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RAPID COMMUNICATION

Associations between the *GNB3* C825T polymorphism and obesity-related metabolic risk factors in Korean obese women

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Abstract
Purpose It is important to identify a 'metabolically unhealthy obese' subset with higher cardiovascular risk among obese individuals. We investigated the associations between the *GNB3* C825T polymorphism and obesity-related metabolic risk factors among Korean obese women.
Methods This study was a sub-investigation of a double-blind randomized controlled trial that examined the additive effect of or list at on weight loss with sibutramine. A sample of 111 obese women were divided into T-carriers (CT/TT) or a homozygous CC group, according to the presence of the 825T allele at *GNB3*. These groups were compared to determine their associations with obesity-related metabolic risk factors, i.e., fasting plasma glucose, serum lipids, serum insulin/insulin resistance, and abdominal fat amounts.
Results The allele frequencies of the *GNB3* polymorphism were C allele = 59.5 % and T allele = 40.5 %. The T allele was found to be significantly associated with greater visceral fat and higher serum lipids, and these significances remained robust after adjusting for potential covariates.
Conclusions The *GNB3* 825T polymorphism is significantly associated with greater visceral fat and higher serum lipids in Korean obese women and it suggests that the *GNB3* C825T is a determinant of obesity-related metabolic traits in this population.

Keywords G-Protein beta 3 subunit · Single nucleotide polymorphism · Metabolic risk factors

Abbreviations

WC	Waist circumference
SNP	Single nucleotide polymorphism
GNB3	Guanine nucleotide-binding protein beta 3 subunit
BMI	Body mass index
TG	Triglyceride
CT	Computed tomography
LDL-C	Low-density lipoprotein cholesterol
PCR	Polymerase chain reaction

Introduction

Obesity is a common health problem and is increasing world wide. Obesity is linked to metabolic derangements, but demonstrates a wide range of metabolic phenotypes. The concept of 'metabolically healthy/unhealthy obese' arose during recent years [1]. Moreover, several studies have shown that the 'metabolically healthy obese' are at reduced risk of cardiovascular diseases and mortality than the 'metabolically unhealthy obese' [2, 3].

Genetic factors contribute to obesity-related metabolic risk factors such as waist circumference (WC), plasma glucose, serum lipids, and insulin levels [4], and thus, it is important to identify genes responsible. Single nucleotide polymorphisms (SNPs) in guanosine nucleotide-binding proteins (G proteins) may be crucial because G proteins control a broad range of biological processes, including

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energy homeostasis and lipid metabolism [5]. The C825T polymorphism of the G protein beta 3 subunit (*GNB3*) gene has been most extensively studied and has been reported to be weakly and inconsistently associated with some obesity-related metabolic risk factors in general population [6–8].

Thus, the aim of this study was to evaluate associations between the *GNB3* C825T SNP and obesity-related metabolic risk factors (fasting plasma glucose, serum lipids, serum insulin/insulin resistance and abdominal fat amounts) in a sample of Korean obese women to identify the susceptible subpopulations.

Methods

Design and subjects

This study was a sub-investigation of a double-blind randomized controlled trial designed to assess the additive effect of or list at on weight loss with sibutramine. Details of the study design were described in a previously published article [9]. Inclusion criteria were women aged 19–49, no history of anti-obesity drug use, and a body mass index (BMI) of $\geq$27 kg/m^2. Exclusion criteria were obesity with endocrine abnormalities, hypercortisolism, uncontrolled hypertension, known history of diabetes mellitus and/or diabetic therapy. This study was conducted with 111 Korean obese women at baseline of randomized controlled trial who satisfied the eligibility criteria. The study was approved by the Institutional Review Board of Gachon University Gil Medical Center.

Anthropometry and assessment of body fatness

Height and weight were measured using an automatic digital stadiometer (in Body BSM330, Biospace, Seoul, South Korea). BMI was calculated by dividing body weight in kilograms by height in meters squared. Based on the World Health Organization protocol, WC was measured at the midpoint between the inferior costal margin and the superior border of the iliac crest on the mid axillary line. Fat mass was measured by Dual-energy X-ray absorptiometry (Lunar Prodigy, Lunar Corp., Madison, CA, USA), and body fat percentage was calculated by dividing the absolute value of fat mass by total body mass. Abdominal adipose tissue was measured by computed tomography (CT; Somatom Sensation 64, Siemens, Erlangen, Germany).

