Impact of GNB3-C825T, ADRB3-Trp64Arg, UCP2-3'UTR 45 bp del/ins, and PPAR γ -Pro12Ala Polymorphisms on *Bofutsushosan* Response in Obese Subjects: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT Obesity is known to be influenced by a number of genes, including the $\beta 3$ subunit of G protein (*GNB3*), $\beta 3$ -adrenergic receptor (*ADRB3*), uncoupling protein 2 (*UCP2*), and peroxisome proliferator activated receptor gamma (*PPAR γ*). The single nucleotide polymorphisms (SNPs) of the above genes, such as GNB3-C825T, ADRB3-Trp64Arg, UCP2-3'UTR 45 bp del/ins, and PPAR γ -Pro12Ala, are associated with obesity and body mass index. The present study evaluates the impact of *Bofutsushosan*, a traditional Eastern Asian herbal medicine with known anti-obesity properties, on obese subjects according to the presence of the above-mentioned SNPs. Upon randomization, the volunteers were allocated to receive *Bofutsushosan* ($n=55$) or placebo ($n=56$) treatments for 8 weeks. Following the treatment schedule, significant reductions in total cholesterol and significant improvement in the Korean version of obesity-related quality of life scale were seen in the *Bofutsushosan*-treated group, but not in placebo. *Bofutsushosan* exerted significant anti-obesity effects on a number of parameters in the carriers of the GNB3-825T allele, but only on waist circumference in the GNB3-C/C homozygote. Significant anti-obesity impact of *Bofutsushosan* was also seen on a number of obesity-indices in both ADRB3-Arg64 carriers and ADRB3-Trp64 homozygotes, as well as in UCP2-D/D carriers, but not in UCP2-D/I+I/I variants. The effect of *Bofutsushosan* was more pronounced in PPAR γ -Pro/Pro genotype compared to PPAR γ -Pro/Ala variants. Thus, the results revealed differential responses of the subjects to the anti-obesity effects of *Bofutsushosan* treatment according to the polymorphism of the vital obesity-related genes. Our study provides new insight into individualized clinical applications of *Bofutsushosan* for obesity.

KEY WORDS: • *Bofutsushosan* • obesity • single nucleotide polymorphism • clinical trial

INTRODUCTION

OBESITY IS ONE OF THE MOST ALARMING global public health problems, contributing to type 2 diabetes, hypertension and cardiovascular diseases, many forms of cancer, and death. Obesity is influenced by genetic and environmental factors and their interactions. Many studies have focused on the associations between specific genes and obesity phenotypes, with 426 findings of positive associations with 127 candidate genes.¹ The most significant observation is that 22 genes are each supported by at least five

positive studies.² Accordingly, *GNB3*, *ADRB3*, *PPAR γ* , and *UCP2* have been found to be encoded by genes associated with obesity and body composition.²

The pharmacogenomics of obesity is a new and challenging area of research, which could be a valuable tool to understand why some patients being treated for obesity respond to pharmacological and/or behavioral interventions, while others do not (cited by Irizarry *et al.*).³ A single nucleotide polymorphism (SNP) is a variation in a single base pair in the DNA sequence of a particular gene, such as insertion, deletion, or substitution of a base, and represents the most common form of genetic variation. For the past few years, SNPs have been used extensively to explore obesity candidate gene regions, selected on functional and/or positional basis. Accordingly, GNB3-C825T, ADRB3-Trp64Arg, UCP2-3'UTR 45 bp del/ins, and PPAR γ -Pro12Ala polymorphisms have been demonstrated to be SNPs that are associated with obesity and body mass index (BMI).^{1,4–18}

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Bofutsushosan, a traditional Eastern Asian herbal medicinal formulation that contains 18 crude drugs, is an effective agent for treating various disease states, especially obesity and metabolic disorders. Previous animal studies have demonstrated the anti-obesity effects of *Bofutsushosan*.¹⁹ Experimental results with rodent models have suggested that *Bofutsushosan* prevents adipogenesis in white adipocytes through the modulation of gene expression,²⁰ and attenuates the weight and size gains of white adipose tissue (WAT), along with upregulation of uncoupling protein 1 (UCP1) mRNA in WAT.²¹ Additionally, a clinical trial on obese Japanese women concluded that *Bofutsushosan* could be a useful herbal medicine in treating obesity with impaired glucose tolerance.²² In our previous study on obese Korean volunteers where we evaluated the impact of *Bofutsushosan* on the Asian type obesity pattern, it was revealed that this herbal formulation significantly decreased body weight, BMI, waist circumference (WC), body fat mass, total cholesterol (T-Chol), and high-density lipoprotein cholesterol (HDL-Chol) in the subjects categorized under "liver Qi stagnation" pattern and WC in the subjects diagnosed with "indigestion" pattern.²³

Although there have been a few clinical reports concerning *Bofutsushosan* treatment, to the best of our knowledge, no studies have been conducted to address the influence of genetic variations of *GNB3*, *ADRB3*, *UCP2*, and *PPAR* γ simultaneously on *Bofutsushosan* response in obese subjects. Genetic polymorphisms greatly account for the interindividual differences in the responses to drug treatment for a particular disease, as well as for adverse drug reactions. For instance, type 2 diabetic patients with the Pro12Ala genotype in the *PPAR2* gene exhibited better therapeutic response to rosiglitazone (a thiazolidinedione class of antidiabetic drug) than did by patients with the Pro12Pro genotype.²⁴ In another study, a change in systolic blood pressure in hypertensive patients treated with irbesartan (an angiotensin II receptor antagonist, which is used primarily for the treatment of hypertension) was found to be related to the ApoA-IV A1449G, ApoA-V C31455T, and ApoB C711T SNPs of the apolipoprotein gene family.²⁵ The presence of the C-allele of the LDLR C16730T SNP of low-density lipoprotein receptor gene was found to be associated with decreased systolic blood pressure in hypertensive patients treated with atenolol (a selective β 1 receptor antagonist, which is used mainly for the treatment of hypertension and angina).²⁵ Furthermore, a clinical study demonstrated that overweight and obese participants with LS or SS but not LL genotype for serotonin transporter (*5-HTT*) gene had greater weight loss with sibutramine (an inhibitor of the reuptake of the neurotransmitters norepinephrine and serotonin, which was used before in the management of obesity) treatment compared to placebo.²⁶ Moreover, it has been revealed that the XmnI and BCL11A SNPs may help to predict the response to hydroxyurea (an antineoplastic drug, which can also reactivate fetal hemoglobin production and thereby is frequently used to treat β -thalassemia) in Iranian β -thalassemia patients.²⁷ It has been suggested that the presence of the XmnI T/T genotype or the BCL11A

rs766432 C allele correlates strongly with response to hydroxyurea.²⁷

The above observations prompted us to find whether any relationship exists between the genetic polymorphisms of *GNB3*-C825T, *ADRB3*-Trp64Arg, *UCP2*-3'UTR 45 bp del/ins, and *PPAR* γ -Pro12Ala and the anti-obesity impact of *Bofutsushosan*. This study, which represents the extension of our previous work, was conducted on obese Korean subjects.²³

SUBJECTS AND METHODS

Subjects

The clinical study presented here was part of the trial conducted by us in Dongguk University Oriental Hospital, Korea, to evaluate the efficacy and adverse effects of *Bofutsushosan* and *Boiogito* according to the oriental obesity pattern.²³ Briefly, a total of 166 obese subjects were enrolled following the initial screening of 169 volunteers who responded to advertisements in local newspapers or the Web site of Dongguk University Oriental Hospital. The volunteers were recruited between August 2009 and September 2010, and the study was completed in November, 2010. A total of 56 subjects were randomly assigned to the placebo group and 55 subjects to the *Bofutsushosan* group (Fig. 1).

