Article

Serotonin Transporter Gene Polymorphisms Predict Adherence to Weight Loss Programs Independently of Obesity-Related Genes

Mana Yatsuda , Miyako Furou , Keiko Kamachi , Kaori Sakamoto , Kumiko Shoji , Osamu Ishihara ,
Yasuo Kagawa *

Article

Serotonin Transporter Gene Polymorphisms Predict Adherence to Weight Loss Programs Independently of Obesity-Related Genes

Mana Yatsuda [1], Miyako Furou [2], Keiko Kamachi [1,2], Kaori Sakamoto [2,3], Kumiko Shoji [2,3], Osamu Ishihara [1,2,3] and Yasuo Kagawa [2,3]

[1] Nutrition Clinic, Kagawa Nutrition University, 3-24-3 Komagome, Toshima, Tokyo 170-8481, Japan; tp.m.yatsuda@eiyo.ac.jp, kamachi@eiyo.ac.jp, ishihara.osamu@eiyo.ac.jp

[2] Institute of Nutrition Sciences, Kagawa Nutrition University, 3-9-21 Chiyoda, Sakado, Saitama 350-0288, Japan; miyakoflowerforest@gmail.com, sakamoto@eiyo.ac.jp, shoji.kumiko@eiyo.ac.jp, kagawa@eiyo.ac.jp

[3] Faculty of Nutrition, Kagawa Nutrition University, 3-9-21 Chiyoda, Sakado, Saitama 350-0288, Japan

* Correspondence: kagawa@eiyo.ac.jp; Tel.: 81-492-82-3618

Abstract: Background/Objectives: Adherence to treatment instructions is essential in managing chronic diseases related to obesity. One gene associated with adherence is the serotonin transporter (5-HTTLPR) gene, which has long (L) and short (S) alleles, resulting in LL, SL, and SS genotypes. Risk alleles for obesity include the R variant of the β3-adrenergic receptor (*β3AR*) and the G variant of uncoupling protein 1 (*UCP1*). This study aimed to evaluate whether the S/L variant of *5-HTTLPR*, the R variant of *β3AR*, and the G variant of *UCP1* are associated with adherence to a weight loss program. To assess the factors influencing adherence, eating behavior was evaluated using the Eating Behavior Questionnaire (EBQ). **Methods**: The study included 56 women with a mean age of 57.3±10 years and a mean BMI of 27.2±5.6 kg/m2. Long-read sequencing was used to analyze S/L mutations. Participants followed a six-month diet and exercise regimen for obesity management. Outcomes were assessed using clinical data and EBQ scores. **Results:** Participants were classified as SS (69.6%), SL (17.9%), or LL (12.5%). The R variant of *β3AR* was present in 34% of participants, with the G variant of *UCP1* in 75%. After the intervention, SS participants showed significantly greater reductions in weight, body fat percentage, and waist circumference than LL participants ($p < 0.05$). Among EBQ items, significant improvements ($p<0.05$) were observed in SS participants for eating as a diversion, feeling of fullness, bad eating habits, unsteady eating patterns, and total EBQ score. In SL participants, only bad eating habits improved, whereas no significant changes were observed in LL participants. Obesity risk alleles did not significantly affect clinical outcomes. **Conclusions:** SS genotype participants demonstrated higher adherence to the weight loss program, leading to improved clinical outcomes and EBQ scores, independent of obesity risk genes.

Keywords: serotonin transporter; obesity; adherence; personality

1. Introduction

1.1. Adherence and Serotonin Transporter Gene

Patient adherence is defined as the degree to which a patient follows treatment recommendations prescribed by a physician or registered dietitian. Adherence is important in the treatment of obesity-related diseases, such as metabolic syndrome, hypertension, type 2 diabetes mellitus, and cardiovascular disease [1].

Behavioral therapy, with high adherence rates, is an important component of lifestyle modification, assuming that obesity-leading behaviors have a strong educational component [1]. Stricter adherence to the Japanese Dietary Guidelines has been shown to reduce all-cause mortality

