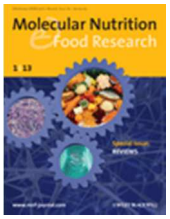


Serotonin-transporter promoter polymorphism modulates the ability to control food intake: effect on total weight loss

Journal:	*Molecular Nutrition and Food Research*
Manuscript ID	Draft
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Bonnet, Gemma; Department of Physiology, University of Murcia, Murcia. Spain. IMIB, Spain. Gómez-Abellán, Purificación; Department of Physiology, University of Murcia, Murcia. Spain. IMIB, Spain. Vera, Beatriz; Department of Physiology, University of Murcia, Murcia. Spain. IMIB, Spain. Sánchez-Romera, Juan Francisco; Department of Psychobiology and Behavioral Neurobiology, University of Murcia, Murcia. Spain. IMIB, Spain Hernández-Martínez, Antonio; Department of Endocrinology and Nutrition, "Virgen Arrixaca" Hospital and University of Murcia. Murcia. Spain Sookoian, Silvia; Department of Clinical and Molecular Hepatology, Institute of Medical Research, A. Lanari-IDIM, University of Buenos Aires-CONCET, Buenos Aires, Argentina. Pirola, Carlos Jose; Department of Molecular Genetics and Biology of Complex Diseases, Institute of Medical Research, A. Lanari-IDIM, University of Buenos Aires-CONCET, Buenos Aires, Argentina. Garaulet, Marta; University of Murcia, Department of Physiology
Keywords:	5-HTTLPR, disinhibition, emotional eating, SLC6A4, weight loss

Title: Serotonin-transporter promoter polymorphism modulates the ability to control food intake: effect on total weight loss

Running title: *SLC6A4,* disinhibition and weight loss

Authors: Gemma Bonnet[1*], Purificación Gómez-Abellán[1*], Beatriz Vera[1], Juan Francisco Sánchez-Romera[2], Antonio M. Hernández-Martínez[3], Silvia Sookoian[4], Carlos Jose Pirola[5], Marta Garaulet[1].

Affiliations: [1]Department of Physiology, University of Murcia, Murcia. Spain. IMIB, Spain. [2]Department of Psychobiology and Behavioral Neurobiology, University of Murcia, Murcia. Spain. IMIB, Spain. [3]Department of Endocrinology and Nutrition, "Virgen Arrixaca" Hospital and University of Murcia. Murcia. Spain. [4]Department of Clinical and Molecular Hepatology and [5]Department of Molecular Genetics and Biology of Complex Diseases, Institute of Medical Research, A. Lanari-IDIM, University of Buenos Aires-CONCET, Buenos Aires, Argentina.

*both authors had similar contributions

Keywords: 5-HTTLPR; disinhibition; emotional eating; SLC6A4; weight loss.

Corresponding author: Marta Garaulet. Department of Physiology, Faculty of Biology, University of Murcia. Campus de Espinardo, s/n. 30100. Murcia, Spain. Phone: +34 868 88 39 30. Fax: +34 868 88 39 63. E-mail: garaulet@um.es.

ABSTRACT

Scope: The biggest challenge for losing weight is the ability to control the amount of food eaten; the tendency to overeat is called disinhibition. Our aims were to determine whether a) the *SLC6A*4-promoter variant (5-HTTLPR) relates to disinhibition; b) this association could affect total weight-loss during a behavioral/dietary treatment for obesity.

Methods and Results: 2961 subjects attended voluntarily five weight-loss clinics; a subsample (n=624) was recruited for *SLC6A4* genotyping. Total weight-loss, Emotional-Eating-Score (EES) and Disinhibition-Score (DS) were examined. We observed: a) reduced ability to control food intake (disinhibition) is implicated in the impairment to lose weight; b) 5-HTTLPR is implicated in disinhibition. S carriers (low-expressing) of the *SLC6A4*-promoter variant had a lower inhibition capacity and showed more failure (1.6 times) to control the amount of food eaten than LL ($P<0.05$); other factors such as eating while bored, overeating after work at night, or craving for specific foods were associated to the *SLC6A4* genotype ($P<0.05$); c) The combination of disinhibition (high DS) and genetics (S carrier) had a higher impact on total weight loss than each factor separately.

Conclusions: Serotonin-transporter-promoter polymorphism is associated with the ability to control food intake and interacts with emotional eating to modulate total weight loss.

Word count: 200

INTRODUCTION

The association between emotional eating and body weight is becoming an area of increased interest and concern in obesity research [1, 2]. Studies suggest that emotional eaters tend to eat more when experiencing negative emotions such as annoyance, irascibility, fear, unhappiness, or boredom [3].

One of the biggest challenges for those trying to lose weight, is the ability to control the amount of food eaten [4]. This tendency to overeat is called disinhibition, and occurs in response to different stimuli or factors that may be *external*, such as social events and palatability of food; *internal*, such as feelings and thoughts, and *genetics* [5]. Different studies have analyzed the relative influence of these factors and assert that disinhibition is strongly heritable [5].

Serotonin is related to disinhibition. The reduction of brain serotonin (5-hydroxytryptamine or 5-HT) may result in hyperphagia [6] while the treatment with serotonin reuptake inhibitors, results in a decrease in disinhibition [6, 7]. It is known that serotonin activity is regulated by the 5-HT transporter (5-HTT) which a) modulates the reuptake of serotonin in serotonergic nerve terminals and b) determines the magnitude of the postsynaptic response to serotonin and signaling quantity [8]. The human 5-HTT is known to be encoded by the serotonin transporter gene (*SLC6A4*). One functional genetic variant, the *SLC6A4*-linked polymorphic region (5-HTTLPR) [9] consists of two common alleles, a short (S) variant with 14 copies and a long (L) variant with 16 copies of a 20-23-bp imperfect repeat sequence [10-13]. Importantly, S allele (SS or SL genotypes) has been associated with a lower *SLC6A4* expression resulting in

a reduced serotonin reuptake and release capability; conversely, L variant is associated with an increase of gene transcription (almost three times more) [14].

The S allele (low-expressing) of the *SLC6A4* promoter variant has been linked to eating disorders [15-17] and obesity [18, 19]. This polymorphism has been described as an independent genetic risk factor for obesity in the general population [18, 19] and in childhood [20]. However, other study has not found this association [21]. Moreover, the emotional status has been implicated in this association [18, 22, 23].

Most of the studies about *SLC6A4* promoter variant and disinhibition have been performed on subjects with mental disorders or diagnosed with BED (binge eating disorders) or other food-related psychological alterations [24-27]. Considering that serotonin influences disinhibition and the ability to control the amount of food eaten, it may be speculated that individuals who carry the S allele of the *SLC6A4* promoter variant could have greater difficulties in weight control than non-carriers during a weight loss treatment, as a result of this genetic predisposition that leads them to eat more.

