele frequency was consistent in subjects with and without further metabolic evaluations.

Clinical characteristics and biochemical findings for CHD patients and control subjects are shown in Table 2. Blood pressure and fasting plasma glucose, insulin, and lipid values did not differ between subjects with and without the mutation in CHD and normal groups. The degree and distribution of obesity, expressed by the BMI and the waist to hip ratio, did not differ significantly between subjects with and without the mutation. Plasma glucose and insulin responses to a 75-g oral glucose challenge were relatively similar between subjects with and without the mutation in CHD and control groups. Despite similar SSPI concentrations, SSPG values were not significantly different between subjects with and without the mutation in the CHD group (11.3 ± 1.2, n = 11 *v* 11.9 ± 0.6 mmol/L, n = 47, *P* = NS) and control group (8.4 ± 0.7, n = 30 *v* 8.2 ± 0.4 mmol/L, n = 91, *P* = NS).

After adjustment for age and BMI, patients with CHD had higher blood pressure, fasting plasma glucose, insulin, triglyceride, and LDL cholesterol, lower HDL cholesterol, larger glucose and insulin responses to oral glucose, and higher SSPG compared with control subjects (data not shown).

DISCUSSION

The allele frequency of Trp64Arg mutation of exon 1 of the β_3-adrenergic receptor gene did not differ significantly between CHD patients and control subjects. Our distribution data are similar to those previously reported in Chinese,[27] Mexican-American,[5] African-American,[5] and Caucasian[6,7] populations, but are lower versus data in Pima Indians[5] and Japanese[9,13,28] and higher versus data in Samoans and Nauruans.[27] Therefore,

Table 1. Genotype and Allele Frequency of the β_3-Adrenergic Receptor Gene Trp64Arg Mutation in Patients With CHD and Controls

	CHD Patients		Controls	
Genotype/Allele	No.	%	No.	%
No. of subjects	137		188	
Male/female ratio	102/35		140/48	
Genotype				
Trp/Trp	111	81.0	139	73.9
Trp/Arg	24	17.5	45	23.9
Arg/Arg	2	1.5	4	2.1
	χ^2 = 2.25		*df* = 2, *P* = .325	
Allele				
Trp	246	89.8	323	85.9
Arg	28	10.2	53	14.1
	χ^2 = 2.18		*df* = 1, *P* = .139	

Table 2. Characteristics (mean ± SEM) of Patients With CHD and Controls According to Genotype of β_3-Adrenergic Receptor

Characteristic	CHD Patients			Controls		
	Arg Mutation	No Mutation	*P*	Arg Mutation	No Mutation	*P*
No. of subjects	26	111		49	139	
Male/female ratio	10/16	25/86		14/35	34/105	
Age (yr)	63 ± 2	61 ± 1	.57	58 ± 1	57 ± 1	.57
BMI (kg/m^2)	25.6 ± 0.6	25.2 ± 0.3	.60	23.3 ± 0.4	23 6 ± 0.3	.65
Waist to hip ratio	0.93 ± 0.01	0.93 ± 0.01	.59	0.91 ± 0 01	0.91 ± 0.01	.84
SBP (mm Hg)	130 ± 3	131 ± 2	.80	121 ± 2	119 ± 1	.33
DBP (mm Hg)	80 ± 2	80 ± 1	.82	77 ± 1	77 ± 1	.80
FPG (mmol/L)	7.0 ± 0.6	6.7 ± 0.2	.62	5.3 ± 0.1	5.5 ± 0.1	.09
FPI (pmol/L)	120 ± 48	108 ± 12	.20	72 ± 12	60 ± 6	.07
Glucose response (mmol · L^{-1} · h^{-1})*	27.8 ± 2.3	32.7 ± 1.2	.07	21 6 ± 0.5	21.3 ± 0.3	.78
Insulin response (pmol · L^{-1} · h^{-1})*	1,278 ± 2,174	1,776 ± 210	.27	1,386 ± 150	1,380 ± 66	.26
Triglyceride (mmol/L)	1.9 ± 0.2	2.0 ± 0.2	.80	1.1 ± 0.1	1.2 ± 0.1	.63
Cholesterol (mmol/L)	5.0 ± 0.2	5.1 ± 0.1	.64	4.6 ± 0.2	4.8 ± 0.1	.36
LDL-C (mmol/L)	3.3 ± 0.2	3.3 ± 0.1	.85	2.6 ± 0.2	2.9 ± 0.1	.08
HDL-C (mmol/L)	0.9 ± 0.1	1.0 ± 0.0	.77	1.2 ± 0.1	1.2 ± 0.0	.79
Total/HDL-C ratio	5.6 ± 0.3	5.7 ± 0.2	.84	4.2 ± 0.2	4.4 ± 0.1	.54

Abbreviation: FPG, fasting plasma glucose; FPI, fasting plasma insulin.