(hazard ratio 0.85, p<0.001) and cardiovascular disease mortality in Japanese adults [2]. The effectiveness of cardiometabolic disease treatment depends on patients' adherence to lifestyle changes [3]. Adherence is defined as the degree to which an individual's behavior matches the physician's recommendations, i.e., demonstrating active patient engagement [3]. Factors influencing adherence are based on personality traits related to dietary habits [4]. These adherence factors are based on patient behavior, which is influenced by genetic background. The serotonin transporter gene is associated with many human behaviors [5]. Thus, one of the authors (Y.K.) reported that adherence to weight loss advice in obese patients is influenced by polymorphisms in the promoter region of the serotonin (5-HT) transporter (*5-HTTLPR*) [6]. The serotonin transporter (5-HTT) is encoded by a gene (*SLC6A4*) on chromosome l7q11.2 [7]. The two most common alleles are the long (L: 16 repeat insertion) and short (S: 14 repeat insertion) variants [7]. The long variant of *5-HTTLPR* has higher transcriptional activity than the short variant [7]. Three single nucleotide polymorphisms in *TPH2* (brain tryptophan hydroxylase), *SLC6A4* (serotonin transporter), and *HTR3B* (serotonin receptor) were found to be significantly associated with obesity in humans [8]. These single nucleotide polymorphisms, which reduced serotonin effects, were in serotonin synthesis (*TPH2*, rs11179071), serotonin transport (*SLC6A4*, rs9903062), and serotonin receptor (*HTR3B*, rs2276307), and caused obesity [8].

1.2. Personality Genes and Behavior

Human behavior, including adherence, depends on personality [4]. Increasing evidence indicates that personality differences among people affect various health outcomes, such as obesity, type 2 diabetes mellitus, and cardiovascular disease. The S variant of *5-HTTLPR* is associated with trait anxiety [9]. According to Cloninger's proposal, personality is composed of temperament and character [10]. Temperament is defined in terms of perception-based habits controlled by the amygdala and other parts of the limbic system, with responses being genetically determined [10]. In contrast, character is defined in terms of concept-based values controlled by the cerebral cortex determined by learning via the hippocampus [10]. Four dimensions of temperament, namely novelty seeking, harm avoidance, reward dependence, and persistence, were added later and are independently heritable [11]. A Five Factor Model of personality has been proposed with genetic analysis of extraversion, neuroticism, agreeableness, conscientiousness, and openness [12]. The Five Factor Model is currently the most widely used classification of personality traits [12]. These personality phenotypes are measured by questionnaires with quantitative scores for each trait [12]. A population-based cohort study based on data from the UK Biobank found that, of the five personality traits, diligence (conscientiousness) was significantly associated with a reduced risk of cardiovascular diseases and type 2 diabetes mellitus [13]. A meta-analysis of genome-wide association studies identified six genetic loci of the Big Five Inventory [14]. Of the genome-wide significant loci, for example, neuroticism was located at variants on chromosome 8p23.1 and in *L3MBTL2* [14]. However, Manhattan plots indicate that the gene locus for diligence or conscientiousness is on chromosome 7 [14] and not the *5-HTTPR* locus on chromosome 17 [7]. Indeed, no significant effect of the serotonin transporter genotypes was observed on any of the dimensions of the Big Five Inventory, NEOFFI [15]. For example, there was no significant difference in the frequency of conscientiousness between SS (31.8%), SL (31.4%), and LL (31.7%) types [15]. Using positron emission tomography of 11C-labelled serotonin analogs, a significant positive correlation was found between self-direction and the availability of 5-HTT, suggesting that a self-confident character may be associated with enhanced serotonin neurotransmission in the L type [16]. Psychological differences in harm avoidance between S and L types were also detected by functional magnetic resonance imaging of the amygdala nucleus [17].

1.3 Ethnic Differences of Genes and Obesity

There are three genotypes (LL, SL, and SS) that are combinations of a long L and a short S [5,17]. Recently, 14 allelic variants have been discovered, but the combined proportion of variants with 15,

19, 20 and 22 repeats were only 3.1% in Japanese participants [18]. Significant ethnic differences in the distribution of genotypes were observed between Japanese and Caucasians: Japanese, SS= 61.9%, SL= 31.4%, LL= 0.8%, others= 6.4%; Caucasian, SS= 20.3%, SL= 48.6%, LL= 31.1%, others=0.0% [18]. Furthermore, there are large ethnic differences in nutritional intake and obesity rates (BMI >30 kg/m², 4.8% for Japanese men and 3.7% for Japanese women, 35.5% for American men and 37.0% for American women) [19]. A detailed analysis of serotonin metabolism and obesity in black adolescents [8] showed significant difference from those in Japanese adolescents [18]. Therefore, special consideration should be given to nutritional studies of the serotonin transporter in Japanese people [19].