To qualify for this study, subjects had to be ethnically Korean, in general good physical condition, a nonsmoker, 18–65 years of age, with a BMI ≥ 25 kg/m², and with a body weight that had remained stable within ± 3 kg during the previous 3 months. The basic aim and principles of the study, possible adverse effects of the treatment, as well as the right to withdraw from the study were thoroughly explained to all screened subjects. The subjects were then requested to read the informed consent forms carefully and sign the forms if they agreed to participate in the study. A physician interviewed and scrutinized the general health conditions of the volunteers. Exclusion criteria included: malignancy, heart, kidney, or liver diseases, history of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) enzyme activities > 2.5 times the normal upper limits, or level of creatinine > 2.0 mg/dL. Subjects receiving medications that may affect the body composition and metabolism, and subjects who had displayed a hypersensitive reaction to a drug were also excluded. Furthermore, pregnancy at any time during the study was also among the exclusion criteria. The detailed inclusion and exclusion criteria of the present study are summarized in Table 1. The medical, scientific, and ethical aspects of the study were approved by the Institutional Review Board of Dongguk University Oriental Hospital.

Study design

The study was a randomized, double-blind, placebo-controlled, 8-week trial. Following their recruitment, the subjects were randomized with the use of a table of random numbers compiled by an independent statistician. The active or *Bofutsushosan* group received *Bofutsushosan* capsules

FIG. 1. Flow chart of the participation history of the subjects. *Following randomization, 55 subjects (who were not part of the present study) received *Boiogito* treatment (for other study) that is not shown in this figure.

(12 capsules a day, each containing 237 mg of *Bofutsushosan* dry extract; Hanpoong Pharm. Co., Seoul, Korea), whereas the placebo group received capsules (12 capsules a day, 250 mg) filled with corn starch. The *Bofutsushosan* formulation is summarized in Table 2. The above formulation and dose of *Bofutsushosan* was selected on the basis of an earlier clinical study on Korean obese subjects performed for 12 weeks,²⁸ which revealed a marked impact on body weight, WC, and BMI of the subjects.

The randomization code was completely double-blinded to the research investigators and participants. The code was transferred to Hanpoong Pharm. Co. where *Bofutsushosan* and placebo capsules were manufactured and labeled according to the code, which was not revealed until the completion of the clinical trial. A serial number was marked on the surface of the box containing *Bofutsushosan* or placebo capsules, and the research pharmacist distributed those capsules to the participants. The capsules were administered orally three times a day, four capsules at one time. At each visit to the hospital, the capsules for a 2-week dose regimen were provided, and the

subjects were asked to revisit once every second week during the 8-week period of the study. The subjects were also asked to return the capsules that they did not take for any reason, and this parameter was counted as a means of determining their adherence to the dose regimen. If final compliance to the treatment schedule was <80%, the data for that subject were excluded due to the violation of the protocol of the clinical trial. The randomization code was unblinded only after all the clinical and laboratory data collection was completed.

Subjects were educated on the dietary guidelines and were asked to consume 20–25 kcal/day per kg body weight, and to note down their total dietary intake within the entire 24 h period just before the day of the visit. Upon each visit, their dietary intake was assessed during an interview and with reference to

TABLE 1. STUDY SELECTION CRITERIA

Inclusion criteria	Exclusion criteria
1. 18–65 years of age	1. Subjects with significant history or currently suffering from cardiovascular disease, hepatic or renal dysfunctions, cancer
2. BMI ≥ 25 kg/m ²	• ALT, AST > 2.5 times normal range
3. Stable body weight ($\leq \pm 3$ kg) in the preceding 3 months	• Creatinine > 2.0 mg/dL
4. Sedentary individuals	• Clinically significant abnormal ECG
5. Otherwise healthy nonsmokers	2. Patient taking drugs that could interfere with the study
6. Subjects signed informed consent forms	3. Known to have allergic responses or sensitivity to any of the medication
	4. Pregnancy

TABLE 2. THE COMPOSITION OF *BOFUTSUSHOSAN* DRY EXTRACT

Crude drug	Content (mg) ^a
Zingiberis Rhizoma Recens	16.7
Glycyrrhizae Radix	83.3
Scutellariae Radix	83.3
Gardeniae Fructus	50.0
Gypsum Fibrosum	83.3
Saposhnikovia Radix	50.0
Rhei Rhizoma	62.5
Natrii Sulfas	31.3
Platycodi Radix	83.3
Atractylodis macrocephalae Rhizoma	83.3
Schizonepetae Herba	50.0
Chuanxiong Rhizoma	50.0
Angelicae Gigantis Radix	50.0
Menthae Herba	50.0
Ephedrae Herba	50.0
Forsythiae Fructus	50.0
Talcum	125
Paeoniae Radix Alba	50.0

^aThe amount of each crude drug required to prepare 237 mg of *Bofutsushosan* dry extract.

the 24 h dietary recall notes. The data were recorded in the case report forms. The subjects were also asked to maintain their current lifestyle, without any new exercise program.

Measurements

The efficacy of treatment was assessed by measuring the changes in body weight, BMI, WC, body fat percent, body fat mass, resting metabolic rate (RMR), and serum clinical parameters, and by completion of the Korean version of obesity-related quality of life (KOQOL) scale. The safety of the treatment was evaluated by measuring the changes in cardiovascular parameters (blood pressure, heart rate, and electrocardiogram), liver enzymatic activities, and kidney function parameters, as well as by assessing the self-reported symptoms of the subjects. The genotypic analyses of the subjects to determine the mutation of particular genes were conducted before starting the treatment schedule.

The subjects' blood pressure was measured at every visit following a subject rest period of more than 10 min using a mercury sphygmomanometer (Sankei, Tokyo, Japan). The pulse rate was measured from the radial artery while the subject was sitting after resting for more than 10 min. The electrocardiogram of each subject was recorded and analyzed in a dedicated test room.

Anthropometric assessments

The height of each subject was measured to the nearest 0.1 cm at their first visit. Body weight was measured to the nearest 0.1 kg using a model GM1000 electro body weight scale (Neo GM Tech, Incheon, Korea) at their first visit and every second week of the study. WC was determined while each subject was in an upright posture by measuring the circumference of the midsection between the lowest costal margin and the highest iliac crest (3 cm top of the anterior superior iliac spine). The same skilled examiner verified the intravariability of the measurements, and the mean of three consecutive measurements was recorded.

Determination of body fat parameters

Body fat percent and body fat mass of the subjects were measured at the first visit and at weeks 4 and 8 using bioelectrical impedance (BIA) with an Inbody 720 unit (Biospace, Seoul, Korea).

