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A Controlled Pharmacogenetic Trial of Sibutramine on Weight Loss and Body Composition in Obese or Overweight Adults

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Abstract

Background/ Aim—Weight loss in response to sibutramine is highly variable. We assessed the association of specific markers of polymorphisms of candidate *a2A* adrenoreceptor, 5-HT transporter and *GNβ3* genes and weight loss with sibutramine.

Methods—We conducted a randomized, double-blind, pharmacogenetic study of behavioral therapy and sibutramine (10 or 15 mg daily) or placebo for 12 weeks in 181 overweight or obese participants. We measured body weight, BMI, body composition, gastric emptying and genetic variation (*α2A* C1291G, *5-HTTLPR*, and *GNβ3* C825T genotypes). ANCOVA was used to assess treatment effects on, and associations of the specific markers of candidate genes with weight loss and body composition.

Results—Sibutramine, 10 and 15 mg, caused significant weight loss ($p = 0.009$); there was a statistically significant gene by dose interaction for *GNβ3* genotype. For each candidate gene, significant treatment effects at 12 weeks were observed ($p<0.017$) for all specific genotype variants (delta weight loss in the 2 sibutramine doses versus placebo): *α2A* CC genotype (Δ ~5kg), *GNβ3* TC/TT genotype (Δ ~6kg), and *5-HTTLPR* LS/SS (Δ ~4.5kg). Gene pairs resulted in significantly greater sibutramine treatment effects on weight (both $p<0.002$): in participants with *5-HTTLPR* LS/SS with *GNβ3* TC/TT, Δ ~6kg and those with *a2A* CC with *GNβ3* TC/TT, Δ ~8kg; however, effects were not synergistic. Treatment with sibutramine also resulted in significantly greater reduction of body fat for specific *α2A* CC and *GNβ3* TC/TT genotype variants individually (both $p<0.02$).

Conclusions—Selection of patients with obesity based on candidate genes may enhance response to multidimensional sibutramine and behavioral therapy.

Keywords

Adrenergic; norepinephrine; serotonin; transporter; SLC6A4

INTRODUCTION

Sibutramine, a noradrenergic and serotonergic reuptake inhibitor approved for the long term treatment of obesity, induces satiety, prevents decline in metabolic rate associated with hypocaloric diets (1), and causes weight loss especially when combined with behavioral therapy (2–4). However, there are large differences in weight loss among individuals treated with sibutramine.

Genetic variations that potentially affect serotonergic and adrenergic functions may influence appetite, glucose and fat metabolism, and responses to psychotropic medication (5–8). This study explored whether genetic variations may account for the differences in weight loss with sibutramine treatment by examining polymorphisms of specific markers of candidate genes that may affect sibutramine's pharmacological actions (Figure 1): α2A adrenoreceptors; the serotonin transporter protein, SERT [or solute carrier 6A4 (SLC6A4)]; and the downstream effects of ligand-receptor interaction mediated through guanine nucleotide binding (G) proteins. G proteins are composed of three different α, β, and γ subunit isoforms which are involved in transduction of ~80% of ligand-receptor interactions; the βγ subunits form a functional monomer. On receptor activation, α and βγ subunits dissociate and modulate several intracellular effector systems.

The specific variations in the three candidate genes were selected because of associations with differences in biological functions. The C-1291G single nucleotide polymorphism (SNP) of

the *α2A*-adrenoreceptor gene is located in the promoter region and may alter gene expression and receptor density. With the *5-HTTLPR* variation located in the promoter of the SLC6A4 gene, the short allele is associated with reduced function of SERT (5), variation in response to SSRI in generalized social anxiety disorder (9) and in tolerability of serotonergic antidepressants (10).

The Gβ3 protein is encoded by the *GNβ3* gene, in which there is a common C825T SNP located on exon 10. The 825T allele results in alternative splicing, formation of a truncated splice variant, Gβ3s, enhanced G-protein activation, association with affective and metabolic disorders (11,12), and altered response to α2-adrenergic activation (13).

Neither of these genetic variants is conclusively associated with obesity per se, though *α2A*-adrenoceptor gene is associated with storage of fat in the abdominal area (14), *5HTTLPR* with change in BMI from adolescence to young adulthood (15), and with obesity /overweight in an adult male population (16). In preliminary pharmacogenetic studies, *GNβ3* CC genotype [111 patients (17)] and *SLC6A4* LS/SS (heterozygous/short) genotype [48 patients evaluated in our laboratory (18)] were associated with greater weight loss with sibutramine treatment than placebo. In our prior study (18), sibutramine-induced weight loss was also associated with delayed gastric emptying of solids measured by radioscintigraphy.

The aim of this study was to examine, in overweight and obese individuals, the influence of specific markers of candidate genes controlling serotonergic and adrenergic mechanisms (specifically, *α2A*-receptor, *5-HTTLPR* and *GNβ3*) on weight loss and body composition change in response to sibutramine or placebo treatment. Secondary aims were to evaluate whether weight at 4 weeks of treatment predicted weight achieved at 12 weeks of treatment, and whether the candidate genes also influenced treatment effects on weight loss at 4 weeks.

METHODS

Study Design and Participants

This was a randomized, double-blind, placebo-controlled, parallel-group study of two-doses (10 and 15 mg) sibutramine in combination with behavioral therapy and its effects on weight loss; we explored the influence on weight and body composition of specific markers indicative of variation in three candidate genes. Although the study was conducted at a tertiary referral center,,the 181 otherwise healthy overweight and obese participants were recruited from among people in southeastern Minnesota. The study was approved by the Mayo Clinic Institutional Review Board. All participants provided signed informed consent.

Eligibility Criteria

Overweight and obese males and females, aged 18–65 years with a BMI of 25–50 Kg/m^2, who were not currently on appetite suppressants, orlistat or MAOI inhibitors. Volunteers who were not on treatment for cardiac, pulmonary, gastrointestinal, hepatic, renal, hematological, neurological, or endocrine conditions were eligible for participation. Individuals with unstable psychiatric disease, weight >300 pounds, blood pressure >140 mmHg systolic and >90 mmHg diastolic, or >105 mm Hg diastolic after a 30 minute rest period were excluded.

Randomization and Interventions

Participants were recruited between July 2006 and September 2007. Participants received placebo or sibutramine, 10 or 15 mg daily, for 12 weeks in a 1:1:1 randomized, parallel-group design. Patient randomization to each treatment group was balanced on gender, *5HTTLPR* variation, and BMI category (overweight versus obese) to ensure balanced randomization in the treatment groups as these are potentially influential covariates, based on our preliminary

study results (18). Treatment allocation was concealed to the participants and investigators. Participants received standardized, structured behavioral therapy for weight management at entry, 4, 8 and 12 weeks later.

Behavioral Therapy

To standardize for potential differences in the behavioral aspects of weight reduction therapy, all participants were given a standard behavioral weight management text, the "LEARN" Manual (19) and were to read one of the 12 sessions, in chronological order, each week. Participants met for 15 minutes with a master's or doctoral level psychologist with training in obesity treatment at study entry and at weeks 4, 8 and 12. The psychologists followed a structured study session outline and documented the content of their visit to ensure adherence to the behavior therapy protocol.

