A Positive Association Between Homeostasis Model Assessment of Insulin Resistance Score and the Trp64Arg Polymorphism of the β3-Adrenergic Receptor Gene in Schizophrenia Patients in Taiwan

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Objective: To investigate the possible association between the Trp64Arg polymorphism of the β3-adrenergic receptor gene and the homeostasis model assessment of insulin resistance (HOMA-IR) index in schizophrenia patients in Taiwan.

Method: A total of 203 inpatients who met *DSM-IV* diagnostic criteria for schizophrenia were recruited from a psychiatry center in Taiwan from September 2002 to August 2003. All patients had been treated with antipsychotics for at least 6 months. The genotyping of the Trp64Arg polymorphism of the β3-adrenergic receptor gene was done by the polymerase chain reaction–restriction fragment length polymorphism method with the restriction enzyme MvaI. The HOMA-IR index was used to indicate the degree of insulin resistance.

Results: After adjusting for sex, age, and body mass index status, the association between the HOMA-IR index and the Trp64Arg polymorphism of the β3-adrenergic receptor gene was still positive (regression coefficient = −0.65, $P = .033$).

Conclusions: The polymorphism of the β3-adrenergic receptor gene may be related to the development of insulin resistance in chronic schizophrenia patients in Taiwan.

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A growing body of evidence suggests a higher prevalence of metabolic abnormalities in schizophrenia patients, such as insulin resistance, hyperglycemia, and new-onset diabetes mellitus or dyslipidemia. For example, the prevalence rate of diabetes among patients with schizophrenia was estimated to be around 15% or an increased risk of 2- to 3-fold in previous reports.[1] Perez-Iglesias et al[2] followed 144 drug-naive schizophrenia patients for 1 year and found a statistically significant increase in the mean values of insulin levels and insulin resistance indexes. Kahn et al[3] evaluated cases of first-episode schizophrenia after the first year of antipsychotic treatment in an open pragmatic randomized controlled trial and reported high rates of hyperglycemia (23.4%) and hypertriglycemia (35%). Green et al[4] described a significant increase in total cholesterol but not in fasting glucose level in a cohort of 263 first-episode patients treated with haloperidol or olanzapine for 2 years. Although the aforementioned studies did not support the hypothesis that schizophrenia is associated with an underlying abnormality in glucose metabolism, there is evidence indicating that a high level of insulin resistance exists prior to the effects of treatment.[5,6] Venkatasubramanian et al found a significantly higher mean insulin resistance score of antipsychotic-naive schizophrenia patients (n = 44) relative to healthy controls matched by age and sex.[7] Identifying and treating insulin resistance in advance may help to prevent potential cardiovascular and diabetes risks of these patients in the future.[8]

The β3-adrenergic receptor (ADRB3) is expressed predominantly in human visceral fat and is the principal mediator of catecholamine-stimulated thermogenesis and lipolysis. Therefore, ADRB3 dysfunction leads to visceral obesity, correlated with insulin resistance through its effect on energy expenditure of fat tissue.[9] In 1995, a missense mutation in codon 64 of the *ADRB3* gene with a replacement of tryptophan to arginine (Trp64Arg) was reported to be associated with insulin resistance or obesity.[10] It was associated with an earlier onset of non–insulin-dependent diabetes mellitus in Pima,[10] central obesity and hyperinsulinemia in Japanese,[11] weight gain in French,[12] and insulin resistance in Finnish populations.[13] Later, in vitro studies on fat cells demonstrated that this Arg carrier did have a 10-fold decrease in ADRB3 receptor sensitivity.[14] A consistent small effect of this *ADRB3* mutation on body mass index (BMI) was replicated in 3 meta-analyses,[15] but the association of

Trp64Arg with insulin resistance was still controversial. Zhan et al[16] used cross-sectional data from 128,052 individuals by meta-analyses and concluded that the association was only significant in the Asian population, albeit to a limited degree, increasing homeostasis model assessment (HOMA) by 0.55 in the Arg gene carriers.