Assessment of RMR

The RMR of the subjects were measured at the first visit, and at weeks 4 and 8 using a MedGem closed circuit indirect

calorimeter (Health Tech, Golden, CO, USA). The measurement was performed following a 30 min rest.

Measurement of serum clinical parameters

The serum biochemical parameters were assessed at baseline and at the end of the study. Levels of glucose and lipid parameters were measured from blood samples collected following a 12 h fast. All serum clinical parameters were analyzed as described previously.⁹

Evaluation of KOQOL scale

Each subject completed a questionnaire to evaluate the KOQOL scale as described previously.²⁹ The questionnaire is relatively simple to complete, but yields data that are highly reliable and accurate. For this measurement, each subject answered 15 questions on six different areas (mental and social health, physical health, job and domestic duty, daily life, sexual relations, and food). Based on the response, the KOQOL scale was determined. This evaluation was performed before and after the treatment schedule.

Genotype analyses

Genomic DNA was isolated from whole blood by proteinase K digestion using a QIAmp DNA blood Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The purity and concentration of DNA in the samples were determined spectrophotometrically. Subsequently, polymerase chain reaction (PCR) was performed to amplify the genomic DNA fragment representing a particular gene polymorphism. The amplification reactions were carried out using a commercial ready-to-use PCR reagent (AccuPower[®] PCR PreMix; Bioneer, Daejeon, Korea) in a total reaction volume of 20 μ L containing PCR PreMix, 1 μ L of cDNA, and specific primers (10 pmol for each). The sequences of the primers (Cosmogenetech, Seoul, Korea) are depicted in Table 3. The optimized annealing temperatures of the primers for PCR reactions were as follows: 60°C (C825T and Trp64Arg), 58°C (UCP2 del/ins), and 54°C (Pro12Ala). The PCR amplification conditions were as follows: a prior incubation step at 94°C for 10 min followed by 35 cycles of amplification encompassing denaturation at 94°C for 60 sec, annealing at the corresponding optimized temperature for 45 sec, extension at 72°C for 60 sec, and a final extension at 72°C for 10 min. The resultant PCR products were then either kept intact (for UCP2 del/ins) or digested with restriction enzymes *Bse*DI (for C825T; Fermentas, Thermo Fisher Scientific, Waltham, MA, USA), *Mva*I (for Trp64Arg; Takara Bio, Shiga, Japan), or *Hha*I

TABLE 3. PRIMER SEQUENCES USED FOR SINGLE NUCLEOTIDE POLYMORPHISMS ANALYSES

SNPs	Forward primer (5'-3')	Reverse primer (5'-3')	Ref.
C825T GNB3	TGA CCC ACT TGC CAC CCG TGC	GCA GCA GCC AGG GCT GGC	31
Trp64Arg ADRB3	CGC CCA ATA CCG CCA ACAC	CCA CCA GGA GTC CCA TCA CC	17,32
UCP2 del/ins	CAG TGA GGG AAG TGG GAG G	GGG GCA GGA CGA AGA TTC	33
Pro12Ala PPAR γ 2	TCT GGG AGA TTC TCC TAT TGG	GTG GAA GAC AAC TAC AAG AG	34

(for Pro12Ala; Takara Bio). Finally, all the products were subjected to electrophoresis in a 1.5% agarose gel. The resulting genotype-specific band patterns were as follows: C825T: T/T, single band of 268 bp; C/T, three bands of 268, 152, and 116 bp; C/C, two bands of 152 and 116 bp; Trp64Arg, Trp64 homozygote: five bands of 99, 62, 30, 12, and 7 bp; Trp64/Arg64 heterozygote (also termed “Arg64 carrier” in this study), six bands of 161, 99, 62, 30, 12, and 7 bp (notably, the 30, 12, and 7 bp fragments were too small to be resolved on the gel); Arg64 homozygote, expected to be represented by four bands of 161, 30, 12, and 7 bp (there were no subjects who were homozygous for Arg64); UCP2 del/ins: D/D, a single band of 457 bp; D/I, two bands of 457 bp and 502 bp; I/I, a single band of 502 bp; Pro12Ala: Pro/Pro, a single band of 154 bp; Pro/Ala, three bands of 154, 132, and 22 bp; Ala/Ala: expected to be represented by two bands of 132 and 22 bp (there were no subjects who were homozygous for Ala).

Statistical analyses

The data are expressed as mean $\pm$ SD. All statistical analyses were carried out using SAS v9.1 (SAS Institute, Cary, NC, USA). Statistical evaluation was performed by the intention to treat (ITT) approach to ensure unbiased comparisons among the experimental groups, and the last observation carried forward (LOCF) approach was used to impute values for the missing data.

Independent *t* tests were employed to determine the level of significance for the following: (1) the differences between the placebo and *Bofutsushosan* groups (a) in the general characteristics of the subjects at baseline (Table 4) and (b) in the changes in general and safety outcome variables of the subjects after intervention (Tables 5 and 11, respectively); (2) the difference in the change of a given outcome parameter between the allelic variants for *ADRB3*, *UCP2*, and *PPAR- γ 2* genes in *Bofutsushosan*-treated subjects (Tables 7–9). One-way analysis of variance (ANOVA) was conducted to determine the level of significance in the difference in the change of a given outcome variable among the SNPs of *GNB3* (Table 6). A paired *t* test was carried out for the comparison between pre- and post-treatment measurements of outcome variables under a particular evaluation (Tables 5–9 and 11). Differences were considered significant at $P < .05$.

RESULTS

Participation history and general characteristics of the subjects at baseline

There were no differences in the general characteristics of the subjects in the two groups at baseline (Table 4) except for T-Chol and HDL-Chol. Accordingly, a general linear model was applied to adjust the treatment effects on the above two cholesterol parameters. During the study, 29 subjects dropped out because of personal choice, noncompliance, or adverse effects of the treatments. Accordingly, 82 subjects completed the study, 42 in the *Bofutsushosan* group, and 40 in the placebo group (Fig. 1).

TABLE 4. BASELINE CHARACTERISTICS OF THE SUBJECTS

	Bofutsushosan (n = 55)	Placebo (n = 56)	P value ^a
Age, years	41.56 $\pm$ 8.62	39.21 $\pm$ 10.12	.191
Height, cm	160.5 $\pm$ 6.79	161.79 $\pm$ 7.85	.357
Body weight, kg	74.53 $\pm$ 10.98	76.86 $\pm$ 11.7	.279
WC, cm	97.63 $\pm$ 7.45	98.99 $\pm$ 7.81	.380
BMI, kg/m ²	29.72 $\pm$ 6.17	29.28 $\pm$ 3.11	.640
Body fat, %	38.33 $\pm$ 4.98	39.02 $\pm$ 5.39	.489
Body fat mass, kg	28.61 $\pm$ 6.14	29.87 $\pm$ 6.83	.309
RMR, kcal/d	1396.73 $\pm$ 383.55	1453.39 $\pm$ 335.02	.414
Fasting BST, mg/dL	99 $\pm$ 9.33	98.36 $\pm$ 9.04	.713
T-Chol, mg/dL	207.69 $\pm$ 39.36	191.84 $\pm$ 40.12	.038*
HDL, mg/dL	55.27 $\pm$ 14.7	50.29 $\pm$ 11.32	.047*
TG, mg/dL	127.35 $\pm$ 58.62	123.02 $\pm$ 67.02	.718
KOQOL	33.51 $\pm$ 7.6	34.52 $\pm$ 8.56	.513

^aBofutsushosan vs. placebo by independent *t* test.