Characterization of Participants at Baseline

Motivational level for exercise—At time of entry to the study, the motivational level for physical activity was assessed using the Stage of Change for Physical Activity Questionnaire (20) to identify the proportion in each treatment group performing regular physical activity.

Psychological assessment of participants—In order to screen for significant psychiatric or psychological dysfunction, we used the Hospital Anxiety and Depression Scale [HADS (21)], the self-administered alcoholism screening test [SAAST, substance abuse (22)], the Questionnaire on Eating and Weight Patterns-Revised (binge eating disorder and bulimia), and the Weight Efficacy Life-Style Questionnaire (WEL). bipotential participants with HADS score of ≥8 on a subscale, or a total score of ≥11, or difficulties with substance or eating disorders were excluded and referred to their primary care doctor for further health care.

Detection of Variations of Candidate Genes

Genomic DNA was isolated using the Gentra Puregene Blood Kit (Qiagen Inc., Valencia, CA). Venous blood DNA was analyzed for *5HTTLPR* (by PCR followed by gel electrophoresis), *α2A* C1291G (restriction site MspI, rs1800544), and *GNβ3* C825T (rs5443 by TAQMAN®, Applied Biosystems, Foster City, CA) genotypes. We have published elsewhere (18,23,24) the methods and frequency distribution of alleles in controls. Assay details and GenBank numbers for each polymorphism are included in the Appendix.

Weight, Blood Pressure and Compliance Measurements

Automated measurements of body weight and blood pressure were recorded in the Clinical Research Unit at the start, monthly, and at week 12, and fasting blood glucose at baseline and week 12. Participants were evaluated by the study coordinator or physician (for ~10 minutes) every 4 weeks for waist, hip and thigh measurements, medical monitoring (symptoms of increased blood pressure and potential medication side effects), adherence to study protocol and medications, and use of prescription or over-the-counter medications. At the end of each 4 weeks' of treatment, compliance to medication intake was assessed by pill count.

Body Composition

Total body fat was measured by dual energy x-ray absorptiometry (Lunar Corp., Madison, WI) in the Mayo Clinical Research Unit (25) at baseline and at week 12.

Gastric Emptying

Prior to treatment and in the last week of the treatment phase, participants underwent a stable isotope breath test to measure gastric emptying (26). The test meal consisted of 100 mg [^{13}C]-

Spirulina platensis, 27 g freeze dried egg mix, 6 saltine crackers, and 6 oz water and had a caloric content of 238 kcal. Breath samples obtained at 45, 90, 120,150, 180, and 240 minutes post-meal were collected and stored in Vacutainers (Becton Dickinson, Franklin Lakes, NJ) and submitted for measurement of $^{13}CO_2$ enrichment by an external reference laboratory. The use of this test was conducted under IND #67,129 (held by ABDiagnostics, LLC, Brentwood, TN). The method for calculating gastric emptying $t_{1/2}$ followed prior studies in our laboratory (26).

Statistical Analyses

Sample size considerations are included in the Appendix.

All analyses used the intent-to-treat principle. The primary endpoint was weight (kg) measured at 12 weeks post-start of treatment; secondary endpoints were body fat composition and gastric emptying of solids. Unadjusted p values are provided; thus, $p<0.017$ suggests significant associations for the three candidate variations with weight loss.

ANCOVA models were used to assess associations of each candidate gene (wildtype versus non-wildtype) with weight loss, that is, to compare the placebo and two sibutramine groups at the end of the treatment period, using as covariates corresponding baseline weight, BMI category, gender, and genetic marker status (wildtype versus non-wildtype). A summary of the actual weight loss (pre-treatment baseline weight and the 12-week post start of treatment weight) was also compiled for each treatment group in subjects who completed the study ("per protocol subset"). In order to adhere to the intent-to-treat paradigm, subjects with missing values had their post-treatment weight imputed using the overall (patients) mean value with a corresponding adjustment in the residual error degrees of freedom of the ANCOVA (i.e., subtracting one degree of freedom for each missing value imputed). A similar analysis was used to assess treatment effects on the 4-week post-start of treatment weight values. In addition, a multiple linear regression model was used to predict the 12-week post-treatment weights using baseline weight, BMI group, gender treatment group, and weight at week 4 post-treatment. The partial r-square values in this analysis provided an estimate of the proportion of variation in week 12 weight values accounted for by week 4 weight values..

Separate analyses with gender, each individual genetic marker, and treatment group by genetic marker interaction term were included as covariates in these separate ANCOVA models for post-treatment weight. The corresponding F-tests in the ANCOVA for these interaction terms provided tests of the hypothesis that genetic marker status influences response (e.g., weight) to treatment.

Since sufficient numbers of patients in specific pairwise combinations of the genetic markers were observed, similar additional ANCOVA models examined whether pairwise combinations of genetic markers affect the weight change response to treatment.

χ^2 tests were used to explore whether adverse events were associated with genetic variations.

Finally, we assessed gastric emptying $T_{1/2}$ and compared the three treatment groups by ANCOVA using baseline $T_{1/2}$ values as covariate.

RESULTS

Demographics, Genotype Distributions and Study Flow

At baseline, the three groups had similar demographic, obesity (weight, BMI, waist), fasting blood glucose and genotype distributions (Table IA and IB). The trial flow is summarized in the supplemental figure at the end of the paper. Adherence with study medication for all

participants over the three months of treatment was 97±0.3% (mean use at months 1, 2 and 3 being 97.7, 96.6 and 96.7% respectively). Thirteen subjects withdrew from the two sibutramine groups, 1 due to hypertension (10mg dose). Ten subjects in the placebo group withdrew from the study, 1 due to hypertension.

Baseline Psychological Assessment

The three study groups were similar in eating behaviors (scores on the Three Factor Eating Inventory of hunger, cognitive restraint and disinhibition - Table 1A) and in the self-report of symptoms of anxiety or depression (by the HADS), difficulties with alcohol usage (SAAST) and motivational level for physical activity.

Weight, BMI, Body Composition and Gastric Emptying after 12 Weeks Treatment

There were statistically significant effects of sibutramine on body weight, BMI and percent fat (based on analysis of body composition). Sibutramine, 10 and 15 mg doses, resulted in significantly lower values for weight, BMI and proportion of body fat (Table IIA, which shows ITT analysis) compared to placebo ($p<0.01$, $p<0.001$, and $p=0.05$, respectively). Table IIA summarizes the least squares mean values that are based on the post-treatment observations (e.g., weight) corrected for baseline differences. There was no significant overall treatment effect of sibutramine on gastric emptying of solids.

The per protocol data (participants completing the therapies) are summarized in Table IIB.