Although a higher insulin resistance may endanger schizophrenia patients with more potential health-related risks, the association between this genetic variant and insulin resistance or obesity in schizophrenia patients is less investigated. Current evidence suggests that the effect of this *ADRB3* mutation on insulin resistance is ethnically heterogeneous. Thus, we investigated the association of the *ADRB3* Trp64Arg variant with insulin resistance and central obesity in schizophrenia patients of Taiwanese ethnic background.

METHOD

Patients

In this cross-sectional study, 203 inpatients who met *DSM-IV* criteria for schizophrenia were recruited from a psychiatry center in Taiwan from September 2002 to August 2003. All patients had been treated with antipsychotics for at least 6 months. The study protocol was approved by the Ethics Committee of the participating hospital (YLH-IRB-93001), and all participants completed the consent forms. A brief medical history, current psychopharmacotherapy, and daily activity level of each study subject were collected by a questionnaire administered by a trained interviewer or chart review. Advice on diet, exercise, and lifestyle was given to all patients. Anthropometrics measurement was done before breakfast. The body weight of each subject was checked again 1 month later. Body mass index (kg/m^2) and waist-hip ratio were calculated for each individual.

Blood Assay and Genotyping

Venous blood samples were collected following routine procedure after an overnight fast. Blood glucose was measured by the hexokinase method, and the serum insulin concentration was assayed by routine chemiluminescent immunoassay in the laboratory of the participating hospital. Homeostasis model assessment was used to assess insulin resistance (HOMA-IR). The HOMA-IR index was calculated according to Matthews et al[17] as follows: (fasting blood sugar in mg/dL) × (serum insulin level in µu/mL) ÷ 405. In addition, we calculated the triglyceride/high-density lipoprotein (HDL) cholesterol ratio as a predictor of insulin resistance using the cutpoint of 3.5 described by McLaughlin et al.[18] The DNA was isolated from peripheral leukocytes using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota). The genotyping of *ADRB3* polymorphism was done by the polymerase chain reaction–restriction fragment length polymorphism method with the restriction enzyme MvaI as described previously.[12]

Statistical Analysis

The statistical analysis was performed with the SPSS for windows, version 12.0 (SPSS Inc, Chicago, Illinois). Data were expressed as mean ± SD. The *t* tests were used to compare genotypes for differences of continuous variables. Multiple linear regression analysis controlled for age, sex, BMI, and exercise level was performed to evaluate the effects of the *ADRB3* gene polymorphism on the HOMA-IR index. The value *P* < .05 was accepted as statistically significant.

RESULTS

Basic Data

A total of 203 inpatients were recruited into the study. The basic data are shown in Table 1. About 30% of patients reached the definition of obesity (BMI ≥ 27[19]), 32.5% reached the definition of insulin resistance (HOMA-IR > 2.5),[20] and 29.6% had triglyceride/HDL ratios > 3.5. Hypercholesterolemia and hypertriglyceridemia were defined as serum cholesterol level > 220 mg/dL and triglyceride levels > 150 mg/dL; 9.4% and 28.6% of our sample fulfilled the criteria of hypercholesterolemia and hypertriglyceridemia, respectively. Also, 15.8% of patients had fasting blood sugar levels over 110 mg/dL, and 14.3% had fasting insulin levels > 15.6 µu/mL. The waist-hip ratio (the circumference of the waist divided by that of the hips > 0.9 for men and > 0.85 for women) is used as a measure

Table 1. Data for 203 Inpatients With Schizophrenia in Taiwan[a]

Variable	Men (n = 106)	Women (n = 97)
Age, y	42.6 ± 12.2	45.0 ± 10.6
Age at onset, y	23.3 ± 8.3	22.3 ± 7.1
Body mass index (kg/m²)	24.3 ± 4.12	25.1 ± 3.8
Education, y	10.0 ± 3.3	8.8 ± 3.8
Fasting blood sugar (mg/dL)	99.9 ± 22.7	100.0 ± 18.3
Insulin (μu/mL)	9.4 ± 6.7	9.6 ± 7.2
HOMA-IR	2.4 ± 1.9	2.4 ± 2.2
HDLs (mg/dL)	47.1 ± 13.1	53.0 ± 14.2
LDLs (mg/dL)	105.7 ± 29.4	100.2 ± 27.2
Total cholesterol (mg/dL)	174.4 ± 32.2	174.1 ± 32.7
Triglycerides (mg/dL)	142.9 ± 91.3	145.7 ± 65.8
Combined glucose intolerance	4.1 ± 1.0	4.0 ± 1.2
Smoking (packs/d)	0.5 ± 0.5	0.1 ± 0.4