* $P < .05$.

WC, waist circumference; BMI, body mass index; RMR, resting metabolic rate; BST, blood sugar test; T-Chol, total cholesterol; HDL, high-density lipoprotein; TG, triglyceride; KOQOL, Korean version of obesity-related quality of life.

Effects of treatments on clinical outcome

Following completion of the study, body weight, WC, BMI, body fat percent, and body fat mass were significantly reduced in both *Bofutsushosan* and placebo groups, although the changes were not significant between groups (Table 5). However, the levels of T-Chol and HDL-Chol after intervention were found to be significantly lower than those at baseline in the *Bofutsushosan* group ($P = .01$ and $.03$, respectively) but not in the placebo group. Moreover, following the treatment schedule, the KOQOL score was not changed significantly in the placebo group, but it was significantly improved in the *Bofutsushosan* group ($P = .02$). Interestingly, after intervention, systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were reduced in both the placebo and *Bofutsushosan* groups, but only significantly in the former group ($P = .01$ and $.049$ for SBP and DBP, respectively). However, the changes in those two parameters did not differ significantly between the groups. In contrast, following intervention, the pulse rate of the *Bofutsushosan* group but not of the placebo group was significantly reduced compared to the baseline.

Impact of *GNB3*-C825T polymorphism on the response to Bofutsushosan treatment

After the completion of the trial, the following C825T polymorphism-specific changes in the clinical outcomes were evident in the *Bofutsushosan* group (Table 6). In the C/C genotype, WC was significantly reduced, whereas in the C/T genotype, body weight ($P = .015$), BMI ($P = .015$), WC ($P = .02$), body fat mass ($P = .015$), T-Chol ($P = .0003$), and HDL-Chol ($P = .02$) were significantly decreased. On the other hand, in the T/T genotype, body fat percent ($P = .04$), body fat mass ($P = .03$), TG ($P = .046$), and DBP ($P = .007$) were significantly reduced.

TABLE 5. CHANGES IN THE OUTCOME VARIABLES OF THE SUBJECTS AFTER INTERVENTION

	Bofutsushosan (n = 55)	Placebo (n = 56)	P value ^a
Body weight, kg			
Week 8 – baseline	–0.86 ± 1.73	–1.05 ± 1.66	.622
P value ^b	.0005*	.0001*	
WC, cm			
Week 8 – baseline	–1.79 ± 2.93	–1.84 ± 2.68	.442
P value ^b	.0003*	.0001*	
BMI, kg/m ²			
Week 8 – baseline	–0.32 ± 0.66	–0.41 ± 0.59	.334
P value ^b	.0008*	.0001*	
Body fat percent			
Week 8 – baseline	–0.67 ± 1.6	–0.68 ± 1.26	.742
P value ^b	.0096*	.0014*	
Body fat mass, kg			
Week 8 – baseline	–0.97 ± 1.65	–0.89 ± 1.25	.401
P value ^b	.0005*	.0001*	
RMR, kcal/d			
Week 8 – baseline	–11.19 ± 451.4	–12.2 ± 495.24	.911
P value ^b	.8731	.8755	
Fasting BST, mg/dL			
Week 8 – baseline	0.14 ± 8.16	0.32 ± 5.5	.783
P value ^b	.9102	.7138	
T-Chol, mg/dL ^c			
Week 8 – baseline	–11.45 ± 27.39	–3.85 ± 19.65	.586
P value ^b	.0098*	.2165	
HDL, mg/dL ^c			
Week 8 – baseline	–2.81 ± 8.24	1.07 ± 7.68	.157
P value ^b	.0327*	.3762	
TG, mg/dL			
Week 8 – baseline	–7.43 ± 59.61	–15.34 ± 59.73	.366
P value ^b	.4239	.1079	
KOQOL			
Week 8 – baseline	–2.69 ± 7.1	–1.31 ± 14.92	.382
P value ^b	.0184*	.5727	
SBP, mmHg			
Week 8 – baseline	–1.9 ± 11.43	–4.76 ± 11.29	.782
P value ^b	.2864	.0102*	
DBP, mmHg			
Week 8 – baseline	–1.24 ± 9.73	–3.76 ± 11.87	.265
P value ^b	.4145	.0494*	
PR, bpm			
Week 8 – baseline	–2.67 ± 8.1	–0.27 ± 9.96	.343
P value ^b	.0389*	.8640	

^aBofutsushosan vs. placebo (by independent *t* test).^bPre- vs. post-treatment within group (by paired *t* test).^cThe treatment effects on this parameter were adjusted using general linear model.**P* < .05.

SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate.

Impact of *ADRB3-Trp64Arg* polymorphism on the response to Bofutsushosan treatment

After the completion of the study, the following Trp64Arg polymorphism-specific changes in the clinical outcomes were noted in the *Bofutsushosan* group (Table 7). In Arg64 carrier,

TABLE 6. CHANGES IN THE OUTCOME VARIABLES OF THE SUBJECTS ACCORDING TO GNB3 POLYMORPHISM

	Genotype ^a	Bofutsushosan		P value ^b
		Pre	Post	
Body weight, kg	C/C	70.53 ± 8.77	69.33 ± 7.6	.12
	C/T	75.33 ± 13.11	74.16 ± 12.58	.0147*
	T/T	74.75 ± 9.04	73.82 ± 9.26	.0705
BMI, kg/m ²	C/C	27.52 ± 1.17	27.1 ± 0.66	.1486
	C/T	29.59 ± 3.16	29.14 ± 2.99	.0155*
	T/T	28.12 ± 2.38	27.79 ± 2.43	.077
WC, cm	C/C	95.8 ± 8.38	93.07 ± 6.4	.0488*
	C/T	97.49 ± 6.99	95.9 ± 7.24	.0206*
	T/T	96.91 ± 7.31	95.19 ± 6.33	.0581
Body fat percent	C/C	36.22 ± 6.36	35.75 ± 6.68	.5142
	C/T	39.2 ± 4.95	38.64 ± 5.18	.1148
	T/T	37 ± 3.74	36.02 ± 4.06	.0433*
Body fat mass, kg	C/C	25.28 ± 4.03	24.5 ± 3.93	.251
	C/T	29.52 ± 6.64	28.54 ± 5.93	.015*
	T/T	27.7 ± 5.04	26.63 ± 5.09	.0325*
RMR, kcal/d	C/C	1318.33 ± 351.53	1448.33 ± 217.39	.4527
	C/T	1434.17 ± 445.61	1382.5 ± 354.71	.6238
	T/T	1450 ± 340.69	1449.17 ± 279.56	.9938
TG, mg/dL	C/C	129.33 ± 56.22	171.33 ± 92.78	.2938
	C/T	122 ± 56.33	108.04 ± 38.32	.2592
	T/T	105 ± 42.93	85.92 ± 33.11	.0462*
T-Chol, mg/dL	C/C	187.33 ± 20.29	195.83 ± 34.14	.6638
	C/T	214.04 ± 46.27	197.46 ± 39.34	.0003*
	T/T	204.5 ± 39.73	193.33 ± 25.8	.2085
HDL-Chol, mg/dL	C/C	48.33 ± 10.21	50.17 ± 7.25	.2257
	C/T	57.08 ± 14.06	52.83 ± 12.17	.023*
	T/T	61.92 ± 19.13	59.67 ± 14.93	.398
Fasting BST, mg/dL	C/C	101 ± 6.99	99.5 ± 10.63	.6539
	C/T	96.5 ± 8.67	96.13 ± 6.73	.7912
	T/T	99.08 ± 8.66	101.08 ± 16.36	.5351
SBP, mmHg	C/C	115 ± 1.298	112.17 ± 13.69	.6535
	C/T	120.33 ± 11.92	119.79 ± 13.08	.7927
	T/T	122.42 ± 14.85	118.25 ± 14.16	.295
DBP, mmHg	C/C	71.67 ± 8.66	75 ± 12.07	.4765
	C/T	75.75 ± 10.18	76.79 ± 8.52	.5608
	T/T	79.58 ± 5.73	71.5 ± 9	.007*†
PR, bpm	C/C	80.67 ± 14.51	76 ± 10.04	.3493
	C/T	76.21 ± 8.71	73.83 ± 9.11	.1687
	T/T	75.33 ± 8.69	73.08 ± 7.22	.275