Influence of Individual Candidate Genes on Weight Following Treatment with Sibutramine

There was a statistically significant gene by treatment (placebo, sibutramine 10mg, and sibutramine 15mg doses) interaction for *GNβ3* genotype, reflecting differential treatment effects in one *GNβ3* genotype (TC/TT) versus the other (CC) genotype ($p=0.018$); this is reflected in the different patterns of the effects of sibutramine compared to placebo in association with the two *GNβ3* genotypes (TC/TT in contrast to CC; see Figure 2, right side). Such statistical interaction of treatment group and genotype was not observed with *α2A* CC versus CG/GG ($p=0.33$) or with *5HTTLPR* LS/SS versus LL ($p=0.64$) genotypes. Thus, the patterns of post-treatment weights with the 2 sibutramine doses and placebo were not sufficiently different for the two variants of *α2A* and *5HTTLPR*.

However, for each candidate gene, significant overall treatment effects were observed in one genotype variant (specifically *α2A* CC [$p=0.009$], *GNβ3* TC/TT [$p<0.001$], and *5HTTLPR* LS/SS [$p=0.014$] genotypes), but not in the corresponding alternative (*α2A* CG/GG, *GNβ3* CC, and *5HTTLPR* LL) genotype. The post-treatment mean weights for specific genotypes and the corresponding p-values from the ANCOVA analyses for treatment effects in these specific subgroups (based on ITT) are shown in Figure 2.

Weight loss on sibutramine (averaged over 10 and 15 mg doses) versus placebo was observed for *α2A* CC (~5kg loss), *GNβ3* TC/TT (~6kg loss), and *5HTTLPR* LS/SS genotypes (~4.5kg). The proportions of participants receiving placebo or sibutramine who lost >5kg weight in association with the genotypes of interest are shown in Table IIC. From the results for sibutramine, the sensitivity and specificity for each individual genotype and the combination of *α2A* CC with *GNβ3* TC/TT genotypes to predict a >5kg weight response to sibutramine can be computed: *α2A* CC (sensitivity 49%, specificity 74%), *GNβ3* TC/TT (sensitivity 56%, specificity 78%), *5HTTLPR* LS/SS (sensitivity 40%, specificity 64%), and the combination of *α2A* CC with *GNβ3* TC/TT (sensitivity 63%, specificity 89%).

Influence of Individual Candidate Genes on Body Composition Following Treatment with Sibutramine

For two of the three candidate genes, significant reduction in the proportion of fat (on body composition) was observed in response to sibutramine compared to placebo for one genotype polymorphism: *α2A* CC and *GNβ3* TC/TT (both p<0.02, ITT results in Table II).

Gene-Gene Combinations and Weight Following Treatment with Sibutramine

Given the significant treatment by *GNβ3* interaction noted above, we explored three way interactions between treatment, *5HTTLPR*, and *GNβ3* (p=0.31), or between treatment, *α2A*, and *GNβ3* (p=0.84); however neither of these three way interactions were significant.

However, there were significant treatment effects (ITT results in Table II and Figure 3) demonstrated for specific combinations of genotypes in which lower post-treatment weights were observed on sibutramine compared to placebo. The three combinations of interest showed the following:

- **a.** For combination of *5HTTLPR* LS/SS with *GNβ3* TC/TT (treatment effects, p<0.002, unadjusted, Figure 3, left panel), post-treatment differences were ~6kg, averaged over two doses of sibutramine. In contrast, in patients with the combination *5HTTLPR* LS/SS and *GNβ3* CC, treatment effects were not observed (p=0.54, Figure 4, left panel).
- **b.** For combination of *α2A* CC with *GNβ3* TC/TT (treatment effects, p<0.001, unadjusted, Figure 3, right panel), post-treatment differences were of ~8kg, averaged over two doses of sibutramine. In contrast, in patients with the combination of *α2A* CC and *GNβ3* CC, no treatment effects were observed (p=0.94, Figure 4, right panel).
- **c.** For combination of *α2A* CC and *5HTTLPR* LS/SS, the unadjusted p value for treatment effects was 0.032 (therefore deemed nonsignificant), while treatment effects in the combination of *α2A* CC/CG and *5HTTLPR* LS/SS were not observed (p=0.24, data not shown).

We also assessed whether the weight loss in response to sibutramine was greater for the combinations of two genes than the sum of the weight loss observed with each one of the two genes. Separate estimates of the effect of 15 mg and 10 mg sibutramine versus placebo are shown for gene combinations and individual genes of interest in Figure 5. In summary, there was no evidence that the combined genes were synergistic in response to sibutramine. The predominant effects in the combinations was attributable to the GNβ3 TC/TT genotype.

Weight Loss at 4 Weeks' Treatment, Influence of Candidate Genes, and Prediction of 12 Week Weight Loss

Overall treatment effects were observed at 4 weeks (p=0.002) with both 10 mg and 15 mg, differing from placebo (p<0.05, adjusted for two tests). Weight loss at 4 weeks was a significant predictor of weight at 12 weeks, even after adjusting for baseline weight, gender, BMI group and treatment group (partial r-square=0.77, implying that 77% of the variation in weight values at 12 weeks could be explained by the weight values at 4 weeks).

Overall sibutramine treatment effect results for the same, specific gene variants at 4 weeks were similar to those observed at 12 weeks for patients with *GNβ3* TC/TT (p<0.001), *5HTTLPR* LS/SS (p=0.002), and *α2A* CC (p<0.002). However, the *GNβ3* by treatment dose interaction, which was significant at 12 weeks, was not significant at 4 weeks (p=0.27).

Adverse Events and Influence of Candidate Genes

Table III lists adverse events which were consistent with known adverse effects of sibutramine. A significantly higher proportion of participants on sibutramine reported dry mouth and constipation at any time during the trial. There were no significant associations of any of the adverse events in Table III and candidate genes.

DISCUSSION

In this double-blind trial, sibutramine in combination with behavioral therapy resulted in the anticipated degree of weight reduction, but it did not retard gastric emptying of solids. Sibutramine reduced the maximum calorie ingestion in a challenge meal in humans (18) and fundic accommodation in dogs (27), suggesting its effects on weight loss may be mediated centrally (e.g., at the appetite center) or peripherally by changing satiation.

The degree of weight loss at 4 weeks predicted weight loss at 12 weeks, consistent with. analysis of pooled data from seven studies of sibutramine-induced weight loss showing that 2-kg weight loss at 1 month has high sensitivity but low specificity as an indicator of long-term success among non-diabetic subjects (28). Our study provides insights on the pharmacogenetics of sibutramine in combination with behavioral therapy in obesity. In addition to the magnitude of weight loss at 4 or 12 weeks (28), which is included in the recommendation for sibutramine prescription, candidate gene variations provide useful markers of response to sibutramine. They may help select obese patients likely to experience improved outcome with this multimodal treatment since the different markers are present in almost 50% of patients..

Effects of Single Gene Variations

In support of the primary aim of the study, variations in the candidate *α2A* receptor, *5HTTLPR* and *GNβ3* genes were individually associated with the degree of weight loss with sibutramine at 12 weeks (the primary goal of the study), as well as at 4 weeks.

These results confirm our preliminary observation (18) that participants with *5HTTLPR* LS/SS (but not LL) genotype had greater weight loss with sibutramine compared to placebo. The LS/SS genotype reduces reuptake of 5-HT (5), potentially complementing the pharmacological effect of the serotonin reuptake inhibition by sibutramine. Pharmacogenetic effects of *5HTTLPR* genotypes are documented in psychiatric disease (9,10,29) and irritable bowel syndrome (30).