[a]Data are presented as mean ± SD.
Abbreviations: HDLs = high-density lipoproteins, HOMA-IR = homeostasis model assessment of insulin resistance, LDLs = low-density lipoproteins.

Table 2. The Correlation of HOMA-IR With Other Continuous Variables

Variable	Correlation Coefficient	P Value[a]
Age	−0.196	.005
Body mass index	0.407	.000
Waist/hip ratio	0.227	.001
LDLs	0.173	.014
Total cholesterol	0.162	.022
Triglycerides	0.265	.000
HDLs	−0.243	.001
Triglycerides/HDLs	0.260	.000

[a]$P < .05$ by Pearson correlation.
Abbreviations: HDLs = high-density lipoproteins, HOMA-IR = homeostasis model assessment of insulin resistance, LDLs = low-density lipoproteins.

of central obesity. About 64.5% of our sample fulfilled the criteria of central obesity. The HOMA-IR index was found to be correlated with age, central obesity, and triglyceride/HDL ratio in our sample (Table 2).

Frequency of the Trp64Arg Allele

The overall frequency of the 64Arg allele was 12.3%. None were Arg/Arg homozygous, 24.6% were heterozygous, and 75.4% were Trp/Trp homozygous.

The Association Between Genetic Polymorphism, HOMA-IR, and Obesity

There was no significant difference of age, age at onset, combined glucose intolerance, gender distribution, or BMI among different genotypes as shown in Table 3. However, there was a statistically significant difference of HOMA-IR index among different genotypes. After adjusting for each participant's sex, age, and BMI status, the association between HOMA-IR index and the Trp64Arg polymorphism of the β3-adrenergic receptor gene was still positive (regression coefficient = −0.65, P = .033). Using BMI ≥ 27 as the criteria of obesity, we found that the

Table 3. Comparisons of Genetic Variants for Variables[a]

Variable	Trp/Trp	Arg/Trp
Sex (male/female), n	79/74	27/23
Age, y	44.2 ± 11.9	42.5 ± 10.3
Age at onset, y	23.1 ± 8.1	22.2 ± 6.7
Combined glucose intolerance	4.1 ± 1.1	4.1 ± 1.0
Body mass index (kg/m²)	24.7 ± 4.1	24.5 ± 3.4
Insulin (μu/mL)	10.0 ± 7.4	8.1 ± 4.7
Smoking (packs/d)	0.3 ± 0.5	0.4 ± 0.5
HOMA-IR[b]	1.9 ± 1.3	2.6 ± 2.2
Triglycerides/HDLs	3.3 ± 3.6	3.1 ± 2.2
Waist/hip ratio	0.9 ± 0.6	0.9 ± 0.8
Body weight change (kg/mo)	1.2 ± 2.8	0.52 ± 2.6

[a]Data are presented as mean ± SD unless otherwise specified.
[b]$P = .025$ by test.
Abbreviations: HDLs = high-density lipoproteins, HOMA-IR = homeostasis model assessment of insulin resistance.

Trp64Arg variant was associated with insulin resistance in the obese group (P = .004 by t test), while no significant association was observed in the nonobese group.

DISCUSSION

The frequency of the Arg carrier of the *ADRB3* gene in our sample was 12.3%. This rate is lower than those reported in Pima Indian and Japanese subjects but is similar to previous reports of 13.9% in other Taiwanese populations.[21,22] In our sample, the 64Arg variant of the *ADRB3* gene was not associated with age at onset of schizophrenia or combined glucose intolerance.