1.4. Serotonin Transporter and Energy Metabolism

Serotonin is present in all species, regulating diverse behavioral and physiological processes to maintain energy balance [20]. Serotonin neurons in the brain are essential for thermoregulation and control the metabolic activity of thermogenic fat [21]. Ablation of serotonin neurons by diphtheria toxin caused loss of thermoregulation by decreasing uncoupling protein 1 (*UCP1*) in adipocytes [21]. Energy expenditure in adults with the SS genotype was lower than that of adults with the SL and LL genotypes, and, as a result, the BMI of adults with SS genotype (26.7 ± 0.2 kg/m²) was higher than that of adults with the SL (26 ± 0.1 kg/m²) and LL (25.4 ± 0.2 kg/m²) genotypes (p<0.0002) [22]. Genetic risk factors for obesity include both psychology-related gene polymorphisms including the S variant of 5-HTTPLPR and metabolism-related gene polymorphisms, including the R variant of *β3*-adrenergic receptor (*β3AR*, rs4994, W64R) [23,24] and the G variant of uncoupling protein 1 (*UCP1*, rs1800592, A3826G) [25,26].

Adipocyte lipolysis by hormone-sensitive lipase is activated by phosphorylation by cyclic AMP-dependent protein kinase A, which is activated by *β3AR* upon catecholamine binding [27]. A prospective cohort study of 373,026 individuals with genetic susceptibility to coronary heart disease found that those at moderate and high genetic risk were at higher risk than those at low genetic risk, using a weighted polygenic risk score calculated by summing the number of risk-increasing alleles at 300 single nucleotide polymorphisms and multiplying them by the corresponding effect estimates [28]. A cross-sectional study of physical activity, television viewing, and sleep duration in 233,110 adults from the UK Biobank found that behavior has a strong influence on cardiovascular disease and type 2 diabetes mellitus [29]. Therefore, to interpret the effects of adherence, it is necessary to assess participant behavior.

1.5 Objective of This Study

In this study, the effect of *5-HTTLPR* polymorphisms on adherence to dietary and exercise instructions for weight loss, as well as on eating behaviors that facilitate adherence, was investigated. Since obesity is influenced by both risk genes (*β3AR* and *UCP1*) and eating behaviors, the impact of these risk genes was also examined. This study was unique in that the adherence patterns of Japanese individuals differ from those of other ethnic groups [7–12] in genetic [18], nutritional [2,19], anthropometric [6], and psychological [5,15] aspects. One of the authors (Y.K.) previously reported that the SS type of *5-HTTLPR* is associated with improvements in fasting blood glucose levels [6]. In a study of 264 patients (excluding those taking medication for diabetes mellitus, hypercholesterolemia, and hypertension), the changes in fasting blood glucose levels were examined after 11 weeks (short term) and 23 years (long term) of nutrition and exercise guidance [6]. Fasting blood glucose levels were significantly lower in the SS group than in the LL+SL group (P=0.01 in the short term, P<0.0001 in the long term) [6]. In the present study, a detailed analysis of anthropometric and blood biochemistry data was conducted to evaluate the effects of *5-HTTLPR*, *β3AR*, and *UCP1* genotypes on adherence to recommendations from the Healthy Diet Course. To enhance adherence to the course, it is essential that participants clearly understand health information related to their condition [30]. Therefore, at the beginning of the course, participants were informed of their *β3AR* and *UCP1* risk alleles, but not their SS, SL, or LL genotypes. Since hypothalamic serotonin has been

reported to regulate eating behavior and body weight [31], differences in eating behaviors were also analyzed using the Eating Behavior Questionnaire (EBQ) to explore the potential mechanisms underlying genotype-specific variations in anthropometric measurements and blood biochemistry.

2. Materials and Methods

2.1. Participants

The participants were 56 Japanese women with a mean age of 57.3 ± 10 years and BMI of 27.2 ± 5.6 kg/m², who participated in lifestyle modification programs at the Nutrition Clinic of the Kagawa Nutrition University, Japan. Relevant clinical information of the participants is summarized in Table 1. The participants voluntarily participated in the weight loss program for approximately 6 months between 2014 and 2017, receiving eight dietary and exercise instruction sessions, a basic knowledge course on lifestyle-related diseases, a 500-kcal lunch, and individual nutritional counseling. The items included anthropometric data (height, weight, body fat, waist circumference, blood pressure) and fasting blood biochemistry. These measurements were performed before and after the intervention, and a questionnaire survey on eating behavior was also completed. Activities of daily living were within the normal range, and all participants were able to attend the clinic without assistance. Results are presented as mean ± standard deviation (SD) values. The experimental plan was approved by the Human Genome Medical Ethical Committee of Kagawa Nutrition University (No. 301-G, May 29, 2014, and revised No. 257-G, October 10, 2015), and conformed to the World Medical Association Declaration of Helsinki (1964, 2000 edition). Prior to the experiment, the purpose and content of this study were fully explained to the participants, and written, informed consent was obtained.