However, results in our current study do not confirm the observation of Hauner et al. that *GNβ3* CC genotype was associated with greater weight loss on sibutramine versus placebo (17). Our study shows that the *GNβ3* TT/TC genotype was associated with greater weight loss with sibutramine than with placebo. The 825T allele is associated with a functionally active splice variant of the G-protein β3 subunit (Gβ3-s) and enhanced intracellular signal transduction. Our hypothesis was that the T allele is associated with greater weight loss since sibutramine inhibits presynaptic reuptake of the neurotransmitters allowing for greater pharmacological action through enhanced intracellular signal transduction.

Effect of Combinations of Gene Variations

Combinations of gene variations were associated with significantly greater weight loss in response to sibutramine than placebo. However, there was no evidence of synergism between combinations of two genotypes on response to sibutramine therapy compared to the effect on weight loss associated with individual genotypes (Figure 5).

Gene-to-gene interactions involving adrenergic genotype variation (chiefly β3 and α2B adrenoceptor genes) were previously associated with obesity phenotype (31–33), but not with pharmacogenetics of obesity therapy.

Potential Effects of Genetic Variation on Adrenergic Mechanisms in Obesity

In animal studies, norepinephrine secretion is strongly associated with eating: α2-adrenoceptors increase and α1-adrenoceptors suppress eating through effects on paraventricular nucleus of the hypothalamus (34). The hypophagic effects of sibutramine and its metabolite are attributed in part to their α1-adrenergic effects (35). Hindbrain adrenergic neurons orchestrate multiple concurrent glucoregulatory responses involved in feeding and appetite control (36), Apart from the central effects, α2A adrenoceptors are involved in the inhibition of subcutaneous fat lipolysis, which favors weight gain (37).

Several lines of evidence suggest that *α2A* C-1291G polymorphism is functionally important through its significant associations with measures of receptor function (38); attention deficit hyperactivity disorder (ADHD); improvement of inattention with methylphenidate treatment in ADHD (39); higher weight gain in schizophrenic patients treated with clozapine and olanzapine (40,41); and personality traits in adolescents (42). The influence of this candidate gene on loss of weight with sibutramine appears unrelated to the gene's association with psychiatric or personality traits since all treatment groups in this study were balanced on all domains.

This study suggests that *α2A* C-1291C genetic variation may result in reduced presynaptic uptake of norepinephrine at α2A adrenoceptors, which may also complement the reuptake inhibition by sibutramine and contribute to the greater weight loss.

Study Strengths and Limitations

The strengths include study design (placebo-controlled, randomized, dose-response study), standardized behavioral treatment, single-center conduct and team to ensure consistency, racial homogeneity of the study population, use of a candidate gene approach to avoid spurious results from multiple comparisons, selection of biomarkers to assess each candidate gene based on biological relevance, and *a priori* sufficient statistical power to assess each gene biomarker of interest based on frequency of the minor allele of each gene and the sample size that was feasible in a single-center study. Moreover, given the preliminary data from the prior study (18), participant randomization was based on gender, BMI and *5HTTLPR* genotype.

There are some limitations of this study. First, the effects on cellular functions of each genotype considered as candidates modulating weight loss in response to sibutramine were not examined. Second, the genotype variations studied may not be the causes but may be in linkage disequilibrium with the causative factor of the altered pharmacogenetics of sibutramine. Third, we have not evaluated each variant separately and had to combine the less prevalent groups for each SNP [SS *5HTTLPR* 20%, GG *α2A* 7% and TT *GNβ3* 3% in controls studied from the same geographical region (18,23,24)]. However, there is evidence of a biological effect for at least one of the genotypes, e.g. the reduced serotonin transporter function in a lymphoblastoid cell line associated with the short allele in *5HTTLPR* (5). Fourth, the sample size was too small to appraise three-way statistical interactions that assess the magnitude of effect in association with variations in three genes. Fifth, we elected to study candidate genes and cannot exclude other potential genetic factors that may impact the response to sibutramine. Sixth, we studied specific markers (e.g. SNPs) of the genes of interest, and further studies with alternative markers (or Tag SNPs) may enhance the predictive accuracy in the response to sibutramine. Finally, the sample size was too small to assess the influence on response to sibutramine of genetic variation in 5-HT_{2A} and 5-HT_{2C} receptors, since prevalence of the minor allele in

controls from the same communities was 15% for 5-HT_{2A} 1438G/A and 35% for 5-HT_{2C} cys23ser (23,24,43). These mechanisms are of interest in view of associations between 5-HT_{2A} (1438 G/A) and eating disorders and anorexia nervosa (44) and with food and alcohol intake in obese people (45), and between 5-HT_{2C} receptor cys23ser substitution in the gene's coding region with weight loss (46).

CONCLUSION

We conclude that the selected markers of the candidate genes are individually significant predictors of weight loss in response to multidimensional sibutramine and behavioral therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

Sample Size Considerations

a. Weight loss in response to sibutramine

Our previous studies (14) with sibutramine suggested the residual pooled standard deviation for change in weight would be 4.0 kg. A two-sample t-test (two-sided $\alpha = 0.025$) with 60 subjects per group provided 80% power to detect an overall (main effect) difference between

an active treatment group and placebo of ~ 2.25 kg. Thus, an entire sample size of 180 patients was planned.

b. Effect of genetic variation on weight response to sibutramine

A stratified randomization procedure was used to assign subjects to treatment groups with balanced proportions of subjects on gender, and *5HTTLPR* polymorphism in each treatment group. Assessing the influence of polymorphisms on treatment differences (interactions) is also a comparison of differences, i.e., sibutramine vs. placebo in those with the genetic polymorphism vs. those without (wildtype). Assuming a similar marginal distribution for *5HTTLPR* as previously observed (35% LL vs. 65% LS/SS), we estimated there would be ~60 patients in the LL strata and 120 patients in the LS/SS strata in the 180 patients planned for the study. Consequently, we anticipated 20 patients per treatment group in the LL strata and 40 patients per treatment group in the LS/SS strata for *5HTTLPR* genotype. Using a pooled estimate of the standard deviation across all strata and treatment groups implies a standard deviation of $\sqrt{2}*4.0$ for a test of differential treatment effects (e.g. placebo vs. an active treatment group in the LL strata vs. the LS/SS strata). Thus, there would have been ~80% power (at a 2-sided α level of 0.025) to detect a differential treatment effect (interaction) between two treatment groups of 4.9 kg. The anticipated analyses (ANCOVA, see below) would have provided similar power for somewhat smaller overall main effects and gene by treatment interactions. Since the minor allele frequency of the other two candidate gene [G allele 43% in *α2A* C1291G (21) and T allele 49% in *GNβ3* C825T (22)] were greater than that of the S allele in *5HTTLPR* polymorphism, there was at least equivalent power to detect an interaction for the other two genes of interest.