The Arg carrier of the *ADRB3* gene was not associated with any surrogate indicator of obesity such as BMI or waist-hip ratio in our sample. Tsai et al[23] found no significant association of this *ADRB3* variant with clozapine-induced weight gain in 87 schizophrenia patients in Taiwan. However, a consistent small effect of the *ADRB3* variant on BMI was demonstrated in at least 3 meta-analyses.[15] Obesity is a complex phenotype that involves multiple genetic variants interacting with environmental and behavior factors. There are many other putative candidate genes of obesity that were not considered in our sample, and this may offer an explanation of inconsistency. Ujike et al[24] identified genetic variants of serotonin-2A (5-HT$_{2A}$) receptors, 5-HT$_{2C}$ receptors, the G-protein β3 subunit, and the *ADRB3* gene as possible genetic risk factors for olanzapine-induced weight gain in 164 schizophrenia patients, and these genetic variants showed additive genetic effects on weight gain.

The *ADRB3* Trp64Arg variant was associated with insulin resistance in our sample. After adjusting for sex, age, and BMI status, the Arg carrier of the *ADRB3* gene was still associated with higher insulin resistance (regression coefficient = −0.65, P = .033). Using BMI ≥ 27 as the criteria of obesity, we found that the association of the Trp64Arg variant and insulin resistance existed

only in the obese group ($P = .004$ by t test) and not in the nonobese group. This is inconsistent with Tsai et al,[21] who surveyed 299 pregnant women and concluded that the *ADRB3* Trp64Arg variant has no effect on obesity but might be a possible determinant of insulin resistance in the Taiwanese population. Nagano et al[25] analyzed 89 Japanese patients with schizophrenia and concluded that the 64Arg allele, with a frequency of 22%, was not associated with obesity, type 2 diabetes mellitus, dyslipidemia, hypertension, or plasma insulin.

Our observation is consistent with the "insulin hypothesis" of obesity-associated insulin resistance.[26] In this regard, insulin resistance is believed to be a result of lipotoxicity. This hypothesis is supported by the well-established role of free fatty acids in the promotion of insulin secretion in pancreatic β cells and findings about GPR40 in the signal translation of free fatty acids in β cells. More important, it is the combination of insulin and free fatty acids that represents a promising mechanism for the initiation of obesity-associated insulin resistance. Under this concept, several hypotheses, including inflammation, diacylglycerol-protein kinase C, and endoplasmic reticulum and oxidative stress, have been developed to specify the cellular and molecular mechanism of lipotoxicity.[26] In fact, Trp64Arg variants of the *ADRB3* gene have already been reported to be associated with increased fasting free fatty acid concentration.[27] However, we did not check free fatty acid levels in our sample.

The Trp64Arg variant was not associated with insulin level in our sample. In the 50 patients who fulfilled the definition of BMI ≥ 27, the Trp64Arg variant was associated with lower fasting insulin levels ($P = .007$). There are reports that the *ADRB3* variant affected insulin secretion directly.[28] Hojlund et al[29] also found that twins heterozygous for the Trp64Arg polymorphism showed significantly lower fasting insulin and glucose concentration compared with their homozygous wild-type cotwins.

There are limitations in our study. First, the effect of antipsychotic drug was not controlled. However, since polypharmacy was prevalent in our sample, it was almost impossible to control the effect of psychopharmacotherapy. We also found that the BMI difference for patients who took first- and second-generation antipsychotics was insignificant statistically (data not listed here).

Also, the number of patients enrolled was small. A larger sample size may be required to test the relationship between obesity and the *ADRB3* gene in schizophrenia patients. Finally, there are many other important molecules such as free fatty acid and insulin-like growth factor-1[7] to be surveyed along with insulin level to clarify the complex association between insulin resistance and the *ADRB3* variant.

Insulin resistance is believed to develop long before other associated diseases, such as diabetes, hypertension, or cardiovascular diseases. Thus, identifying and treating insulin resistance in advance has great preventive value.[8] There is evidence suggesting that interventions such as weight loss, exercise, and increased dietary fiber intake may be beneficial to improve insulin sensitivity or even diabetes progression.[8] Through early detection, these effective interventions can be initiated as soon as possible in genetically susceptible patients to achieve better preventive effects.

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