^aDistribution (number of patients): C/C, 7; C/T, 28; T/T, 18; unidentified, 2.^bPre- vs. post-treatment (by paired *t* test).**P* < .05.†Significant difference (*P* < .05) among the allelic variants (by one-way ANOVA).

body weight (*P* = .009), BMI (*P* = .016), T-Chol (*P* = .015), and HDL-Chol (*P* = .048) were significantly reduced. In the Trp64 homozygote, body weight (*P* = .02), BMI (*P* = .02), WC (*P* = .001), body fat percent (*P* = .001), body fat mass (*P* = .007), and DBP (*P* = .0206) were significantly decreased. Notably, a significant depletion in fasting glucose was noticed in the Arg64 carrier when the change in this parameter due to *Bofutsushosan* treatment was compared between Arg64 carrier and Trp64 homozygote.

TABLE 7. CHANGES IN THE OUTCOME VARIABLES OF THE SUBJECTS ACCORDING TO *ADRB3* POLYMORPHISM

	Genotype ^a	Bofutsushosan		P value ^b
		Pre	Post	
Body weight, kg	Arg64	75.16 ± 13.45	73.97 ± 12.34	.009*
	Trp64	74.69 ± 9.32	73.99 ± 9.74	.0219*
BMI, kg/m ²	Arg64	29.53 ± 3.65	29.1 ± 3.46	.0157*
	Trp64	30.06 ± 7.54	29.79 ± 7.64	.0222*
WC, cm	Arg64	96.66 ± 7.63	94.79 ± 7.46	.2446
	Trp64	98.84 ± 7.09	95.67 ± 6.38	.0001*
Body fat %	Arg64	39.63 ± 5.59	39.06 ± 5.73	.6331
	Trp64	37.69 ± 4.55	36.29 ± 4.48	.001*
Body fat mass, kg	Arg64	29.9 ± 7.7	28.47 ± 6.97	.1078
	Trp64	28.13 ± 4.86	26.63 ± 4.16	.0007*
RMR, kcal/d	Arg64	1363.33 ± 390.02	1402.78 ± 313.23	.5657
	Trp64	1448.44 ± 374.08	1417.08 ± 320.67	.4641
TG, mg/dL	Arg64	128.67 ± 60.88	124 ± 65.82	.3917
	Trp64	125.81 ± 59.69	100.83 ± 41.34	.0671
T-Chol, mg/dL	Arg64	203.33 ± 32.47	191.67 ± 28.09	.015*
	Trp64	208.41 ± 43.39	199.33 ± 38.93	.1235
HDL-Chol, mg/dL	Arg64	53.9 ± 13.69	52.61 ± 10.69	.0484*
	Trp64	56.69 ± 15.69	55.75 ± 14.1	.2849
Fasting BST, mg/dL	Arg64	98.38 ± 8.29	95.22 ± 5.78	.1183 [†]
	Trp64	99.63 ± 10.24	100.13 ± 13.1	.196
SBP, mmHg	Arg64	118.43 ± 13.79	117.83 ± 11.23	.7191
	Trp64	120.28 ± 11.85	118.58 ± 15.04	.2931
DBP, mmHg	Arg64	73.52 ± 10.69	76.17 ± 8.97	.1892
	Trp64	77.53 ± 7.38	74.17 ± 9.56	.0206* [†]
PR, bpm	Arg64	77.33 ± 9.03	73.67 ± 8.07	.0528
	Trp64	75.91 ± 9.49	74.13 ± 9.12	.323

^aDistribution (number of patients): Arg64, 21; Trp64, 32; unidentified, 2.

^bPre- vs. post-treatment (by paired *t* test).

**P* < .05.

[†]Significant difference (*P* < .05) between the allelic variants (by independent *t* test).

Impact of *UCP2-del/ins* polymorphism on the response to Bofutsushosan treatment

Only a very small number of subjects (*n* = 2) were I/I carriers in the *Bofutsushosan* group. Accordingly, the clinical outcome variables of the I/I genotypic group was combined with that of D/I genotypic group to evaluate the impact of *Bofutsushosan* on I allele carriers.

After the completion of the study, following del/ins polymorphism-specific changes in the clinical outcomes were observed in the *Bofutsushosan* group (Table 8). In the D/D genotype, body weight (*P* = .002), BMI (*P* = .003), body fat percent (*P* = .01), body fat mass (*P* = .001), T-Chol (*P* = .04), and HDL-Chol (*P* = .01) were significantly reduced. On the other hand, in the D/I + I/I genotype, no significant difference in any of the outcome variables was evident. Notably, the degree of depletion in WC in the D/D genotype was also found to be significantly greater than that in the D/I + I/I genotype. On the other hand, the decrease in SBP in the D/I + I/I genotype was significantly greater compared to that in the D/D genotype.

Impact of *Pro12Ala* polymorphism of *PPAR*γ2 on the response to Bofutsushosan treatment

After the completion of the study, the following *Pro12Ala* polymorphism-specific changes in the outcomes were observed (Table 9). In the Pro/Pro genotype, body weight (*P* = .0007), BMI (*P* = .001), WC (*P* = .0007), body fat percent (*P* = .01), body fat mass (*P* = .0006), T-Chol (*P* = .03), and HDL-Chol (*P* = .01) were significantly decreased, while in the Pro/Ala genotype, HDL-Chol was significantly increased (*P* = .0488). Notably, significant depletion in TG and DBP was noticed in the Pro/Pro and Pro/Ala genotypes, respectively, when the changes in these two parameters were compared between the genotypes.