Detection of Polymorphisms of Candidate Genes

1. Analysis of the $\alpha 2_A$ -1291 (C–G) Genbank M23533, rs1800544 was examined using restriction fragment length polymorphism (RFLP) at the MspI site, and validated by direct sequence. Primers to amplify the MspI region (in the promoter) as follows: *sense* 5'TCACACCGGAGGTTACTTCCCTCG3'; *antisense* 5'TCCGACGACA GCGCGAGTT 3'.

2. Analysis of the α_{2C} adrenoreceptor polymorphism Del 322–325, (Genbank #J03853) (a 12 base pair deletion from nucleotide 964 in the region coding for the third intracellular loop of the receptor) used the restriction fragment patterns of NciI, and confirmed by direct sequencing with the primers: *sense* 5'CCACCATCGTCGCCGTGTGGCTCATCT 3'; and *antisense* 5'AGGCCTCGCGGCAGATGCCGTACA 3'.

3. 5HTTLPR Polymorphisms (Genbank #X76753) was identified by PCR-based fragment length polymorphisms and confirmed by direct sequencing. We used oligonucleotide primers flanking the 5HTTLPR long polymorphic region corresponding to nucleotide positions 1671 (sense 5'GCCGCTCTGAATGCCAGCAC 3'), and 2219 (antisense 5' GGAGGAACTGACCCCTGAAAACTG 3') to generate 528 and/or 572 base pair PCR amplified fragments.

GNβ3 (Genbank #AY631782, rs5443) - was determined by Taqman® SNP Genotyping Assay C_2184734_10 (Applied Biosystems, Foster City, CA). The cycling conditions were 95°C 10 minutes, then 40 cycles of 95°C 15 sec, 60°C 1 min. The SNP analysis is done using the Sequence Detection software v1.3.1 (Applied Biosystems, Forest City, CA) The *sense* and *antisense* primers used for validation by direct sequence of the C825T (rs5443) polymorphisms were 5' TGACCCACTTGCCACCCGTGC 3' and 5'GCAGCAGCCAGGGCTGGC 3'.

All polymorphisms were confirmed by random direct sequencing. Samples were sequenced by Mayo Clinic's DNA Sequencing Core Facility using Applied Biosystems BigDye® terminator v1.1 cycle sequencing chemistry and analyzed on Applied Biosystems 3730XL DNA Analyzer. Where there was not a restriction site available, as in the case of the SERT-transporter, polymorphisms were confirmed by direct sequence alone.

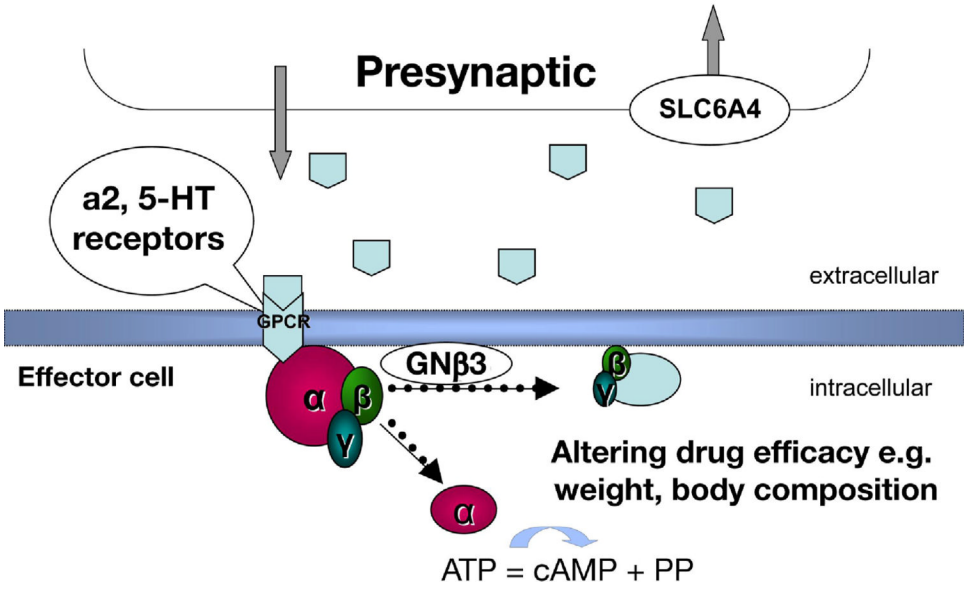

Figure 1. Conceptual diagram

shows the potential interaction between α2A adrenergic mechanisms, G protein translation and serotonin reuptake through SLC6A4 in mediating the effects of the norepinephrine and serotonin reuptake inhibitor, sibutramine.