Considering these questions, the aims were to determine whether a) one allelic promoter variant of the serotonin transporter gene (*SLC6A4*) relates to emotional eating, or to the ability to control the amount of food eaten in an overweight/obese population subjected to a behavioral/dietary treatment for obesity; b) this association could affect total weight loss.

SUBJECTS AND METHODS

Participants

2961 subjects (80% of women) attended voluntarily five different weight-loss clinics in Spain looking for dietetic and behavioral treatment based on the fundaments of a Mediterranean diet ONTIME (Obesity, Nutrigenetics, Timing, Mediterranean; registered at clinicaltrials.gov as NCT02829619). From the total population, a subsample of 624 overweight and obese subjects (BMI: 31.7 ± 5.7 kg/m^2; age: 39 ± 13) was selected for *SLC6A4* genotyping. This subsample was comprised by those subjects who a) attended to one of our weight-loss clinic sites (in the town of Murcia, Spain) and b) had responded to the set of variables that were needed for the outcome of interest. The study was blinded for the researchers, the medical doctor, and the nutritionists, who did not know the genetic variant of each participant.

All subject who came to the clinics to lose weight were included in the study except for those receiving treatment with thermogenic, lipogenic, or contraceptive drugs; diabetes mellitus, chronic renal failure, hepatic diseases, or cancer diagnosis; bulimia diagnosis, prone to binge eating, or undergoing treatment with anxiolytic or antidepressant drugs; or under the age of 14 or above 75 years (exclusion criteria). Participants' data were codified to guarantee anonymity. All procedures were conducted in accordance with good clinical practice.

Characteristics of the treatment

Subjects attended 60-minute therapy sessions once *per* week. The mean duration of the program varied depending on each weight-loss goal. When the weight-loss goal was achieved, the subjects continued completing a maintenance period for five months maximum. The program sessions were led by certified nutritionists. Dietetic treatment

was based on the principles of the Mediterranean diet and the distribution of macronutrient components adhered to the recommendations of the Spanish Society of Community Nutrition [28, 29].

The characteristics of the weight reduction program (Garaulet method©) have been described elsewhere [29]. Briefly, during the initial 4 months, subjects attended a weekly 60-min therapy session in support groups (n=10), followed by a 5-month maintenance period. The dietary energy content ranged from 1200–1800 kcal per day for women and 1500–2000 kcal per day for men to induce an approximate loss of 0.5–1 kg per week, in order to achieve a total weight loss of 10% of the initial weight, as recommended by the Spanish Society of the Study of Obesity (SEEDO) [30].

Total weight loss and physical activity assessment

Anthropometric measurements were assessed at baseline, and weight was monitored weekly. Subjects were weighed on a digital body weight scale which measured to the nearest 0.1 kg, they were barefoot and wore light clothing. We measured the subject's height at baseline, by a Harpenden digital stadiometer (with a rank of 0.7–2.05). Participants were told to stand in a relaxed upright position with their head oriented in the Frankfurt plane. Initial BMI was calculated using baseline measurements as weight (kg)/height^2 (m). We measured their initial body fat with bioelectrical impedance; for this measuring we used TANITA TBF-300 equipment (Tanita Corporation of America, Arlington Heights, IL, USA).

The International Physical Activity Questionnaire (IPAQ) was administered with assistance from a nutritionist to assess physical activity (PA) during the 7-days prior to

enrollment [31]. The IPAQ has been validated internationally and in a Spanish population, in which good correlation with accelerometer data were obtained [31, 32]. A total PA score reflecting intensity and time was calculated in MET (metabolic equivalent of task) minutes per week for the four IPAQ domains combined.

Emotional Eating Questionnaire (EEQ)

In order to assess emotional habitual dietary intake eating behavior, we used the Emotional Eating Questionnaire (EEQ). This is a questionnaire that has been validated for overweight and obese subjects [33]. The questionnaire includes 10 different items which have been created to assess to what extent emotions affect eating behavior: 1. Do the weight scales have a great power over you? Can they change your mood? 2. Do you crave specific foods? 3. Is it difficult for you to stop eating sweet things, especially chocolate? 4. Do you have problems controlling the amount of certain types of food you eat? 5. Do you eat when you are stressed, angry or bored? 6. Do you eat more of your favorite food and with less control when you are alone? 7. Do you feel guilty when eat "forbidden" foods, like sweets or snacks? 8. Do you feel less control over your diet when you are tired after work at night? 9. When you overeat while on a diet, do you give up and start eating without control, particularly food that you think is fattening? 10. How often do you feel that food controls you, rather than you controlling food? Each item was answered with a score of 0 to 3. From the sum of these items we calculated an emotional eating score (EES) which ranged from 0 (non-emotional eaters) to 30 (very emotional eaters) [33].

In the current study, subjects were dichotomized into emotional and non-emotional eaters. As a cut-off point, we used the median emotional score of the total population (<12 non-emotional; ≥12 emotional) [34].

Other life style characteristics such as dietary intake, were determined as previously reported [35].

Disinhibition Score (DS)

A DS score obtained in a previous population [33] was further applied in the current studied population as follows: for every subject the answer of each of the six disinhibition-related questions of the EEQ (0 to 3) was multiplied by its load in the factor analysis [33]. The sum of this product of the six questions was multiplied by the percentage of total variance in the factor analysis (25.6) to obtain a final punctuation as follows: Disinhibition score (DS) = (Question 1: Do you feel less control over your diet when you are tired after work at night? * 0.759) + Question 2: Do you eat more of your favorite food and with less control when you are alone? * 0.664) + (Question 3: Do you eat when you are stressed, angry or bored? * 0.649) + (Question 4: When you overeat while on a diet, do you give up and start eating without control, particularly food that you think is fattening? * 0.610) + (Question 5: How often do you feel that food controls you, rather than you controlling food? * 0.559) + (Question 6: Do you have problems controlling the amount of certain types of food you eat?*0.554))*25.6.

DNA isolation and SLC6A4 genotyping

Genetic analyses were carried out on genomic DNA extracted from white blood cells using a standard extraction method (Qiagen, Valencia). The genotyping for the 43-bp deletion–insertion polymorphism in the *SLC6A4* promoter was performed by hot-start polymerase chain reaction (PCR) using Molecular Biology grade reagents by the previously reported method (Kawasaki). Following protocol was used: initial denaturation at 95°C for 2 min; 30 cycles of a) denaturation at 95°C for 20 s, b) hybridization at 64°C for 10 s, c) polymerization at 72°C for 14 s; and a final polymerization at 72°C for 2 min. PCR was carried out in a total volume of 10 µl containing 0.2 µl of genomic DNA, 0.2 µM of each primer, 4.2 µl of double-distilled water and 5 µl of KOD Hot Start DNA Polymerase. The PCR products were separated by electrophoresis in a 2.5% agarose gel in TAE 1%.