Table 1. Participants' Characteristics (n=56)**.**

	Mean	± SD
Body weight (kg)	66.3	± 13.8
BMI (kg/m²)	27.2	± 5.6
Body fat percentage (%)	35.3	± 6.1
Waist circumference (cm)	93.4	± 13.9
Systolic blood pressure (mmHg)	133	± 18
Diastolic blood pressure (mmHg)	79	± 12
Triglycerides (mg/dl)	108	± 65
Total cholesterol (mg/dl)	219	± 38
LDL cholesterol (mg/dl)	129	± 34
Fasting blood glucose (mg/dl)	94	± 22
HbA1c (%)	5.8	± 0.6

Abbreviation: BMI, body mass index; HbA1c, Hemoglobin A1c.

2.2. Dietary and Exercise Advice

The participants took the Kagawa Nutrition University Healthy Diet Course. This course consisted of both dietary and exercise instruction. Our dietary guidance is based on the "Four-Food-Group Point Method" [32], which restricts energy intake without reducing protein intake to avoid gluconeogenesis and weight rebound. To reduce their body weight without sarcopenia, the participants were instructed to consume a mixture of foods belonging to four major food groups as follows: 240 kcal each of group I (milk, dairy products, and egg), group 2 (protein sources: meat, fish and beans), and group 3 (mineral, vitamin, and fiber sources: vegetables and fruits), and then their total energy intake was adjusted by varying their intake of group 4 (energy source: grains, oil, and sugar) [32]. More than 1,000 patients have participated in the Healthy Diet Course in our clinic and demonstrated excellent long-term weight control [33]. In this study, the Healthy Diet Course lasted 6 months, during which diet and exercise advice was repeated 8 times. Ten typical 500-kcal meals were

provided during the course. At the start of the course, health literacy education was provided, including the importance of weight management by daily weight recording and information about risk alleles of *β3AR* and *UCP1*.

2.3. Genotyping

For genetic testing to identify polymorphisms of *5-HTTLPR*, *β3AR* and *UCP1*, blood was collected from the median cubital vein under fasting conditions with the consent of the participants. DNA was purified using an automated magnetic particle DNA extraction device (Magtration System 6GC; Precision System Science, Chiba, Japan) (34). Long-read sequencing was performed at Takara Bio Inc. (Nojihigashi 7-4-38, Kusatsu, Shiga, Japan) according to the methods reported [35,36]. The promoter region of serotonin transporter gene (*5-HTTLPR*) was located in the first exon in the long arm of chromosome 17, as reported [35]. Participants were classified into SS, SL and LL types of *5-HTTLPR*, and the measured items of each group were compared. Obesity-related gene polymorphisms of *β3AR* and *UCP1* were genotyped using an automated polymorphism analyzer by Kagawa et al. [37] after isolating DNA [34].

2.4. Anthropometry

Height was measured to the nearest 0.1 cm using a digital height gauge (A&D AD-6227), and weight was measured to the nearest 0.1 kg using a digital scale digital height gauge digital scale (TANITA DC-430A, Japan), with 0.6 kg subtracted to account for clothing weight, and the data were used for the analysis. Waist circumference was measured to the first decimal place at the umbilicus. Body fat percentage was measured to the nearest 0.1% using the DEX method (Discovery QDR W type, manufactured by Toyo Medic Co., Ltd.). Body fat mass was calculated using the following formula:

Body fat mass (kg) = body weight (kg) × body fat percentage (%)

Blood pressure was measured using a fully automatic blood pressure monitor (A&D automatic blood pressure monitor TM-2655P).

2.5. Blood Biochemistry

The blood biochemistry values used in the analysis were obtained by drawing blood from the median cubital vein early in the morning, separating the serum, and outsourcing the analysis to LSI Medience Corporation. The blood data included fasting blood glucose, hemoglobin A1c (hereafter referred to as HbA1c), triglycerides (TG), LDL cholesterol (LDL-C) (direct method), and total cholesterol (TC). The methods used for these biochemical analyses are all described in the "National Health and Nutrition Survey Japan, 2019 Edition".

2.5. Eating Behavior Questionnaire

Eating behavior was assessed using the Eating Behavior Questionnaire (EBQ) recommended by the Japan Society for the Study of Obesity. Eating behavior was assessed by aggregating the scores of each item into seven categories: (1) perception of constitution and weight, (2) motivation for eating, (3) eating as diversion, (4) feeling of fullness and hunger, (5) bad eating habits, (6) contents of meals, and (7) unsteady eating pattern. (38). The questionnaire consisted of 66 items, and participants were asked to respond to each item on a scale of: "not at all like me" (1 point), "sometimes like me" (2 points), "tendency to be like me" (3 points), and "very like me" (4 points) [38]. The subtotal scores of all categories were combined into a total score. Higher scores in each category and the total score indicate poorer eating behaviors. An octagonal diagram was created by expressing the scores in each category as a percentage of the 100% out of 4 points [38,39]. The EBQ was administered before and after the Healthy Diet Course.