Adverse effects of the treatments

The adverse effects of the treatments on the subjects are summarized in Table 10. During the entire period of our study, a total of 19 adverse events were reported by 15 subjects. The most prevalent symptom was gastrointestinal problems such as dyspepsia and epigastric pain. In the

TABLE 8. CHANGES IN THE OUTCOME VARIABLES OF THE SUBJECTS ACCORDING TO *UCP2* POLYMORPHISM

	Genotype ^a	Bofutsushosan		P value ^b
		Pre	Post	
Body weight, kg	D/D	74.88 ± 10.08	73.92 ± 10.02	.0018*
	D/I + I/I	74.87 ± 13.5	74.15 ± 12.74	.1291
BMI, kg/m ²	D/D	30.24 ± 7.16	29.88 ± 7.24	.0027*
	D/I + I/I	28.84 ± 2.85	28.6 ± 2.64	.1464
WC, cm	D/D	97.83 ± 7.28	94.47 ± 7.17	<.0001*†
	D/I + I/I	98.33 ± 7.64	97.14 ± 5.69	.9738
Body fat %	D/D	38.47 ± 5.11	37.4 ± 5.2	.0099*
	D/I + I/I	38.43 ± 5	37.65 ± 5.34	.5913
Body fat mass, kg	D/D	28.94 ± 6.54	27.56 ± 6.27	.0011*
	D/I + I/I	28.55 ± 5.17	27.12 ± 3.6	.2127
RMR, kcal/d	D/D	1449.21 ± 325.62	1399.66 ± 318.97	.4825
	D/I + I/I	1327.33 ± 492.03	1436.15 ± 312.85	.3643
TG, mg/dL	D/D	131.05 ± 63.19	120.38 ± 56.36	.7846
	D/I + I/I	116.53 ± 49.76	89.31 ± 41.7	.122
T-Chol, mg/dL	D/D	206.89 ± 39.65	197.62 ± 36.04	.0421*
	D/I + I/I	205.13 ± 39.25	192.54 ± 31.99	.1113
HDL-Chol, mg/dL	D/D	55.13 ± 15.87	52.59 ± 12.07	.014*
	D/I + I/I	56.73 ± 12.31	58.46 ± 13.63	.7338
Fasting BST, mg/dL	D/D	98.29 ± 9.9	98.21 ± 12.39	.4004
	D/I + I/I	101.27 ± 8.13	97.62 ± 6.21	.265
SBP, mmHg	D/D	119.03 ± 12.55	118.69 ± 14.27	.42
	D/I + I/I	120.87 ± 12.92	117.31 ± 11.69	.5079†
DBP, mmHg	D/D	75.5 ± 9.24	75.52 ± 8.6	.5645
	D/I + I/I	77.07 ± 8.42	73.92 ± 10.87	.5807
PR, bpm	D/D	77.13 ± 9.78	74.86 ± 9.55	.0861
	D/I + I/I	74.8 ± 7.78	71.85 ± 5.68	.2798

^aDistribution (number of patients): D/D, 38; D/I, 13; I/I, 2; unidentified, 2.

^bPre- vs. post-treatment (by paired *t* test).

**P* < .05.

†Significant difference (*P* < .05) between the allelic variants (by independent *t* test).

Bofutsushosan group, 15 adverse cases included dyspepsia and epigastric pain (*n* = 7), headache (*n* = 2), diarrhea (*n* = 3), nausea and vomiting (*n* = 2), and palpitations (*n* = 1). In the placebo group, four adverse cases included dyspepsia and epigastric pain (*n* = 3) and headache (*n* = 1). Notably, although there were no significant adverse effects that might greatly affect subject health, four subjects were dropped from the trial: two from the *Bofutsushosan* group (one case of palpitations and one case of dyspepsia) and two from the placebo group (one case of epigastric pain and one case of dyspepsia; Fig. 1).

Safety evaluation

The evaluation of the safety of the proposed treatments was based on the examination of liver- and kidney-related clinical indices of the subjects through blood test at both baseline and the end of the study. The outcome of this analysis is depicted in Table 11. Accordingly, *Bofutsushosan* treatment significantly decreased the activity of both ALT (*P* = .01) and AST (*P* = .02), whereas those two parameters were not significantly altered in the placebo group.

However, the changes in ALT and AST activities in the *Bofutsushosan* group did not differ significantly from that in the placebo group. Notably, the changes in the level of blood urea nitrogen and creatinine were insignificant in both placebo and *Bofutsushosan* groups, and the differences between the groups were also insignificant.

DISCUSSION

The present 8-week, randomized, placebo-controlled, double-blind clinical trial evaluated the anti-obesity impact of *Bofutsushosan*, as well as the association between the polymorphisms in obesity-related genes and the response of the subjects to *Bofutsushosan* treatment.

Both *Bofutsushosan* and placebo treatment resulted in a significant reduction in body weight, WC, BMI, body fat percent, and body fat mass. Although there were no significant differences in the above parameters between the placebo and *Bofutsushosan* groups, the data still revealed the following. First, in the *Bofutsushosan* group, there was a significant reduction in the level of T-Chol, but not in the placebo group. Second, following the completion of

TABLE 9. CHANGES IN THE OUTCOME VARIABLES OF THE SUBJECTS ACCORDING TO *PPAR-γ2* POLYMORPHISM

	<i>Genotype</i> ^a	<i>Bofutsushosan</i>		<i>P value</i> ^b
		<i>Pre</i>	<i>Post</i>	
Body weight, kg	Pro/Pro	74.74 ± 11.18	73.83 ± 10.83	.0007*
	Pro/Ala	72.42 ± 9.48	72.04 ± 9.84	.4971
BMI, kg/m ²	Pro/Pro	29.84 ± 6.38	29.51 ± 6.42	.0011*
	Pro/Ala	28.46 ± 3.57	28.3 ± 3.63	.4997
WC, cm	Pro/Pro	97.41 ± 7.48	95.33 ± 7	.0007*
	Pro/Ala	99.9 ± 7.55	94.83 ± 3.88	.2529
Body fat percent	Pro/Pro	38.46 ± 4.92	37.65 ± 5.3	.0108*
	Pro/Ala	36.96 ± 6.01	35.27 ± 2.71	.6304
Body fat mass, kg	Pro/Pro	28.75 ± 5.97	27.66 ± 5.65	.0006*
	Pro/Ala	27.2 ± 8.36	24.27 ± 2.6	.5254
RMR, kcal/d	Pro/Pro	1405.2 ± 393.65	1424.1 ± 317.29	.9526
	Pro/Ala	1312 ± 279.05	1240 ± 240	.7511
TG, mg/dL	Pro/Pro	122.5 ± 52.27	112.59 ± 55.28	.8844 [†]
	Pro/Ala	175.8 ± 98.47	187 ± 16	.1888
T-Chol, mg/dL	Pro/Pro	206.28 ± 39.46	195.72 ± 35.42	.0284*
	Pro/Ala	221.8 ± 39.59	200.33 ± 24.01	.2013
HDL-Chol, mg/dL	Pro/Pro	54.64 ± 14.94	53.13 ± 12.06	.0101*
	Pro/Ala	61.6 ± 11.22	71 ± 10.15	.0488* [†]
Fasting BST, mg/dL	Pro/Pro	98.9 ± 9.71	98.03 ± 11.13	.8444
	Pro/Ala	100 ± 4.18	98 ± 5.29	.849
SBP, mmHg	Pro/Pro	119.12 ± 12.39	118.44 ± 12.8	.5772
	Pro/Ala	122.4 ± 16.18	116 ± 23.64	.2415
DBP, mmHg	Pro/Pro	75.46 ± 9.36	75.85 ± 8.45	.8583
	Pro/Ala	76.8 ± 4.32	64.33 ± 14.64	.2049 [†]
PR, bpm	Pro/Pro	76.76 ± 9.09	74.28 ± 8.77	.053*
	Pro/Ala	71.6 ± 10.16	69.33 ± 3.79	.5352