Subjects were genotyped with the classic biallelic classification (S and L alleles) [36]. This classification was performed using size discrimination for the LL (556-bp), for the LS (556-bp and 513-bp) and SS (513-bp). Primers used to detect the variant were 5'-CAA GCT TGT TGG GGA TTC TCC-3' (forward) and 5'-AGA GGG ACT GAG CTG GAC AAC-3' (reverse). Biallelic genotyping revealed the following *SLC6A4* allele frequencies: L= 50.4% and S= 49.6%.

We also used a triallelic classification of the subject (S, L_G and L_A alleles) [36]. The triallelic *SLC6A4* polymorphism-genotyping was performed after a restriction digestion with MspI enzyme following PCR amplification during one hour. The MspI enzyme cuts the CCGG sequence, leading to the following alleles: S, L_G and L_A, resulting in six genotypes: SS, SL_G, SL_A, L_GL_G, L_GL_A and L_AL_A. Genotypes were grouped according to their functionality [37] as low and medium activity (S-liked

subjects): SS, SL_G, SL_A, L_GL_A and L_GL_G, and as high activity: L_AL_A. Triallelic genotyping showed the following *SLC6A4* allele frequencies: low and medium activity (S-liked) = 82.9% and high-activity (L_AL_A carriers) = 17.1%.

The genotype information was blinded for the researchers, the medical doctor, and the nutritionists, who did not know the genetic variant of each participant.

Food intake hormones (leptin and ghrelin)

Venous blood samples were taken from the subjects after fasting overnight. Blood collection, plasma separation and processing, as well as biochemical analyses for plasma food intake hormones (leptin and ghrelin) were performed. Basal plasma leptin levels were measured using a gamma counter (DPC Gambyt, city and country) and RIA kits from Mediagnost Laboratory (Reutlinge, Germany) with a sensitivity of 0.5 ng/ml and intra assay CV of 8.3%. Total ghrelin levels were measured using a RIA from Mediagnost Laboratory (Reutlinge, Germany) with a sensitivity of 2 ng/ml and intra-assay CV of 4.0% at 1.2l g/L. A gamma counter (DPC Gambyt) was used for the quantification of this hormone.

Statistical analyses

-For potential associations between emotional eating, energy intake and total weight loss

We analyzed the association of emotional eating score (EES) and disinhibition score (DS) with energy intake (kcal) and weight loss (kg and %) in the total population of n=2961 subjects, by a linear regression analysis and by univariate linear regression

models (ANCOVA). We adjusted analyses for potential confounders including sex, age, study number (different dates in which patient data were collected), nutritional center and obesity when required (when total weight loss was involved in the statistical analysis).

-For genetic associations with emotional eating and weight loss

We tested different genetic inheritance models, and applied a dominant model to the concluding analyses for *SLC6A4*; consequently, minor S allele carriers or S-liked (biallelic: SS + SL; triallelic: SS + SL_G + SL_A + L_GL_A + L_GL_G) were compared against major L allele homozygotes (biallelic: LL; triallelic: L_AL_A). SNP data was analyzed for genotype and allele frequency determination by applying chi-squared statistics. In the current population, genotype frequency was consistent with Hardy–Weinberg equilibrium (P>0.05).

We looked for associations between *SLC6A4,* emotional eating and disinhibition using ANCOVA as previously described, and similar analyses were performed for total weight loss and maintenance period duration.

-For gene-emotional eating interactions for total weight loss

We studied gene-emotional eating and gen-disinhibition interactions for total weight loss. Predicted values of total weight loss according to *SLC6A4* genotypes were plotted against EES and DS, respectively, and evaluated continuously. Further analyses of gene-emotional eating interactions were done with EES and the DS considered as categorical (low or high) taking into account the median value. We adjusted analyses for sex, age, study number and nutritional center and obesity when required.

-To assess the particular relevance of each of the EEQ questions

A discriminate analysis was performed in order to distinguish which of the different questions of the EEQ were able to correctly classify subjects attending to their individual genotype (S or LL), and these questions were treated as predictors. The discriminant variables were entered stepwise according to the Wilks' lambda criterion. The univariate F-tests were then calculated in order to determine the importance of each independent variable in forming the discriminant functions. Examining the Wilk's Lambda values for each of the predictors revealed how important the independent variable was to the discriminant function, with smaller values representing greater importance.

We also fitted logistic regression models to estimate the odds ratios (ORs) and 95% CIs of the emotional eating behaviors associated with the *SLC6A4* promoter variant. Chi-square tests were used to test differences in allele frequency with eating while bored.

All statistical analyses were performed using SPSS statistical software (v. 20). A two-tailed *P*-value of 0.05 was considered statistically significant.

RESULTS

Emotional eating associates with energy intake and total weight loss

Emotional eating (EES) and disinhibition scores (DS) were significantly associated with total weight loss (**Figure 1**) in 2961 subjects (73% of women) who attended to different clinics to lose weight. Characteristics of this population are described in **Table 1**. Those emotional eaters (**Figure 1A**), and those who had a low ability to control food intake

(high DS) (**Figure 1B**), had higher intake of calories (~100Kcal/day more) and tended to lose less weight during the treatment (~700g less) (**Figure 1C and 1D**).

Further regression analyses showed similar significant associations between total weight loss and both EEQ score and disinhibition score (Beta: -0.046; EEM: 0.018; P=0.009; Beta: -0.004; EEM: 0.002; *P*=0.036). Moreover, the adherence to the program was 68% and differed significantly between high emotional eaters 49% and low emotional eaters (*P*=0.042).

Genetics associates with emotions but not with total weight loss

In order to discover whether the *SLC6A4* promoter variant was implicated in these associations, 624 overweight and obese subjects (BMI: 31.7 ± 5.7 kg/m^2; age: 39 ± 13) who attended one of the five weight-loss clinics involved in the study, were recruited for *SLC6A4* analysis (79% women). The frequency of the S allele was of 49.6% (Table 1). We first explored whether the S/L variant of the *SLC6A4* was associated with emotional eating as assessed by the EEQ. We observed that S carriers (low-expressing) were more emotional (significantly higher EES, 12.4±6.6) than homozygote carriers of the major allele (LL) (11.3±6.4) (*P*=0.037) (**Figure 2A**). Similar results were obtained for disinhibition, towards a higher score in S than in LL carriers (*P*=0.036) (**Figure 2B**).