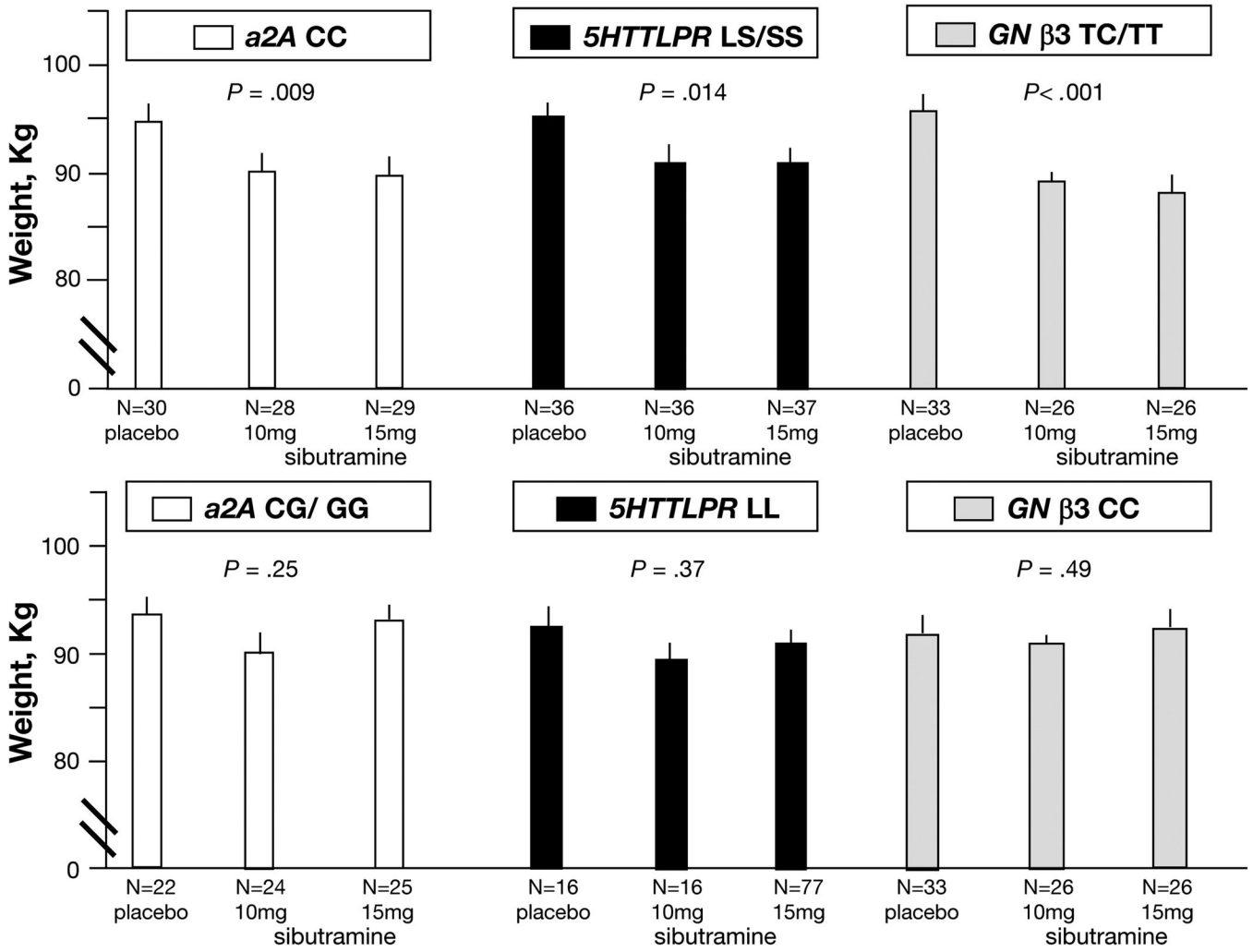

Figure 2. Relationship between 12-Week Post-Treatment Body Weight and Sibutramine Dose Based on Single Genotypes

P-values indicate overall treatment effects for specific genotype. Least squares (adjusted for baseline weight and other covariates) means ± SE are plotted, unadjusted p-values from ITT analyses based on ANCOVA models. N refers to number of patients with complete data in each group. Upper panel identifies significant effects of sibutramine on post treatment weight in patients with the single genetic makeup indicated. Lower panel shows the corresponding treatment group values of post-treatment weight in patients with the alternative genetic variations for these single genes, and in which no significant treatment effects were detected.

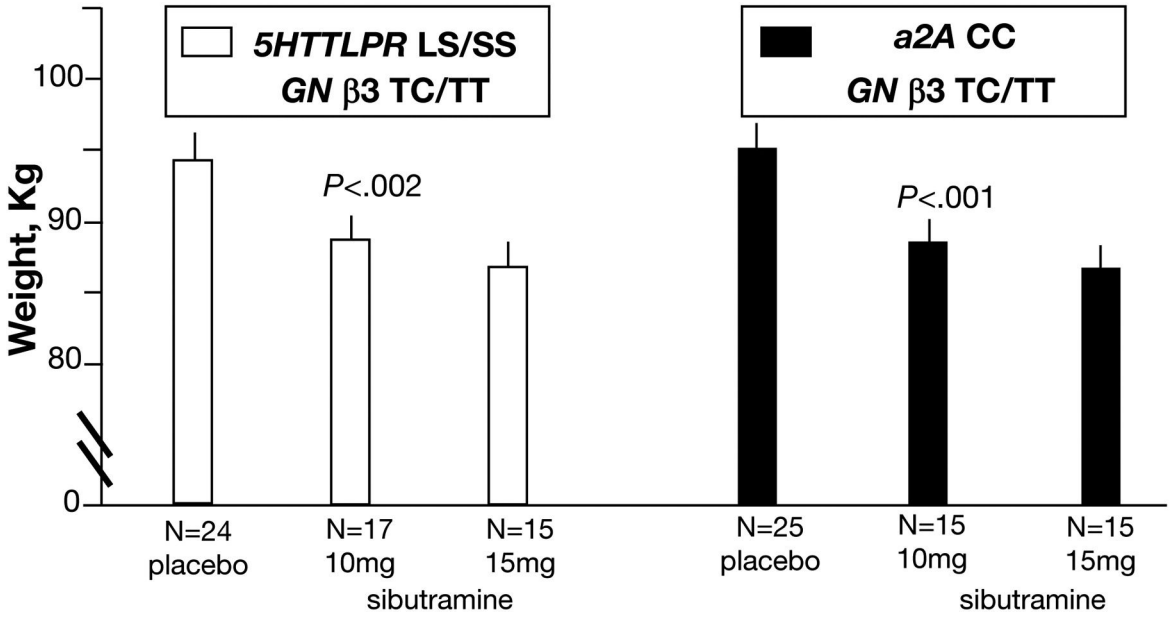

Figure 3. Relationship between 12-Week Post-treatment Body Weight and Sibutramine Dose Based on Gene Combinations

P-values indicate overall treatment effects for specific genotype combination. Least squares (adjusted for baseline weight and other covariates) means ± SE are plotted, unadjusted p-values from ITT analyses based on ANCOVA models. N refers to number of patients with complete data in each group. There were significant effects of sibutramine on post treatment weight in patients with the specified genetic combination indicated.

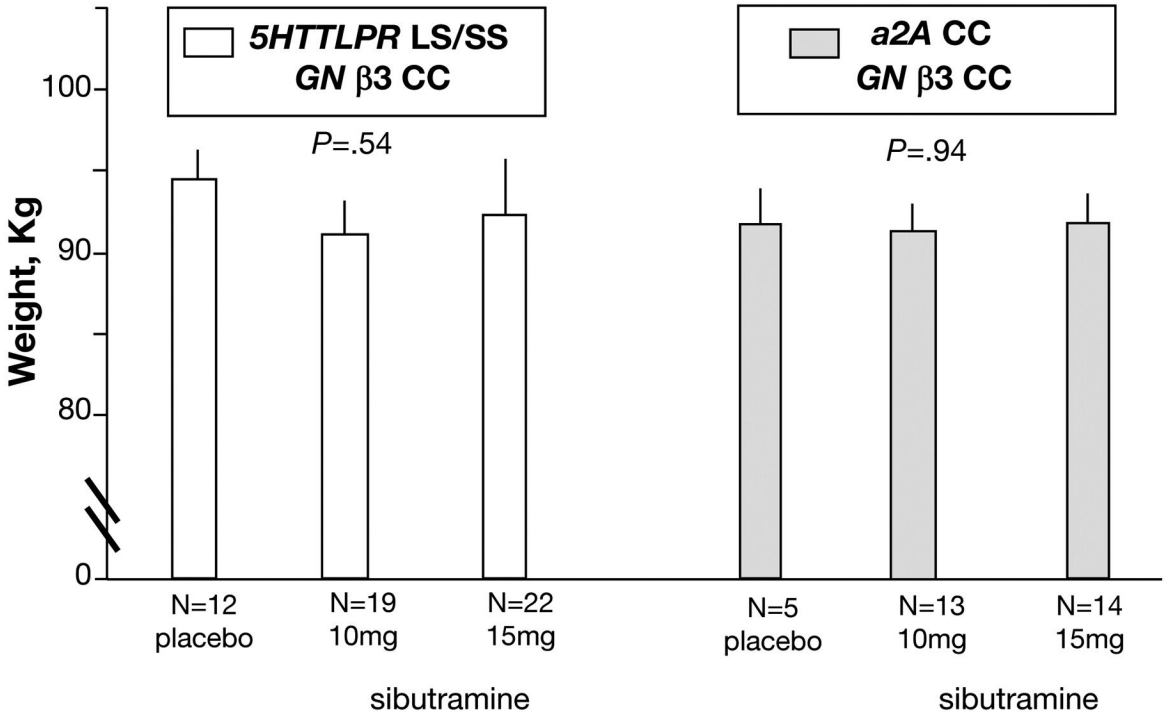

Figure 4. Relationship between 12-Week Post-treatment Body Weight and Sibutramine Dose Based on Gene Combinations with *GNβ3* Variations

P-values indicate overall treatment effects for specific genotype combination. Least squares (adjusted for baseline weight and other covariates) means ± SE are plotted, unadjusted p-values from ITT analyses based on ANCOVA models. N refers to number of patients with complete data in each group. Treatment effects were not detected in these combinations. The lack of effects of these gene combinations with *GNβ3* CC genotype (data in Figure 4) should be contrasted the significant effects of *5HTTLPR* LS/SS or *α2A* CC with *GNβ3* TC/TT (data provided in Figure 3).