2.6. Statistical Analysis

The Shapiro-Wilk test was used to confirm the normality of the distributions of age, number of classes, physical findings, and eating behavior scores, and the Leven test was used to confirm homogeneity of variance. The significance level was set at 5% using IBM SPSS. Normally distributed data are shown as mean ± standard deviation values, and non-normally distributed data are shown as median (25th-75th percentiles) values. Breakdowns of age and number of classes, menopause, motivation for class, obesity and obesity-related disease patients, and types of obesity-related gene polymorphisms are shown as numbers and percentages. To compare before and after the intervention, paired t-tests were used for normally distributed data, and the Wilcoxon signed rank-sum test was used for non-normally distributed data. To compare eating behavior scores before and after the intervention, paired t-tests were used for normally distributed data, and the Wilcoxon signed-rank test was used for non-normally distributed data.

3. Results

3.1. Genotypes

Genotyping showed that, of a total of 56 participants, 39 (69.6%) were SS, 10 (17.9%) were SL, and 7 (12.5%) were LL. Allele frequencies were within the Hardy-Weinberg equilibrium range. The β3-adrenergic receptor (*β3AR*, rs4994, W64R) polymorphisms were WW (66%), WR (29%), and RR (5%), and the uncoupling protein 1 (*UCP1*, rs1800592, A3826G) polymorphisms were AA (25%), AG (43%), and GG (32%). The distribution of *β3AR* genotypes among the SS, SL, and LL genotypes was similar to the overall distribution. However, the distribution of the GG genotype of *UCP1* in SL and LL was 60% and 57%, respectively.

3.2. Anthropometry and Blood Biochemistry

Table 2 shows the changes in the measured values of all participants before and after the intervention. After the intervention, body weight, BMI, body fat percentage, waist circumference, systolic and diastolic blood pressures, and HbA1c were all significantly decreased (Figure 1). However, the reductions in triglycerides, total cholesterol, LDL-cholesterol, and fasting blood glucose were not significant. When the participants were divided into LL type, SL type, and SS type, body weight, body fat percentage, and waist circumference decreased in the following order: SS type > SL type > LL type (Table 3). In particular, the SS type showed a significant decrease (all p <0.001) in the above measured values compared with the LL type, which showed no significant change. Furthermore, multiple comparisons were performed on body weight and body fat loss rates for each gene polymorphism of the serotonin transporter LL, SL, and SS. The SS type had a significantly higher reduction rate in body weight (p=0.05) and body fat (p=0.05) than the LL type (Figure 2).

Table 2. Changes in measurements before and after intervention (overall, n=56).

	Before intervention		After intervention		p value
	Mean	± SD	Mean ± SD		
Body weight (kg)	66.3	± 13.8	63.6	± 13.6	<0.001
BMI (kg/m²)	27.2	± 5.6	26.1	± 5.6	<0.001
Body fat percentage (%)	35.3	± 6.1	34.5	± 5.8	0.004
Waist circumference (cm)	93.4	± 13.9	90.3	± 13.6	<0.001
Systolic blood pressure (mmHg)	133	± 18	122	± 16	<0.001

Diastolic blood pressure (mmHg)	79	± 12		72	± 11	<0.001
Triglyceride (mg/dl)	108	± 65		103	± 45	0.456
Total cholesterol (mg/dl)	219	± 38		219	± 31	0.845
LDL cholesterol (mg/dl)	129	± 34		129	± 27	0.884
Fasting blood glucose (mg/dl)	94	± 22		91	± 18	0.115
HbA1c (%)	5.8	± 0.6		5.8	± 0.6	0.003