No significant associations were found between the *SLC6A4* promoter and total weight loss in the population studied (*P*>0.05). On the other hand, when we studied the potential association between *SLC6A4* genotype and the duration of the maintenance period, our results showed that S carriers quitted the maintenance program before (S carriers: 5.1±0.3 weeks *vs.* LL: 6.5±0.5 weeks; *P*=0.011) and these results were maintained after adjusting for initial BMI (*P*=0.010).

Gene-emotional eating interactions for total weight loss

Subsequent analyses identified significant gene-emotional eating interactions for total weight loss at the *SLC6A4* promoter after triallelic classification of the genetic variant (*P*=0.036) (**Figure 3A**). In general, those S-liked carriers for *SLC6A4* who were high emotional eaters lost significantly less weight than the other participants of the treatment (S-liked and low-emotional or L_AL_A carriers). Interestingly, we also found a significant gene-disinhibition interaction for total weight loss at the *SLC6A4* promoter after biallelic classification (*P*=0.029) (**Figure 3B**).

After categorizing the population in high and low emotional eaters, those classified as emotional eaters who also were S carriers lost ~2kg less than L carriers during the treatment [Total weight loss (kg): (Mean ± SD) (6.81 ± 6.24 *vs* 8.66 ± 8.89)] for S and LL carriers respectively (*P*=0.013; *P* for interaction=0.010). Similar differences were found between S and L carriers among those who had a low ability to control food intake (high DS) [Total weight loss (kg): (Mean ± SD) (6.40 ± 6.20 *vs* 8.47 ± 8.71)] for S and LL carriers respectively (*P*= 0.013; *P* for interaction=0.012).

Gene-emotional eating interactions for leptin

In addition, we identified significant gene-emotional eating interactions for leptin plasma values at the *SLC6A4* promoter. In general those S carriers who exhibited high emotional eating and low ability to control food intake (high DS), showed lower levels of leptin (22.53±1.42 ng/ml) when compared with LL (30.89±2.87 ng/ml), which suggests a less anorexic effect in S carriers. These significant gene-emotions

interactions were found both in biallelic and triallelic classification (P=0.010 and P=0.003, respectively) and also for the initial leptin/body fat ratio (biallelic classification: P=0.010 and triallelic classification: P=0.004). No significant association was found for ghrelin (S carriers: 993.7 ± 73.4 pg/ml and LL: 1153.6 ± 127.0 pg/ml; P>0.05) and neither for interaction (P>0.05).

The relevance of the ability to control food intake

The discriminate model demonstrated that, out of the 10 items included in the analysis, only "the ability to control food intake", was able to reliably classify subjects into two *SLC6A4* genotypes groups, S carriers and LL, in a 46% of the cases.

When analyzing each of the 10 items from EEQ independently by logistic regression analyses (**supplemental table 1**), we found again that the question related to the lack of "control in amount of food eaten" was relevant in the analyses. Indeed, S carriers had 1.6 times more failure to control the amount of food eaten than LL. S carriers also felt less control over their diet when they arrived home tired at night, and craved for specific foods more frequently (1.4 times) than LL. Logistic regression analyses also demonstrated that S carriers had 1.5 times higher odds of eating when they were bored compared to LL.

DISCUSSION

One of the main problems in weight loss treatments is the dramatic inter-individual variability in response to the treatment [29, 38]. The reason why these differences occur is difficult to predict. In the present study we confirmed that a) the reduced ability to

control food intake or to inhibit the temptation to eat (disinhibition) is directly implicated in the impairment to lose weight; b) Genetics is implicated in the reduced ability to control food intake. Indeed, S allele (low-expressing) carriers of the *SLC6A4* promoter variant had a lower inhibition capacity and showed more failure to control the amount of food eaten than LL (high expressing carriers); c) The combination of these two factors i.e., disinhibition (having low inhibition capacity) and genetics (to be S-liked carriers) related to a lower effectiveness of the treatment and showed a higher impact on total weight loss (2 kg of difference) than disinhibition (~700g) or genetics (none effect) separately.

In agreement with our results, several studies have shown that emotional eaters, who tend to eat in response to negative emotions, also tend to have more barriers and greater difficulty in losing weight [28, 34, 39-45]. Disinhibition corresponds to a tendency to lose control over one's eating behavior and to ingest excessively large quantities of food in response to a variety of cues and circumstances [33].

Being under dietary treatment has been considered one relevant external factor that may influence disinhibition [5]. In the current study developed during a weight loss treatment, those participants who had low inhibition capacity had an implicit preference for higher energy foods (they ate ~100 more calories per day). Moreover, subjects with low inhibition capacity lost ~700g less weight during 20 weeks of treatment than did people with high inhibition capacity.

Several studies have shown that disinhibition is the best predictor of food intake, since those individuals with a high disinhibition score consume an elevated amount of

food [46, 47]. High disinhibition has also been associated with a lower quality diet (high-fat foods, processed meat, sweet or carbonated drinks) [48].

Genetics may be involved in the recurring tendency to overeat in response to one's environment or mood. In fact, Konttinnen *et al.* suggested that genetic predispositions to obesity may partly exert their effects through appetitive traits reflecting lack of control over eating or eating in response to negative emotions [42]. In this sense, our data from *SLC6A4* promoter showed that this genetic variant was associated with emotional eating and more specifically with disinhibition. S carriers showed significantly higher DS compared with LL.

Previous studies performed in experimental animal models with genetic depletion of brain serotonin 5HT have indicated that serotonin mediates behavioral disinhibition in the mammalian brain [49]. Moreover, in humans the administration of serotonin reuptake inhibitors (IRS) [7] reduced food intake [6].

Interestingly, our results show that carriers of the short allele (S) at *SLC6A4* had 1.6 times more failure to control the amount of food eaten than non-carriers. Indeed, out of the 10 questions that comprise the EEQ, the question "Do you have problems controlling the amount of certain types of food you eat?" was the first explaining the variability of the sample and was also able to correctly classify subjects into two *SLC6A4* genotypes groups (S carriers and LL) in a 46% of cases. Furthermore, this question was implicated in the interaction between *SLC6A4* genotype and emotional eating for weight loss. It is well known that the serotonin system is involved in the satiating effect of food, modulating the gastric emptying of solids and the secretion of different gastric peptides such as the postprandial peptide YY [50]. However, and in

agreement with a previous study [51], we did not find any association between *SLC6A4* genetic variant and the appetite gastric peptide ghrelin, while those S carriers who displayed higher EES or DS, showed lower plasma levels of leptin (anorexic hormone), suggesting that they had a higher seeking behavior for food than LL carriers. A recent study has shown that low levels of leptin are associated with an increased sadness, which might motivate the search for food [52].