Laboratory tests

All blood samples were obtained from each subject in the morning after a 12-hour overnight fast. Blood glucose levels were measured using a glucose oxidase procedure. Serum total cholesterol, triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) were measured enzymatically. Serum insulin was measured using radioimmunoassay kit (Coat-A-Count Insulin; Diagnostic Products Corp., Los Angeles, CA, USA). Insulin resistance was calculated by dividing the product of fasting plasma insulin level (in micro units per milliliter) and fasting plasma glucose level (in millimoles per liter)by 22.5 (homeostasis model assessment of insulin resistance [HOMA-IR] equation) [10].

Gene analysis

For genetic analysis, DNA was extracted using the 96 Genomics Blood Kit (NucleoGen, Siheung, South Korea), according to the manufacturer's protocol. Polymerase chain reaction (PCR)was performed using an amplification protocol consisting of cycles of denaturation, annealing, and extension. The *GNB3* 825C > T (rs5443) polymorphism was confirmed by directed sequencing using the Big Dye Terminator Cycle Sequencing Kit v3.1 and a 3730XL DNA Analyzer (ABI, Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Subjects were divided into T-carriers (CT/TT) and a homozygous CC group, according to the presence of the 825T allele at *GNB3*. To estimate associations between the *GNB3* C825T SNP and obesity-related metabolic risk factors, the groups were compared using the independent *t* test. The adjusted means of serum lipids and visceral fat areas of the two groups were compared, using regression models with adjustment for covariates (serum lipids: age, body percentage; visceral fat area: age, body fat percentage, and serum insulin level). All statistical tests were conducted using the statistical package R software V2.9.2 (R Development Core Team 2009). *P* values of <0.05 were considered statistically significant for all tests.

Results

The genotype frequencies of the *GNB3* polymorphism were CC = 34.2 %, CT = 50.5 %, TT = 15.3 % and allele frequencies were C allele = 59.5 % and T allele = 40.5 %. These allele frequencies concurred with previously reported values [11], when evaluated for Hardy–Weinberg equilibrium using a χ^2 goodness-of-fit test.

A summary of obesity-related metabolic risk factors according to the presence of the T allele is presented in Table 1. No significant difference in age was evident

Table 1 Obesity-related metabolic risk factors according to the presence of the 825T allele at *GNB3*

	GNB3 genotype		P^a
	With T allele ($n = 73$) (CT/TT)	Without T allele ($n = 38$) (CC)	
Age (years)	37.1 ± 0.9	35.5 ± 1.3	0.30
Body mass index (kg/m^2)	31.8 ± 0.5	30.7 ± 0.5	0.15
Waist circumference (cm)	96.0 ± 1.0	93.5 ± 1.4	0.14
FPG (mg/dL)	90.8 ± 1.3	91.1 ± 1.3	0.89
Serum lipids (mg/dL)			
Total cholesterol	205.7 ± 3.4	185.4 ± 5.9	<0.01
Triglyceride	152.6 ± 9.1	118.2 ± 10.6	0.02
LDL-C	129.9 ± 2.7	116.4 ± 3.8	<0.01
Serum insulin (μU/mL)	9.8 ± 0.8	8.0 ± 0.7	0.13
Insulin resistance[b]	2.2 ± 0.2	1.8 ± 0.2	0.12
Body fat percentage, %[c]	41.2 ± 0.4	40.6 ± 0.6	0.39
Abdominal fat area [2d], cm			
Subcutaneous	312.1 ± 10.8	301.2 ± 14.3	0.55
Visceral	127.0 ± 4.7	104.5 ± 5.7	<0.01

Data are expressed as means ± standard deviations

GNB3 guanine nucleotide-binding protein beta 3 subunit, *FPG* fasting plasma glucose, *LDL-C* low-density lipoprotein cholesterol

[a] *P* values were calculated using the independent *t* test

[b] Calculated by dividing the product of the fasting plasma insulin level (in micro units per milliliter) and the fasting plasma glucose (in millimoles per liter) by 22.5