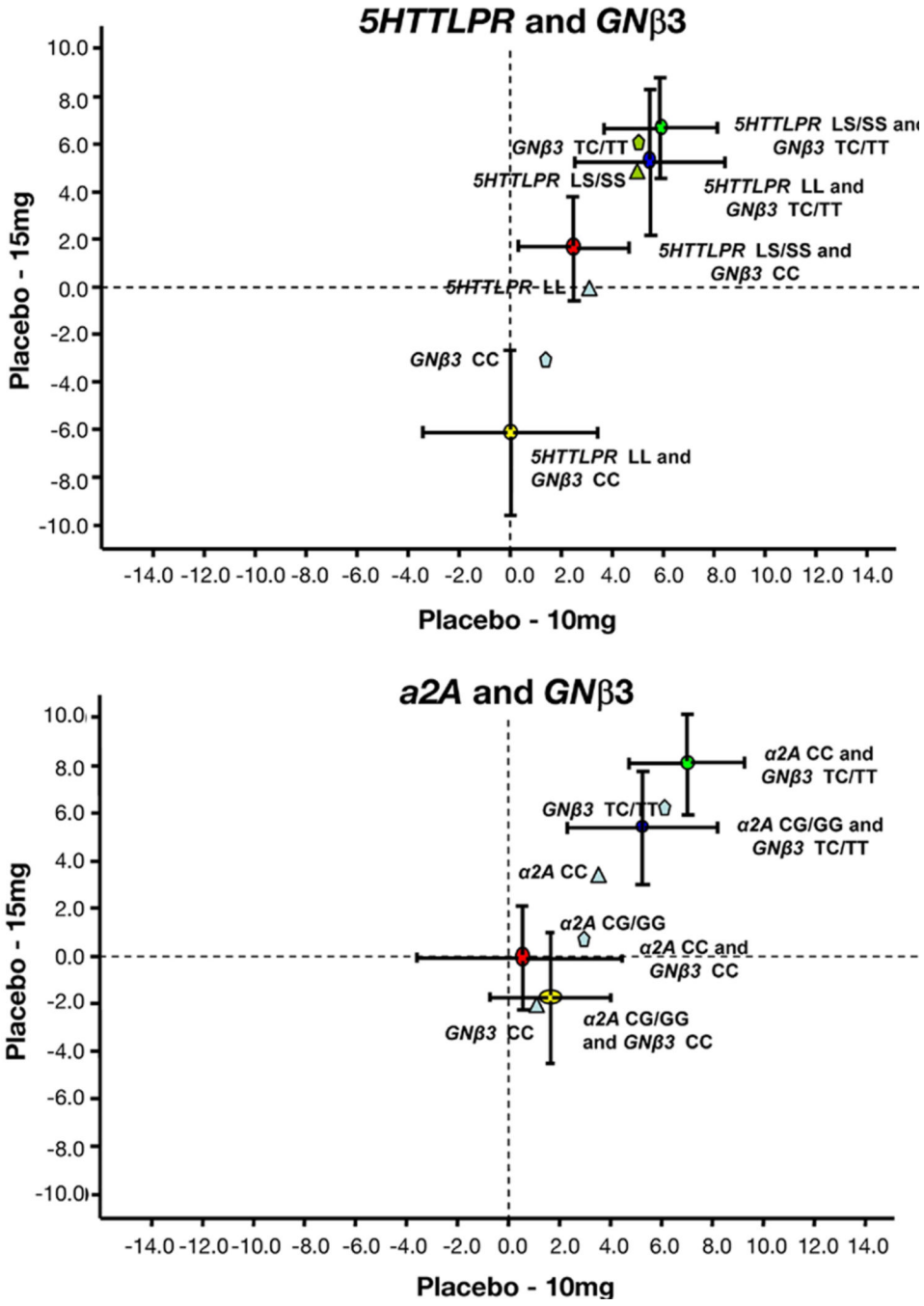

Figure 5. Estimates of treatment effect of sibutramine, 15 mg, vs. placebo (Y-axis) and sibutramine, 10 mg, vs. placebo (X-axis) in association with combined versus individual genotype variation

Note the differences in the responses to each dose of drug relative to placebo are not significantly greater for the different combinations of two genotype variations (means+SEM) relative to weight loss with individual genotype variations (mean data shown for clarity).

Table I

Table IA. Demographics and Psychological Characteristics at Baseline (mean ± SE unless otherwise noted)

	Placebo (N=62)	Sibutramine, 10 mg (N=58)	Sibutramine, 15 mg (N=61)
# obese / # overweight	51/11	48/10	50/11
Age, y	41.3 ± 1.4	42.2 ± 1.4	44.8 ± 1.3
Gender (%Female)	87%	91%	89%
Waist circumference, cm	105.1 ± 1.6	102.3 ± 1.5	101.9 ± 1.5
Weight, kg	95.9 ± 2.1	95.6 ± 2.2	93.1 ± 2.2
BMI, kg/m^2	34.5 ± 0.6	34.8 ± 0.7	33.9 ± 0.6
Fasting blood glucose, pre	94.6 ± 1.3	95.6 ± 2.2	93.2 ± 2.1
Anxiety (HAD score)	4.4 ± 0.3	5.0 ± 0.4	4.5 ± 0.4
Gastric emptying, T ½, min	77.9 ± 2.5	82.1 ± 3.1	82.4 ± 2.5
Depression (HAD score)	3.1 ± 2.3	3.3 ± 0.3	3.0 ± 0.3
SAAST score	2.2 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
Hunger	5.7 ± 0.4	5.4 ± 0.5	5.4 ± 0.4
Disinhibition	8.9 ± 0.5	8.8 ± 0.5	8.8 ± 0.4
Cognitive Restraint	9.8 ± 0.6	9.1 ± 0.6	10.6 ± 0.7
% of participants performing regular physical activity	40	31	41

Table IB. Distribution of Genotypes (single and combined) by Treatment Groups

Note that randomization was balanced on *5HTTLPR* genotype. The randomization also resulted in similar distributions of single and combined genotypes, particularly for *α2A* and *5HTTLPR*.