Other factors such as eating while bored, overeating after work at night, or craving for specific food were also associated to the *SLC6A4* genotype. In previous studies, we have demonstrated that eating while bored is one of the main barriers for weight loss effectiveness [29]. Now we show that a genetic component is implicated in this behavior, S carriers of the *SLC6A4* promoter variant had 1.5 times higher odds of eating when they were bored compared to LL.

Multiple studies have demonstrated that genetics may help to develop more effective and individually tailored weight loss therapeutic strategies [34, 53]. However, in the current population, data from *SLC6A4* promoter showed that this genetic variant was not independently associated with total weight loss, although it had a weak impact on the duration of the maintenance program. These results are in agreement with those obtained by Defrancesco *et al*. [54], who found no influence of this genotype on weight loss, current weight or weight before surgery in a severe obese population although the psychosocial state after bariatric surgery was associated with the serotonin-transporter promoter polymorphism.

It has been proposed that gene-emotional eating interactions may better explain inter-individual differences in weight loss effectiveness than emotions or genetics

separately [34]. The complex interactions between individual factors (as determined by genetics) and the way in which individuals relate to environments (where food is widely available as a strong reward that promotes eating) may help to explain inter-individual differences in weight loss responses. In the current study, the effectiveness of the program was significantly reduced among those S carriers of the *SLC6A4* promoter who had also a low ability to control food intake (high DS). These results, although novel, are not surprising; previous studies have shown that the association between sibutramine administration and weight loss is mediated by significant changes in eating behaviors towards a decrease in disinhibition [6]. Humans eat for many reasons, including rewarding qualities of foods. Indeed, some individuals have difficulties in controlling food intake motivated by hedonics rather than by energy needs [12]. The overconsumption of readily available and highly palatable foods contributes probably to the impairments in weight loss with obesity treatments. For all these reasons, disinhibition together with a particular genetic predisposition, as being S carrier of the *SLC6A4* promoter, could be related to the difficulty in losing weight.

Implementing behavioral interventions designed to diminish disinhibition may be helpful to improve the effectiveness of weight loss treatments [55]. It is considered that reducing these "negative" dietary behaviors may be even more important for weight loss than improving "positive" ones. Current data suggest that this type of intervention should be implemented specifically in S carriers of the *SLC6A4* promoter.

Beside the serotonin-transporter promoter polymorphism (5-HTTLPR), the *SLC6A4* has other polymorphic loci that may affect its expression or function; however, numerous reports show that this variant of the 5´regulatory region of the gene is almost

entirely responsible for the associations with several behavioral traits [18]. In addition, this polymorphism displays a unique DNA secondary structure that has the potential to regulate the transcriptional activity of the associated 5HTT promoter [8].

In conclusion, *SLC6A4* interacts with emotional eating and disinhibition behaviors to modulate total weight-loss. Carrying out pretreatment psychosocial assessment, and combining it with genetic assessment, could provide helpful tailored weight-loss recommendations to obese and overweight individuals.

Acknowledgements: This study was supported by grants from Spanish Government of Economy and Competitiveness (SAF2014-52480-R), and European Regional Development Fund (ERDF).

Competing interests: The authors have declared that no competing interests exist.

REFERENCES

[1] Evers C, M. S. F., de Ridder DTD *Personality & social psychology bulletin* 2010, pp. 792–804.

[2] Fusterforteza, G., [Prevention of Bubonic Plague (Majorca - 1820)]. *Anales de la Real Academia de Farmacia* 1964, *30*, 292-342 CONTD.

[3] Brieva, A. J., Aste, O. E., Kuhn, O. S., [Contribution on the Pharmaceutical Chemistry of Carisoprodol]. *Anales de la Real Academia de Farmacia* 1965, *31*, 8-29.

[4] Rolls, B. J., What is the role of portion control in weight management? *Int J Obes (Lond)* 2014, *38 Suppl 1*, S1-8.

[5] Bryant, E. J., King, N. A., Blundell, J. E., Disinhibition: its effects on appetite and weight regulation. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2008, *9*, 409-419.

[6] Hainer, V., Kabrnova, K., Aldhoon, B., Kunesova, M., Wagenknecht, M., Serotonin and norepinephrine reuptake inhibition and eating behavior. *Annals of the New York Academy of Sciences* 2006, *1083*, 252-269.

[7] Reas, D. L., Grilo, C. M., Pharmacological treatment of binge eating disorder: update review and synthesis. *Expert opinion on pharmacotherapy* 2015, *16*, 1463-1478.

[8] Lesch, K. P., Mossner, R., Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? *Biol Psychiatry* 1998, *44*, 179-192.

[9] Collier, D. A., Stober, G., Li, T., Heils, A., *et al.*, A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. *Molecular psychiatry* 1996, *1*, 453-460.

[10] Andersen, L. B., Harro, M., Sardinha, L. B., Froberg, K., *et al.*, Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *Lancet* 2006, *368*, 299-304.

[11] Heils, A., Teufel, A., Petri, S., Stober, G., *et al.*, Allelic variation of human serotonin transporter gene expression. *Journal of neurochemistry* 1996, *66*, 2621-2624.

[12] Hu, X. Z., Lipsky, R. H., Zhu, G., Akhtar, L. A., *et al.*, Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *American journal of human genetics* 2006, *78*, 815-826.

[13] Van Someren, E. J., Swaab, D. F., Colenda, C. C., Cohen, W., *et al.*, Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. *Chronobiology international* 1999, *16*, 505-518.

[14] Iordanidou, M., Tavridou, A., Petridis, I., Arvanitidis, K. I., *et al.*, The serotonin transporter promoter polymorphism (5-HTTLPR) is associated with type 2 diabetes. *Clinica chimica acta; international journal of clinical chemistry* 2010, *411*, 167-171.

[15] Calati, R., De Ronchi, D., Bellini, M., Serretti, A., The 5-HTTLPR polymorphism and eating disorders: a meta-analysis. *The International journal of eating disorders* 2011, *44*, 191-199.

[16] Monteleone, P., Santonastaso, P., Mauri, M., Bellodi, L., *et al.*, Investigation of the serotonin transporter regulatory region polymorphism in bulimia nervosa: relationships to harm avoidance, nutritional parameters, and psychiatric comorbidity. *Psychosomatic medicine* 2006, *68*, 99-103.

[17] van Strien, T., van der Zwaluw, C. S., Engels, R. C., Emotional eating in adolescents: a gene (SLC6A4/5-HTT) - depressive feelings interaction analysis. *Journal of psychiatric research* 2010, *44*, 1035-1042.