^aDistribution (number of patients): Pro/Ala, 5; Pro/Pro, 50.^bPre- vs. post-treatment (by paired *t* test).**P* < .05.[†]Significant difference (*P* < .05) between the allelic variants (by independent *t* test).

treatment schedule, the KOQOL score was significantly improved in the *Bofutsushosan* group, but not in the placebo group. Thus, our findings are in keeping with earlier reports that have substantiated the anti-obesity effect of *Bofutsushosan*.^{19–22}

Notably, after completion of the trial, significant reductions in both SBP and DBP were observed in the placebo group, but not in the *Bofutsushosan* group, although the differences in those changes between the groups were insignificant. So far, no detailed studies have been conducted to evaluate the impact of *Bofutsushosan* treatment on the blood pressure of obese subjects. Indeed, changes in blood pressure after placebo administration have been seen in the

placebo groups of a number of placebo-controlled trials.^{30,31}

It has been suggested that certain conditions that may influence the clinical BP outcome are psychological factors such as the medical environment or the relationship between the patient and the physician,³⁰ including verbal suggestions.³¹ It has also been opined that the verbal suggestions-induced fall of SBP is specifically mediated by autonomic nervous system efferents that are involved in the regulation of blood pressure, although the precise mechanism behind this is yet to be understood.³¹ Accordingly, without further detailed studies, no definite conclusion can be drawn from our study on the observed placebo effects on the subjects' blood pressure.

TABLE 10. ADVERSE EVENTS REPORTED IN THE STUDY

<i>Treatment group</i>	<i>Dyspepsia, epigastric pain</i>	<i>Headache</i>	<i>Diarrhea</i>	<i>Nausea, vomiting</i>	<i>Insomnia</i>	<i>Palpitations</i>	<i>Total</i>
<i>Bofutsushosan</i> (<i>n</i> = 55)	7	2	3	2		1	15
Placebo (<i>n</i> = 56)	3	1					4
Total	10	3	3	2		1	19

TABLE 11. CHANGES IN THE SAFETY OUTCOME VARIABLES OF THE SUBJECTS

	Bofutsushosan (n = 55)	Placebo (n = 56)	P value ^a
ALT at baseline, IU/L	22.36 ± 16.86	22 ± 17.16	
Week 8 – baseline	–3.57 ± 8.72	–1.88 ± 8.89	.732
P value ^b	.0113*	0.1838	
AST at baseline, IU/L	21.31 ± 10.38	19.66 ± 7.69	
Week 8 – baseline	–2.14 ± 5.94	–1.17 ± 5.5	.573
P value ^b	.0243*	.1801	
BUN at baseline, mg/dL	14.02 ± 4.15	12.99 ± 3.53	
Week 8 – baseline	–0.09 ± 3.43	0.05 ± 3.18	.181
P value ^b	.8688	.9223	
Creatinine at baseline, mg/dL	0.69 ± 0.13	0.67 ± 0.1	
Week 8 – baseline	0 ± 0.08	–0.01 ± 0.08	.305
P value ^b	.7476	.49	

^aBofutsushosan vs. placebo (by independent *t* test).^bPre- vs. post-treatment within group (by paired *t* test).**P* < .05.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urine nitrogen.

The family of genes responsible for obesity includes those expressing heterotrimeric G proteins, which are the vital components of the intracellular signaling pathway and which have a central role in adipogenesis. A common C → T substitution at nucleotide 825 of the gene encoding the $\beta 3$ subunit of the G protein, commonly known as GNB3 C825T polymorphism, is associated with a number of pathophysiological conditions including obesity.^{32,33} It has been revealed that BMI, waist girth, hip girth, subscapular skinfold thickness, triceps skinfold thickness, and body fat percent are significantly higher in subjects with the T/T genotype compared to subjects with the C/T or C/C genotypes.^{32,33}

Bofutsushosan treatment produced significant anti-obesity effects on a number of obesity-related clinical parameters in the carriers of the GNB3 825T allele, whereas in C/C homozygotes, the same treatment produced significant anti-obesity impact only on WC. Thus, our results demonstrate a more targeted anti-obesity impact of *Bofutsushosan* on the T allele, which is a clinically important finding. These findings are also comparable to an earlier study,²⁶ where the 825(T/T + C/T) genotype, but not the 825C/C genotype, was associated with greater weight loss upon treatment with sibutramine, a weight-reducing drug that inhibits presynaptic reuptake of serotonin and norepinephrine, thereby causing accumulation of these neurotransmitters in between the synapses. Accordingly, we could not exclude the possibility of a similar scenario in *Bofutsushosan* treatment, as ephedrine, the active ingredient of *Ephedra* in *Bofutsushosan*, is an amphetamine-type central nervous system stimulant that augments the release of norepinephrine from its storage vesicles.³⁴ However, since *Bofutsushosan* is a multiherbal formulation rather than a single entity, no definite conclusion can be made on this postulation. Future in-depth studies (trials and observational studies) are needed to

ascertain the mode of drug–gene interaction between *Bofutsushosan* and the C825T polymorphism.

ADRB3, which is a G protein coupled receptor, plays an important role in the regulation of thermogenesis and lipolysis in adipose tissue, as well as delivery of free fatty acids into the portal vein. A missense mutation in the codon 64 of *ADRB3* that leads to the replacement of tryptophan by arginine (Trp64Arg) in the first intracellular loop of the receptor is associated with abdominal obesity and insulin resistance, as well as early development of type 2 diabetes.^{35,36} Our study revealed that the anti-obesity effects of *Bofutsushosan* are directed differentially among the Arg64 and TRP64 carriers of *ADRB3* gene. This is relevant to an earlier clinical report that elucidated the effects of TRP64Arg polymorphism on multiple-dose rosiglitazone response in patients suffering from type 2 diabetes,³⁷ where, although the Arg64 genotype had greater attenuated serum TG and lesser attenuated LDL-Chol, as well as smaller enhancement of adiponectin levels compared with the TRP64 carriers, there were no significant differences in most diabetes-related clinical parameters between these two genotypes. Additionally, in an earlier clinical study, no difference in the $\beta 1/\beta 2/\beta 3$ AR-agonist isoproterenol- or *ADRB3*-specific agonist CGP-12117-induced lipolysis was seen among the omental fat tissues harvested from the Trp64/TRP64, TRP64/Arg64, or Arg64/Arg64 variants.³⁸ Taking all this into consideration, we hypothesize that the differential anti-obesity effects of *Bofutsushosan* among the Trp64 and Arg64 carriers in our study might be attributable to the active ingredient ephedrine, as this compound is a nonspecific adrenergic agonist and, accordingly, its mode of action in the subjects might be differentially affected by a base mutational change of only one member of the adrenoceptor family. However, further detailed studies are required to understand the mode of drug–gene interaction between *Bofutsushosan* and Trp64Arg polymorphism.