Genotypes:	Placebo (n=62)	Sibutramine, 10 mg (n=58)	Sibutramine, 15 mg (n=61)	P values[§]
α2A				0.62
α2A (CC), n (%)	36 (58)	29 (50)	31 (51)	
α2A (GC/GG), n (%)	26 (42)	29 (50)	30 (49)	
GN β3				0.08
GNβ3 (CC), n (%)	22 (35)	31 (53)	32 (52)	
GNβ3 (TC/TT), n (%)	40 (65)	27 (47)	29 (48)	
5HTTLPR				0.92
5HTTLPR (LL), n (%)	20 (32)	17 (29)	18 (29)	
5HTTLPR (LS/SS), n (%)	42 (68)	41 (71)	43 (71)	
α2A and *GN β3*				0.25
α2A (CC) and GN β3 (TC/TT), n(%)	28(45)	15(26)	16(26)	
α2A (CC) and GN β3 (CC), n(%)	8(13)	14(24)	15(25)	
α2A (CG/GG) and GN β3 (TC/TT),n (%)	12(19)	12(21)	13(21)	
α2A (CG/GG) and GN β3 (CC),n(%)	14(23)	17(29)	17(28)	
5HTTLPR and *GN β3*				0.31
5HTTLPR (LS/SS) and *GN β3* (TC/TT),n(%)	28(45)	17(29)	18(30)	
5HTTLPR (LS/SS) and *GN β3* (CC),n(%)	14(23)	24(41)	25(41)	
5HTTLPR (LL) and *GN β3* (TC/TT),n(%)	12(19)	10(17)	11(18)	
5HTTLPR (LL) and *GN β3* (CC),n (%)	8(13)	7(12)	7(11)	
α2A and *5HTTLPR*				0.41
α2A (CC) and *5HTTLPR* (LS/SS), n (%)	21(34)	19(38)	24(31)	
α2A (CC) and *5HTTLPR* (LL), n(%)	15(24)	10(17)	7(11)	
α2A (CG/GG) and *5HTTLPR* (LS/SS), n(%)	21(34)	22(38)	19(31)	
α2A (CG/GG) and *5HTTLPR* (LL), n (%)	5(8)	7(12)	11(18)	

Three-Factor Eating Questionnaire was used to measure hunger, disinhibition, cognitive restraint; SAAST score is derived from responses to self-administered alcoholism screening test, based on 35 questions. As would be expected based on the randomization, there were no significant differences in the three groups.

[§]Chi-square test for association between genotype (or genotype combination) and treatment group assignment.

Table II

Table IIA. Effect of Sibutramine Treatment on Weight, BMI, and Body Fat Composition and Influence of Genotype on Effect of Sibutramine Treatment on Proportion of Fat [values are least squares means ± SEM (adjusted for covariates) from ANCOVA analyses]

	Placebo	Sibutramine, 10 mg	Sibutramine, 15 mg	p †
Weight, kg *	94.3±1.2	90.5±1.3	91.3±1.2	0.009
BMI, kg/m^2	34.4±0.4	33.0±0.4	33.0±0.4	0.001
Gastric emptying $T_{1/2}$, min	87.6±4.6	84.9±4.8	79.5±4.6	ns
Dexa body composition, lean	43.6±0.8	43.1±0.8	42.7±0.7	ns
Dexa body composition, % fat	51.2±0.8	49.7±0.8	50.0±0.8	0.051
Dexa % fat by genotype: *α2A* CC	51.6±0.9	49.8±0.9	49.1±0.9	0.016
Dexa % fat by genotype: *α2A* CG/GG	50.9±0.9	49.8±0.9	51.0±0.9	ns
Dexa % fat by genotype: *5HTTLPR* LL	51.3±1.0	49.4±1.1	50.9±1.1	ns
Dexa % fat by genotype:*5HTTLPR* LS/SS	51.3±0.9	50.0±0.9	49.7±0.9	ns
Dexa % fat by genotype: *GNβ3* CC	51.1±1.0	50.4±1.0	50.8±0.9	ns
Dexa % fat by genotype: *GNβ3* TC/TT	51.5±0.9	49.3±0.9	49.3±1.0	0.014

Table IIB. Per Protocol Observations of Weight, BMI and Gastric Emptying in the Therapeutic Trial

	Placebo	Sibutramine, 10 mg	Sibutramine, 15 mg
N=	52	52	54
Weight, kg, at baseline	97.4±2.3	95.9±2.3	94.4±2.2
Weight, kg, at end of 12 weeks' treatment	96.1±2.4	91.4±2.4	89.6±2.3
BMI, kg/m^2 at baseline	35.3±0.7	35.0±0.8	34.3±0.6
BMI, kg/m^2 at end of 12 weeks' treatment	34.8±0.7	33.4±0.8	32.5±0.6
Gastric emptying $T_{1/2}$, min at baseline	77.8±3.0	81.2±3.4	82.8±2.8
Gastric emptying $T_{1/2}$, min at end of 12 weeks' treatment	87.1±5.1	87.2±5.1	81.3±3.0

Table IIC. Proportion (%) ± SE (%) of Patients who Lost >5kg Weight in 12 Weeks of Treatment with Placebo (PLA) or Sibutramine (SIB) Based on Genotype of Interest (per protocol results, "collapsing" over other genotype)

	***α2A* CC**	***α2A* CG/GG**	***GNβ3* TC/TT**	***GNβ3* CC**	***5HTTLPR* LS/SS**	***5HTTLPR LL***	***α2A CC* + *GNβ3* TC/TT**	***α2A CG/GG* + *GNβ3 CC***
PLA	13% ± 6%	18% ± 8%	9% ± 5%	26% ± 10%	14% ± 6%	19% ± 10%	4% ± 4%	14% ± 10%
SIB †	49% ± 7%	26% ± 6%	56% ± 7%	22% ± 6%	40% ± 6%	36% ± 6%	63% ± 9%	11% ± 6%

† From ITT analyses based on ANCOVA models (genotype specific values unadjusted for multiple gene specific comparisons)

* Effect of genotype on treatment-induced weight loss is shown in figure 2. Individual candidate genes were associated with ~5kg weight loss, and two pairwise combinations of these candidate genes were associated with ~7kg during three months' treatment with sibutramine (see figure 2).

† (summarized across 2 doses)

Table III

Adverse Events Noted at Any Time during Therapeutic Trial in More than 5% Participants for Any Treatment Group (values listed are percentages of patients randomized in each treatment group) and Participants with Elevated Blood Pressure

There were no significant associations between candidate genes and adverse events.

	Placebo	Sibutramine 10 mg	Sibutramine 15 mg	p-value[§]
Dry Mouth	24	59	70	<0.001
Constipation	8	24	34	0.006
Insomnia	8	24	25	0.068
Headache	11	5	13	0.28
Anxiety/Restlessness	2	7	0	0.061[†]
Palpitations	0	7	8	0.82
Elevated BP[*]	0	2	2	1.0[†]

[*] In addition, 1 participant randomized to 10 mg sibutramine withdrew because of elevated BP.

[§] Chi-square test for association with treatment group

[†] Fisher's Exact Test