[18] Sookoian, S., Gemma, C., Garcia, S. I., Gianotti, T. F., *et al.*, Short allele of serotonin transporter gene promoter is a risk factor for obesity in adolescents. *Obesity (Silver Spring)* 2007, *15*, 271-276.

[19] Sookoian, S., Gianotti, T. F., Gemma, C., Burgueno, A., Pirola, C. J., Contribution of the functional 5-HTTLPR variant of the SLC6A4 gene to obesity risk in male adults. *Obesity (Silver Spring)* 2008, *16*, 488-491.

[20] Miranda, R. C. K., Genro, J. P., Campagnolo, P. D. B., Mattevi, V. S., *et al.*, Biallelic and triallelic approaches of 5-HTTLPR polymorphism are associated with food intake and nutritional status in childhood. *The Journal of nutritional biochemistry* 2017, *43*, 47-52.

[21] Mergen, H., Karaaslan, C., Mergen, M., Deniz Ozsoy, E., Ozata, M., LEPR, ADBR3, IRS-1 and 5-HTT genes polymorphisms do not associate with obesity. *Endocrine journal* 2007, *54*, 89-94.

[22] Pine, D. S., Goldstein, R. B., Wolk, S., Weissman, M. M., The association between childhood depression and adulthood body mass index. *Pediatrics* 2001, *107*, 1049-1056.

[23] Hasler, G., Pine, D. S., Kleinbaum, D. G., Gamma, A., *et al.*, Depressive symptoms during childhood and adult obesity: the Zurich Cohort Study. *Molecular psychiatry* 2005, *10*, 842-850.

[24] Ardovini, C., Caputo, G., Todisco, P., Dalle Grave, R., Binge eating and restraint model: Psychometric analysis in binge eating disorder and normal weight bulimia. *European Eating Disorders Review* 1999, *7*, 293-299.

[25] d'Amore, A., Massignan, C., Montera, P., Moles, A.*, et al.*, Relationship between dietary restraint, binge eating, and leptin in obese women. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 2001, *25*, 373-377.

[26] Goldfein, J. A., Walsh, B. T., Devlin, M. J., Lachaussée, J. L., Kissileff, H. R., Eating behavior in binge eating disorder. *International Journal of Eating Disorders* 1993, *14*, 427-431.

[27] Grave, R. D., Todisco, P., Oliosi, M., Marchi, S., Binge eating disorder and weight cycling in obese women. *Eating Disorders* 1996, *4*, 67-73.

[28] Corbalán, M. D., Morales, E., Baraza, J. C., Canteras, M., Garaulet, M., *Revista Española de Obesidad* 2009, pp. 144–154.

[29] Corbalan, M. D., Morales, E. M., Canteras, M., Espallardo, A.*, et al.*, Effectiveness of cognitive-behavioral therapy based on the Mediterranean diet for the treatment of obesity. *Nutrition* 2009, *25*, 861-869.

[30] Serra-Majem, L., Aranceta, J., Nutritional objectives for the Spanish population. Consensus from the Spanish Society of Community Nutrition. *Public health nutrition* 2001, *4*, 1409-1413.

[31] Craig, C. L., Marshall, A. L., Sjostrom, M., Bauman, A. E.*, et al.*, International physical activity questionnaire: 12-country reliability and validity. *Medicine and science in sports and exercise* 2003, *35*, 1381-1395.

[32] Roman-Vinas, L, S.-M., M, H., L, R.-B., International Physical Activity Questionnaire: reliability and validity in a Spanish population. *Eur. J. Sport Sci.* 2010, *10*, 297-304.

[33] Garaulet, M., Canteras, M., Morales, E., Lopez-Guimera, G*., et al.*, Validation of a questionnaire on emotional eating for use in cases of obesity: the Emotional Eater Questionnaire (EEQ). *Nutricion hospitalaria : organo oficial de la Sociedad Espanola de Nutricion Parenteral y Enteral* 2012, *27*, 645-651.

[34] Lopez-Guimera, G., Dashti, H. S., Smith, C. E., Sanchez-Carracedo, D*., et al.*, CLOCK 3111 T/C SNP interacts with emotional eating behavior for weight-loss in a Mediterranean population. *PloS one* 2014, *9*, e99152.

[35] Trichopoulou, A., Kouris-Blazos, A., Wahlqvist, M. L., Gnardellis, C*., et al.*, Diet and overall survival in elderly people. *BMJ (Clinical research ed.)* 1995, *311*, 1457-1460.

[36] Wendland, J. R., Martin, B. J., Kruse, M. R., Lesch, K. P., Murphy, D. L., Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. *Molecular psychiatry* 2006, *11*, 224-226.

[37] Enoch, M. A., Gorodetsky, E., Hodgkinson, C., Roy, A., Goldman, D., Functional genetic variants that increase synaptic serotonin and 5-HT3 receptor sensitivity predict alcohol and drug dependence. *Molecular psychiatry* 2011, *16*, 1139-1146.

[38] Bandin, C., Martinez-Nicolas, A., Ordovas, J. M., Madrid, J. A., Garaulet, M., Circadian rhythmicity as a predictor of weight-loss effectiveness. *Int J Obes (Lond)* 2014, *38*, 1083-1088.

[39] Blair, A. J., Lewis, V. J., Booth, D. A., Does emotional eating interfere with success in attempts at weight control? *Appetite* 1990, *15*, 151-157.

[40] Elfhag, K., Rossner, S., Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2005, *6*, 67-85.

[41] Karlsson, J., Hallgren, P., Kral, J., Lindroos, A. K.*, et al.*, Predictors and effects of long-term dieting on mental well-being and weight loss in obese women. *Appetite* 1994, *23*, 15-26.

[42] Konttinen, H., Llewellyn, C., Wardle, J., Silventoinen, K.*, et al.*, Appetitive traits as behavioural pathways in genetic susceptibility to obesity: a population-based cross-sectional study. *Scientific reports* 2015, *5*, 14726.

[43] Lavery, M. A., Loewy, J. W., Identifying predictive variables for long-term weight change after participation in a weight loss program. *J Am Diet Assoc* 1993, *93*, 1017-1024.

[44] Sherwood, N. E., Jeffery, R. W., Wing, R. R., Binge status as a predictor of weight loss treatment outcome. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 1999, *23*, 485-493.

[45] Teixeira, P. J., Silva, M. N., Coutinho, S. R., Palmeira, A. L.*, et al.*, Mediators of weight loss and weight loss maintenance in middle-aged women. *Obesity (Silver Spring)* 2010, *18*, 725-735.

[46] Van Strien, T., Cleven, A., Schippers, G., Restraint, tendency toward overeating and ice cream consumption. *The International journal of eating disorders* 2000, *28*, 333-338.