Abbreviations: BMI, body mass index; HbA1c, Hemoglobin A1c. Statistical significance before and after the intervention was tested using the Wilcoxon signed-rank test.
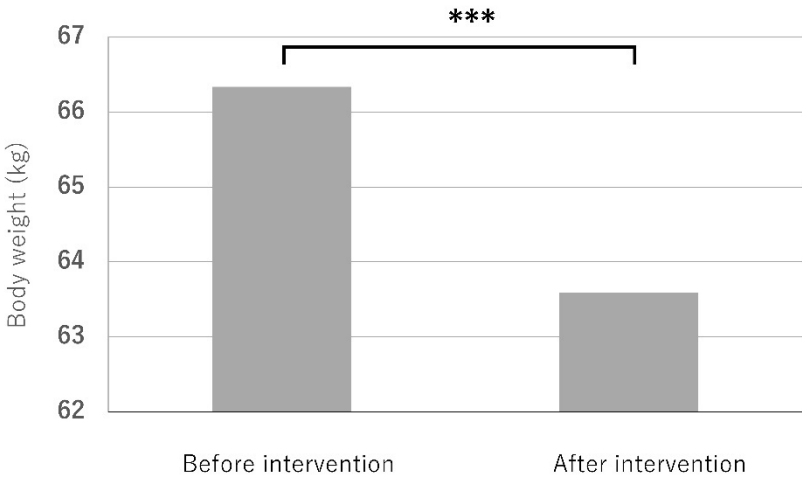
Figure 1. The body weight changes in all subjects from before to after the course (n=56). Body weight decreases from 66.3 kg before the intervention to 63.6 kg after the intervention. The results of the Wilcoxon signed-rank test for paired samples show a significant difference with ***p<0.001.

Table 3. Effects of serotonin transporter gene polymorphism on before and after intervention measurements.

	LL type (n=7)			SL type (n=10)			SS type (n=39)		
	Before	After		Before	After		Before	After	
	Mean±SD	Mean±SD	p value	Mean±SD	Mean±SD	p value	Mean±SD	Mean±SD	p value
Body weight (kg)	69.7±19.8	70.1±22.3	1.000	62.1±12.7	57.2±8.3	0.007	67.5±13.4	64.1±12.5	<0.001
BMI (kg/m²)	28.6±7.6	28.9±8.8	0.917	25.4±4.1	24.5±3.8	0.009	27.4±5.6	26.0±5.2	<0.001
Body fat percentage (%)	33.9±5.6	35.0±5.5	0.910	34.8±6.9	34.2±6.2	0.333	35.7±6.0	34.4±5.9	<0.001
Waist circumference (cm)	98.0±15.8	97.0±18.3	0.672	88.3±11.4	87.0±9.3	0.306	93.9±14.0	90.0±13.5	<0.001
Systolic blood pressure (mmHg)	131±22	123±23	0.116	134±21	123±17	0.058	133±17	122±15	<0.001
Diastolic blood	76±11	72±16	0.128	76±11	66±11	0.038	80±12	73±9	<0.001

pressure (mmHg)								
Triglycerides (mg/dl)	99±43	93±32	0.612	93±54	103±66	0.919	113±70	104±48	0.433
Total cholesterol (mg/dl)	191±25	192±16	0.866	222±33	231±38	0.906	223±40	221±29	0.759
LDL cholesterol (mg/dl)	107±23	111±15	0.446	133±30	137±31	0.415	132±36	131±27	0.794
Fasting blood glucose (mg/dl)	90±9	86±3	0.207	114±30	101±29	0.014	89±16	89±15	0.816
HbA1c (%)	5.6±0.2	5.5±0.3	0.026	6.2±0.9	6.1±1.1	0.356	5.8±0.5	5.7±0.5	0.045

Statistical significance before and after the intervention was tested using the Wilcoxon signed-rank test.
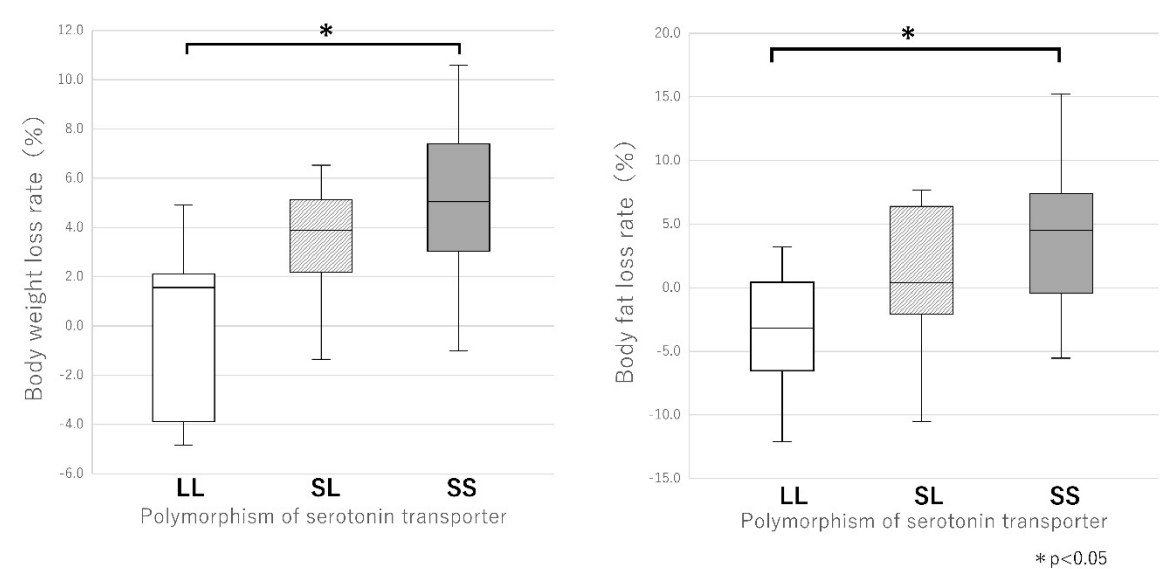
Figure 2. Body weight loss and body fat loss rates for each gene polymorphism of the promoter of serotonin transporter. Regarding the weight loss rate, the results of the Kruskal-Wallis test for independent samples show that the adjusted significance probability for the LL/SS pair is 0.006, for the LL/SL pair is 0.303, and for the SL/SS pair is 0.577. Regarding the weight loss rate, the significance probability for the LL/SS pair is 0.017, for the LL/SL pair is 0.371, and for the SL/SS pair is 0.875.