[c] Measured by dual-energy X-ray absorptiometry

[d] Measured by abdominal computed tomography

Table 2 Adjusted means of serum lipids and visceral fat according to the presence of the 825T allele at *GNB3*

	GNB3 genotype		P^a
	With T allele ($n = 73$) (CT/TT)	Without T allele ($n = 38$) (CC)	
Serum lipids (mg/dL)			
Total cholesterol	204.7 ± 3.6	187.5 ± 5.0	<0.01
Triglyceride	152.7 ± 8.4	118.0 ± 11.7	0.02
LDL-C	129.3 ± 2.7	117.6 ± 3.7	0.01
Visceral fat area[b] (cm^2)	124.1 ± 3.9	110.0 ± 5.5	0.04

Data are expressed as adjusted means ± standard deviations

GNB3 guanine nucleotide-binding protein beta 3, *LDL-C* low-density lipoprotein cholesterol

[a] *P* values were determined using regression models with adjustment for covariates (serum lipids: age, body fat percentage; visceral fat area: age, body fat percentage, and serum insulin level)

[b] Measured by abdominal computed tomography

between the CT/TT and CC groups. The *GNB3* 825T allele was significantly associated with greater visceral fat and higher levels of total cholesterol, TG, and LDL-C. However, there was no evidence of associations between the *GNB3* C825T polymorphism and other obesity-related measures (BMI, WC, fasting plasma glucose, serum insulin/insulin resistance, and body fat percentage). After adjusting for covariates, the adjusted means of visceral fat areas ($P = 0.04$) and serum lipids ($P < 0.01$ for total cholesterol, $P = 0.02$ for TG, and $P = 0.01$ for LDL-C) of the two groups still showed significant differences (Table 2).

Discussion

In this study, the *GNB3* 825T polymorphism was found to be significantly associated with greater visceral fat and

higher levels of serum lipids. To the best of our knowledge, this is the first study to report the relation between the *GNB3* C825T polymorphism and abdominal visceral fat.

Catecholamines have pronounced lipolytic properties, and catecholamine-induced lipolysis is markedly dependent on body regions (i.e., subcutaneous vs. Visceral). Catecholamine-induced lipolysis in visceral adipose tissue is increased, especially in the obese, whereas there is lipolytic resistance to catecholamines in subcutaneous adipose tissue [12]. Furthermore, it has been reported that the *GNB3* 825T polymorphism is possibly associated with blunted lipolytic response due to diminished function of Gprotein-coupled adrenergic receptors [13, 14]. Therefore, the *GNB3* 825T polymorphism can cause abdominal visceral fat accumulation, comparable to our study results. Although the mechanisms where by the *GNB3* C825T polymorphism affects lipid metabolism is not fully understood, previous studies suggested that LDL receptors are modulated by *GNB3* variants [15, 16]. In agreement with our results, a Japanese study showed that subjects with the T allele had higher levels of total cholesterol than CC homozygotes [17]. However, Danishand Taiwanese studies produced conflicting results, showing no association with serum lipids and higher levels of total cholesterol and T Gamong the carriers of CC variant, respectively [6, 18]. One possible explanation for the discrepancies is that these studies were conducted in populations that differed in terms of age, gender, ethnicity, and baseline obesity. Furthermore, the studies also had different sample sizes and study designs.

No association between the T allele and insulin resistance was found. One study found that insulin resistance was significantly higher in carriers of the T allele in the male subjects with abdominal fat distribution (waist-to-hip ratio >0.9) [7]. This discrepancy suggests a sex- and body fat distribution-specific association of the T allele with insulin resistance.

There are some limitations in this study. First, our findings were obtained from obese Korean women and may not be applicable to other populations. Second, the relatively small size of the sample does not allow definite conclusions. Third, other polymorphisms in the *GNB3* gene could be in linkage disequilibrium with the C825T polymorphism [19]. Therefore, the C825T polymorphism itself might only be a marker of other as-yet-unidentified genetic variants involved in the metabolic alterations. In conclusion, our findings suggest that the *GNB3* 825T may determine obesity-related metabolic traits in Korean obese women. Future association studies are needed in larger sample sizes to confirm the results of this study.

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Conflict of interest The authors have no conflicts of interest.

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