[47] Westenhoefer, J., Broeckmann, P., Munch, A. K., Pudel, V., Cognitive control of eating behaviour and the disinhibition effect. *Appetite* 1994, *23*, 27-41.

[48] Rauscher, G. H., Hawley, S. T., Earp, J. A., Baseline predictors of initiation vs. maintenance of regular mammography use among rural women. *Preventive medicine* 2005, *40*, 822-830.

[49] Angoa-Perez, M., Kane, M. J., Briggs, D. I., Sykes, C. E.*, et al.*, Genetic depletion of brain 5HT reveals a common molecular pathway mediating compulsivity and impulsivity. *Journal of neurochemistry* 2012, *121*, 974-984.

[50] Rothrock, S. G., Green, S. M., Harding, M., Bervel, D.*, et al.*, Plain abdominal radiography in the detection of acute medical and surgical disease in children: a retrospective analysis. *Pediatric emergency care* 1991, *7*, 281-285.

[51] Jarrell, H., Hoffman, J. B., Kaplan, J. R., Berga, S.*, et al.*, Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys. *Physiology & behavior* 2008, *93*, 807-819.

[52] Cadorniga Carro, R., [Formation of complexes between surface-active substances and alkaloids]. *Anales de la Real Academia de Farmacia* 1962, *28*, 27-52.

[53] Bandin, C., Martinez-Nicolas, A., Ordovas, J. M., Ros Lucas, J. A.*, et al.*, Differences in circadian rhythmicity in CLOCK 3111T/C genetic variants in moderate obese women as assessed by thermometry, actimetry and body position. *Int J Obes (Lond)* 2013, *37*, 1044-1050.

[54] Defrancesco, M., Liebaert, J., Kemmler, G., Aigner, F., *et al.*, Psychosocial state after bariatric surgery is associated with the serotonin-transporter promoter polymorphism. *Eating and weight disorders : EWD* 2013, *18*, 311-316.

[55] Hansen, O. P., Hippe, E., [Pernicious anemia. Patients with misleading Schilling and absence of macrocytic anemia]. *Ugeskrift for laeger* 1974, *136*, 2859-2863.

Author contributions: GB-R collected the data, recruited the study participants, extracted the DNA and performed the *SLC6A4* genotyping. PG-A recruited the study participants, performed statistical analysis, analyzed the data and wrote the manuscript. BV extracted the DNA, performed statistical analysis and analyzed the data. JFSR performed the *SLC6A4* genotyping. AMH-M wrote and corrected the paper. SS and CJP designed the research. MG designed the research, analyzed the data, wrote the paper, and had primary responsibility for the final content. None of the authors declared a conflict of interest. All the authors have read and approved the final manuscript.

FIGURE LEGENDS

Figure 1. Associations between emotional eating, energy intake and total weight loss. We analyzed the association of emotional eating score and disinhibition score with energy intake (kcal) and total weight loss (kg) in the total population (*n*=2961). Those emotional eaters (high emotional eating score) (Figure 1A), and those who had a low ability to control food intake (high disinhibition score) (Figure 1B), had a higher intake of calories (~100Kcal/day more) and lost less weight during the treatment (~700g less)

(Figure 1C and 1D). A linear regression analysis by univariate linear regression models (ANCOVA) was performed. We adjusted analyses for potential confounders including sex, age, study number, nutritional center and obesity when required.

Figure 2. *SLC6A4* associates with emotions. *SLC6A4* genotypes were associated with emotional eating score (EES) (Figure 2A) and disinhibition score (DS) (Figure 2B) in a subsample of total population (n=624). A dominant model to the concluding analyses for *SLC6A4* was performed. The S carriers (SS + SL) were significantly more emotional (high EES, P=0.037, Figure 2A) and had significantly lower ability to control food intake (high DS, P=0.036, Figure 2B) than homozygote carriers of the major allele (LL). A linear regression analysis by univariate linear regression models (ANCOVA) was performed. We adjusted analyses for potential confounders including sex, age, study number, nutritional center and obesity when required.

Figure 3. Gene-emotional eating interactions for total weight loss. We studied gene-emotional eating (Figure 3A) and gen-disinhibition (Figure 3B) interactions for total weight loss. A dominant model for *SLC6A4* was performed; consequently, minor S allele carriers or S-liked (biallelic: SS + SL; triallelic: SS + SL_G + SL_A + L_GL_A + L_GL_G) were compared against major L allele homozygotes (biallelic: LL; triallelic: L_AL_A). Predicted values of total weight loss according to *SLC6A4* genotypes were plotted against emotional eating score (Figure 3A) and disinhibition score (Figure 3B) respectively, and evaluated continuously. In general, those S-liked or S carriers for *SLC6A4* who were high emotional eaters (3A) and had high disinhibition (3B) lost significantly less weight than the other participants of the treatment. We adjusted analyses for sex, age, study number and nutritional center, and obesity when required.

L_AL_A (in 3A) or LL (in 3B) carriers are indicated by gray circles and S-liked (in 3A) or S (in 3B) carriers are represented by black circles.

Table 1. General characteristics of the total population studied.

	Total population ***n*** **= 2961**
Age (y)	40 ± 14
Women, *n* (%)	2380 (80.4)
Anthropometric	
BMI (kg/m^2)	31.2 ± 5.6
Body fat (%)	36.9 ± 6.9
Weight loss	
Weight loss (kg)	6.8 ± 5.9
Weight loss (%)	7.8 ± 6.3
Weight loss rate (kg)	0.5 ± 0.5
Treatment weeks	20.3 ± 18.3
Maintenance weeks	5.2 ± 3.5
Emotional eating score	12.0 ± 6.3
Disinhibition score	4.47 ± 2.58
Dietary intake	
Total Energy (kcal/day)	1929.8 ± 672.9
Proteins (g)	82.5 ± 31.2
Carbohydrates (g)	198.0 ± 85.6
Fats (g)	91.9 ± 41.3
Proteins (%)	17.5 ± 4.9
Carbohydrates (%)	41.2 ± 10.9
Fats (%)	42.3 ± 9.8
Food-Intake Hormones	
Ghrelin (pg/ml)	1059.0 ± 998.2
Leptin (ng/ml)	19.6 ± 14.1
Physical activity (METs/min/week)	3575 ± 6629
Genotypes (n, (%))	***n*** **= 624**
LL	144 (23.1)
L_AL_A	107 (17.1)
L_AL_G	36 (5.8)
L_GL_G	1 (0.2)
LS	342 (54.8)
SS	138 (22.1)
Abbreviations used: BMI; Body mass index. METs: Metabolic Units.	