Because the numbers of LL and SL types were significantly smaller than those of the SS type, the reduction rates of body weight and body fat in the LL+SL type were compared with those of the SS type (Figure 3). Again, the SS type had significantly higher reduction rates of both body weight (p=0.05) and body fat (p=0.05) than the LL+SL type (Figure 3). After the intervention, in the LL+SL type, the reductions in body weight (p=0.049), systolic pressure (p=0.013), diastolic pressure (p=0.07), fasting blood glucose (p=0.005), and HbA1c (p=0.045) reached significant levels, whereas BMI, body fat, waist circumference, triglyceride, total cholesterol, and LDL cholesterol were not significant. When comparing the reduction in waist circumference, the SS type showed a significantly higher reduction rate than the SL type (p=0.05), LL type (p=0.05) (Figure 4), and the LL+SL type (p<0.05) (Figure 5)
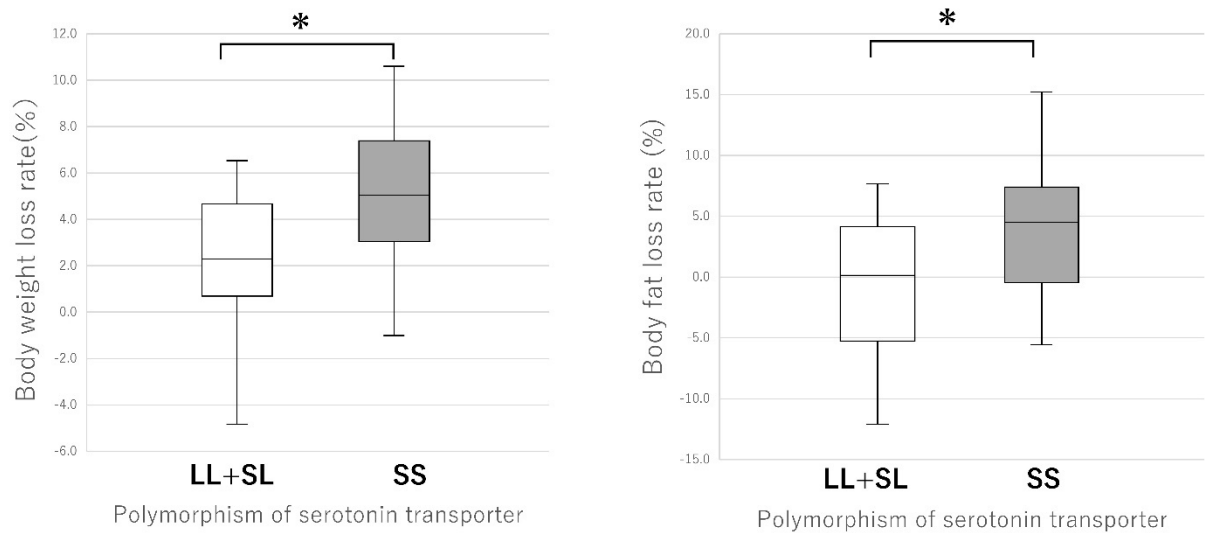
Figure 3. Body weight loss and body fat loss rates for LL+SL and SS genotypes of the promoter of serotonin transporter. Results are expressed as mean ± SD values. The results of the Mann-Whitney U test for independent samples indicate a significant difference. * p<0.05 with respect to LL+SL types.
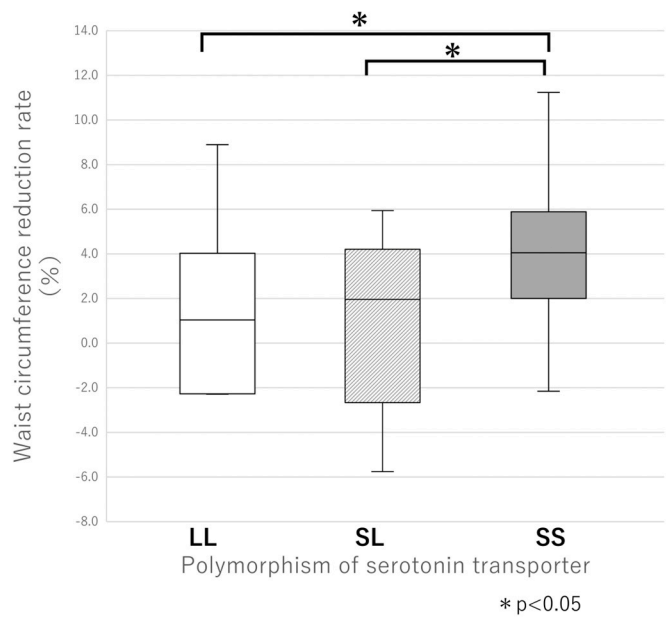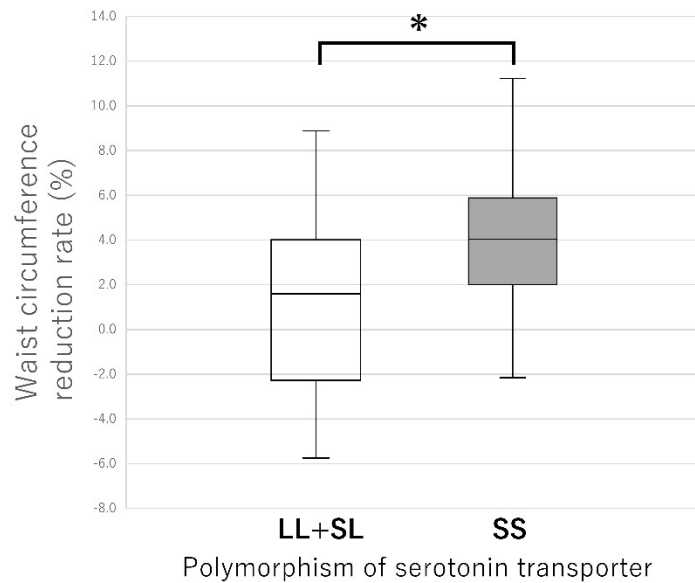