Uncoupling protein 2 (*UCP2*), which is ubiquitously expressed in adult human tissues, plays an important role in energy balance, body weight regulation, and thermoregulation. A 45 bp del/ins polymorphism in the 3'-untranslated region (UTR) of exon 8 of the *UCP2* gene³⁹ is one of the common *UCP2* gene variants in humans. It has been reported that the 3' del/ins polymorphism in the *UCP2* gene is associated with obesity and BMI in different populations.¹ The I/I genotype was shown to be associated with increased BMI in South Indian women.⁴⁰ It has been hypothesized that D/I polymorphism may influence *UCP2* mRNA stability, which, in turn, could affect the expression of this protein and determine body weight by modulating energy expenditure and thermogenesis.⁴⁰ Any impairment in *UCP2* mRNA stability could compromise a subject's ability to remove excess calories through thermogenesis, particularly in a person who is inclined to obesity from other genetic or environmental influences.⁴⁰ This hypothesis is partially supported by the observation of decreased *UCP2* mRNA levels in the visceral fat of obese, but not lean, subjects.⁴¹

In the present study, *Bofutsushosan* treatment resulted in significant improvement in a number of obesity-related

clinical parameters in the subjects with the UCP2 D/D genotype, while the same treatment did not produce significant change in any of the studied parameters in the UCP2 D/I + I/I variants. Thus, our results suggest that the anti-obesity effects of *Bofutsushosan* on the subjects are specifically disposed to the D/D genotype of 3'UTR UCP2 polymorphism. Notably, previous studies have revealed that β_3 -adrenergic agonists could induce the expression of UCP2 gene in a number of tissues,^{42,43} and perhaps thereby exert a thermogenic anti-obesity effect. The above findings suggest that in our study, probably ephedrine in *Bofutsushosan*, an indirect β_3 -adrenergic agonist, might induce UCP2 gene expression. However, this event could not be presently translated into the synthesis of UCP2 mRNA in D/I or I/I, but not in D/D carriers, most likely because of the destabilization of the newly produced UCP2 transcript in the former two genotypes. Accordingly, *Bofutsushosan* treatment might result in an augmented production of UCP2 protein in D/D carriers, thereby exerting a more pronounced anti-obesity impact on the D/D genotype compared to the other two UCP2 SNP variants studied.

The nuclear hormone receptor, *PPAR* γ , is a transcriptional factor that plays a major role in adipogenesis,⁴⁴ as well as in the induction of transcription of specific anti-obesity target genes.⁴⁵ A number of genetic polymorphisms in the *PPAR* γ gene have been discovered. Among them, Pro12Ala variant, a missense mutation, is most prevalent in the *PPAR* γ 2 isoform involving a C→G substitution at nucleotide 34 resulting in the replacement of a proline by alanine at residue 12 of the *PPAR* γ 2 protein.⁴⁶ Functionally, the Ala variant of *PPAR* γ 2 has been demonstrated to have a reduced affinity for the response element and a lower capacity for activating target genes.⁴⁷ Several studies have aimed to find a relationship between the Pro12Ala polymorphism and metabolic syndrome, but the results have been equivocal. However, an extensive meta-analysis using data from 30 independent studies with a total number of 19,136 subjects revealed that BMI was significantly higher in Ala allele carriers compared with Pro allele homozygotes.¹⁷ In female Korean subjects, body weight, BMI, and waist-to-hip ratio were significantly higher in Ala allele carriers than in noncarriers,⁴⁸ consistent with the results of the above-mentioned meta-analysis.¹⁷ In our study, the Ala/Ala genotype was absent among the subjects, and the results presented here are derived from the Pro/Pro and Pro/Ala variants, which revealed that the anti-obesity impact of *Bofutsushosan* was more pronounced in the wild type Pro/Pro genotype than in mutated Pro/Ala variant where the functionality of *PPAR* γ 2 is thought to be impaired.⁴⁷ This suggests a possible interaction between *PPAR* γ and the ingredients of *Bofutsushosan*, especially *Ephedra*, although no definite conclusion can be made on this postulation. However, our hypothesis is in keeping with earlier studies,^{49,50} suggesting a cross-talk of the *PPAR* γ pathway with signaling cascade of cAMP, the cellular content of which can be modulated by *Ephedra*.

Gastrointestinal problems, which were the most common adverse events reported in our study, were noticed in both

the placebo and *Bofutsushosan* groups, but predominantly in the latter group. It has been opined that *Bofutsushosan* treatment may cause or aggravate anorexia, epigastric distress, nausea, vomiting, abdominal pain, soft feces, and diarrhea in the subjects.⁵¹ Indeed, in a clinical trial, 9 out of 127 obese patients receiving *Bofutsushosan* treatment experienced side effects, including diarrhea, abdominal pain, and abdominal discomfort.⁵² In another clinical study on obese Japanese females, the *Bofutsushosan*-treated group showed more improvement in constipation compared to the placebo group.²² However, in a preclinical study using a mouse model, diarrhea was found to be associated with *Bofutsushosan* treatment in the control (nonobese) group, but not in the disease (obese and diabetic) group.¹⁹ Moreover, repeated oral administration of *Bofutsushosan* in Sprague Dawley rats for 13 weeks did not produce any adverse effects in the animals in either sex up to and including doses of 2000 mg/kg/ per day.⁵³ Thus, taking the above into consideration, no definite conclusion can be drawn on the possibility that gastrointestinal problems are a consequence of *Bofutsushosan* treatment. Furthermore, our safety assessment revealed that *Bofutsushosan* treatment did not produce any toxic or adverse impact on hepatic or renal function of the subjects. In our opinion, *Bofutsushosan* and/or certain subject-specific intrinsic factors as well as diet status might play an important role in contributing the gastrointestinal disorders in the volunteers. Furthermore, we cannot exclude the possible contribution of the SNPs to the adverse response to *Bofutsushosan* treatment. Many adverse drug reactions can be associated with SNPs in genes that regulate aspects of drug disposition. The members of these genes encompass drug metabolizing enzymes, which include phase 1 enzymes such as cytochrome P450 superfamily, phase 2 enzymes such as microsomal epoxide hydrolase, and receptors and transporters such as multidrug resistance protein 1 (*MDR1*) P-glycoprotein. Further studies are needed to investigate whether SNPs of the above genes and those we studied may contribute to the adverse response to *Bofutsushosan* treatment.

In conclusion, differential responses of the obesity indices to *Bofutsushosan* treatment were revealed according to the GNB3-C825T, ADRB3-Trp64Arg, UCP2-3'UTR 45 bp ins/del, and *PPAR* γ -Pro12Ala SNPs of the subjects. Our results provide new insights into the clinical efficacy of *Bofutsushosan*.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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