Supplemental table 1. Associations of *SLC6A4* genotypes with emotional eating behaviors.

SLC6A4 gene	**Do you have problems controlling the amount of certain types of food you eat?**						
	Yes		No			Failure to control portion size	
	n	%	n	%	*P* value	OR (95% CI)	*P* value
S carriers	184	29.53	295	47.35	**0.023**	1.560 (1.033-2.356)	**0.034**
LL	40	6.42	104	16.69			
	Do you feel less control over your diet when you are tired after work at night?						
	Yes		No			Out of control in food when they arrived home tired	
	n	%	n	%	*P* value	OR (95% CI)	*P* value
S carriers	163	26.16	316	50.72	**0.042**	1.565 (1.022-2.396)	**0.039**
LL	36	5.79	108	17.34			
	Do you crave specific foods?						
	Yes		No			Having cravings for specific foods	Yes
	n	%	n	%	*P* value	OR (95% CI)	*P* value
S carriers	187	30.16	290	46.77	0.076[1]	1.426 (0.951-2.140)	0.086
LL	46	7.42	97	15.65			
	Are you liable to eat when bored?						
	Yes		No			Eating while bored	
	n	%	n	%	*P* value	OR (95% CI)	*P* value
S carriers	313	50.81	159	25.81	**0.050**[1]	1.533 (1.035-2.271)	**0.033**
LL	84	13.64	60	9.74			

[1] *P* value Chi-square unilateral; ORs were calculated for combined groups of S carriers compared with LL subjects. Adjusted for sex, age, study number and Garaulet center.

Figure 1


1A
P-value < 0.001
Energy intake (kcal)
2.200
2.000
1.800
1.600
1885
1998
*
Low
High
Emotional Eating Score
1B
P-value < 0.001
Energy intake (kcal)
2.200
2.000
1.800
1.600
1905
2017
*
Low
High
Disinhibition Score
1C
P-value = 0.058
Total weight loss (kg)
8
7
6
7.5
6.9
*
Low
High
Emotional Eating Score
1D
P-value = 0.019
Total weight loss (kg)
8
7
6
7.6
6.9
*
Low
High
Disinhibition Score

Figure 2

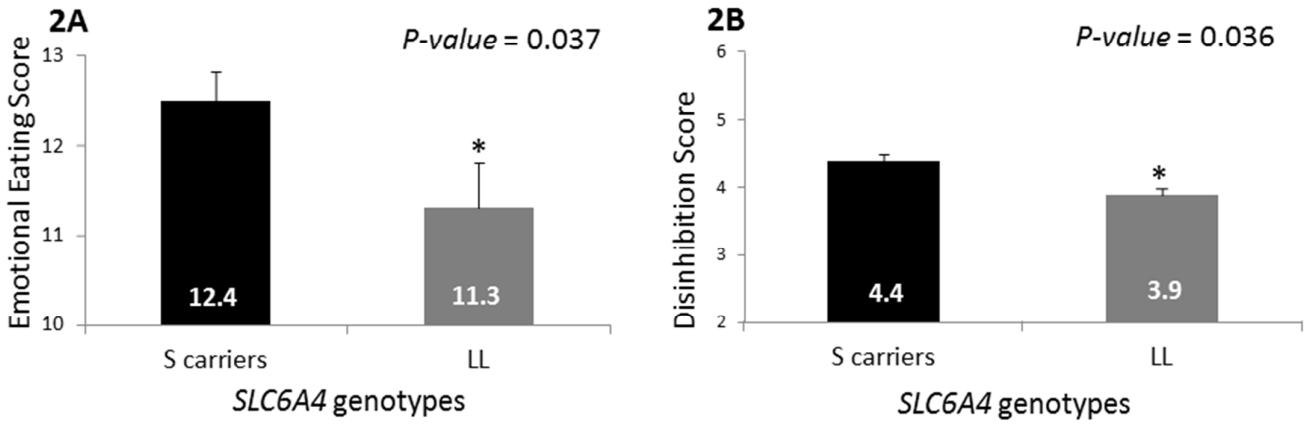
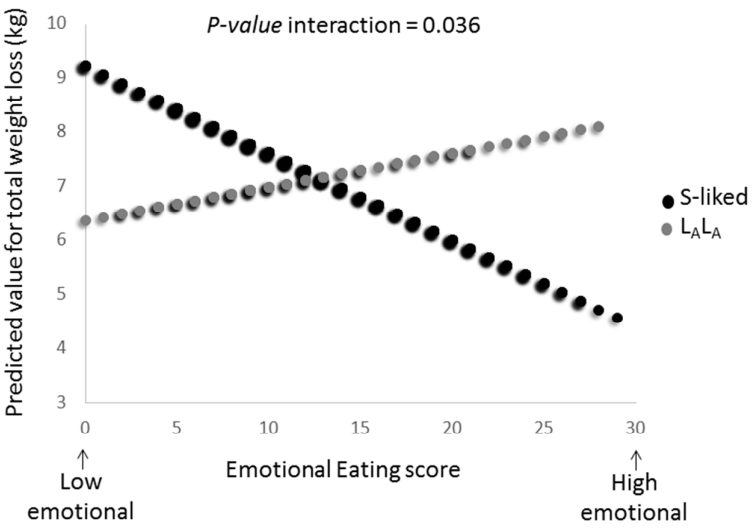
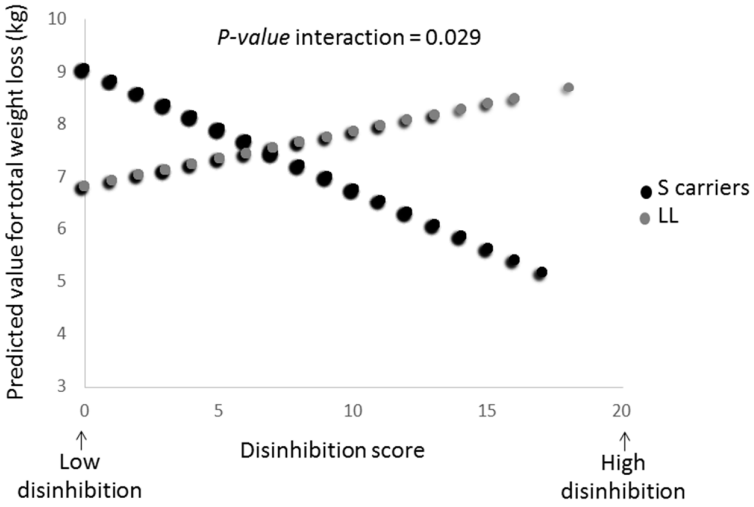


Figure 3

3A



3B



Graphical Abstract