*CHD, n = 11 with Arg mutation and n = 47 without mutation; controls, n = 30 with Arg mutation and n = 91 without mutation.

Trp64Arg polymorphism is unlikely to play a major role in the development of CHD in our population. These results are in contrast to a recent report by Higashi et al,[13] who demonstrated that the frequency of this mutation was higher in Japanese patients with CHD (0.247) versus a group of control subjects (0.145). The reason for the difference between studies is not clear, but three possibilities can be considered. First, there may be differences in the factors affecting CHD development. Nevertheless, these two groups of CHD subjects were all nondiabetic and had relatively similar age, degree of obesity, and lipoprotein levels. A second possibility is a racial difference, ie, in genetic and environmental background. The allele frequency of Trp64Arg polymorphism was reported to be higher in the Japanese population (0.19 in 5,430 people) than in Chinese subjects[29] either in the current study (Table 1) or a previous one (0.12 in 104 people).[27] Third, the sample size was not sufficient to clarify this issue. A larger sample size is needed to understand the genetic variant in the development of CHD in different populations.

The principal functions of the β_3-adrenergic receptor are to mediate lipolysis in fat cells and thermogenesis in brown adipose tissue.[3,4] Some investigators have reported the existence of functional β_3-adrenergic receptors in human omental adipocytes that enhance the delivery of free fatty acid to the portal vein and cause retention of visceral fat and insulin resistance.[30] Accordingly, previous studies have shown an increased weight gain in obese heterozygous carriers and healthy white homozygous carriers of this mutation, and a reduced resting metabolic rate in type 2 diabetic subjects homozygous for this mutation.[7,31] However, inconsistent results were obtained from a subsequent study over a 12-year period, which showed that this mutation was not associated with the BMI, visceral fat, resting metabolic rate, or changes in body weight and fat.[32] Furthermore, a recent study by Li et al[33] showed that the β_3-adrenergic receptor carrying this mutation displayed a normal lipolytic function in response to a partial agonist (CGP12177) in visceral fat obtained from both obese and non-obese subjects. Thus, it has been suggested that the Trp64Arg mutation in the heterozygous form does not play a major role in regulating β_3-adrenergic function in visceral obesity and in the susceptibility to obesity.[34]

It has been reported that subjects with Trp64Arg mutation are likely to have hyperinsulinemia and insulin resistance.[6,28] However, in the present study, no significant relations between this polymorphism and these phenotypes were detected in either CHD patients or control subjects. These findings are in accordance with a recent report from Rissanen et al,[8] who demonstrated that this mutation is not associated with type 2 diabetes mellitus or the insulin resistance syndrome in nondiabetic subjects. Another report in a Japanese population also indicated that this mutation was not associated with hyperinsulinemia or insulin resistance in hypertensive subjects.[9] Therefore, it was postulated that despite the location of the Trp64Arg polymorphism in the first intracellular loop of the β_3-adrenergic receptor, which may be critical for its proper function in signal transduction,[5,35] a positive association does not necessarily indicate a causative gene defect in the putative site, but can also result from other genetic abnormalities in neighboring regions or from chance.[8] Recently, Candelore et al[36] introduced a Trp to Arg substitution in the β_3-adrenergic receptor gene in Chinese hamster ovary (CHO) cells by site-directed mutagenesis. They found no change in the binding or adenylate cyclase activation of this mutation expressed in CHO cells. On the other hand, there is another report showing that the absolute amount of accumulated cyclic adenosine monophosphate was lower in CHO cells transfected with the mutated β_3-adrenergic receptor gene versus the wild-type receptors.[37] It was even suggested that the Trp to Arg substitution could result in decreased activation of the hormone-sensitive lipoprotein lipase and hence decrease lipolysis.[29] All of these findings suggest that the influence of β_3-adrenergic receptor gene mutation on the insulin resistance syndrome remains to be determined.

Although it was not our main purpose, we again confirmed

our data and other findings[10-12] that nondiabetic normotensive CHD subjects, as a group, are glucose-intolerant, hyperinsulinemic, and insulin-resistant compared with non-CHD individuals.

We conclude that Trp64Arg polymorphism of the β_3-adrenergic receptor gene does not likely play a major role in the development of CHD in the Chinese population. In addition, it appears to have no association with the insulin resistance syndrome in either CHD or non-CHD subjects.

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