Figure 4. Reduction in waist circumference in each genotype of the promoter of serotonin transporter gene polymorphism. The results of the Kruskal-Wallis test for independent samples show that the adjusted significance probability for the LL/SS pair is 0.045, for the LL/SL pair is 0.906, and for the SL/SS pair is 0.031.

Figure 5. Reduction in waist circumference in the L/L+S/L type and the S/S type of the promoter of serotonin transporter gene polymorphism. Results are expressed as mean ± SD values. The results of the Mann-Whitney U test for independent samples indicate a significant difference. * p<0.05 with respect to LL+SL types.

The effects of polymorphisms in *β3AR* (WW, WR and RR) on body weight (P=0.568), body fat (P=0.246), and waist circumference were all insignificant. The effects of polymorphism in *UCP1* (AA, AG, and GG) on the change of those anthropometric data were also insignificant, except for the change in waist circumference of AA vs AG (p=0.05). The effects of the combinations of SS, SL, and LL and *β3AR* (WW, WR and RR) and *UCP1* (AA, AG and GG) on the change of body weight, body fat and waist circumference were all insignificant.

3.3. Eating Behavior Scores

Comparison of eating behavior scores of the SS, SL, and LL participants is summarized in Figure 6. In the SS group, the items that improved significantly after the intervention were eating as diversion, feeling of fullness, bad eating habits, unsteady eating pattern, and overall (Figure 6, right). In contrast, in the SS group, there was no improvement in perception of constitution, motivation of eating, and contents of meals (Figure 6, right). Only bad eating habits improved significantly in the SL group (Figure 6, middle), and no items improved in the LL group (Figure 6, left). Because the number of participants of the SL and LL types was smaller than that of the SS type, the LL+ SL group was compared with the SS group (Figure 7, right) (Table 4). In the LL + SL group, none of the items improved (Figure 7, left) (Table 4). In addition, the tested risk gene variants of *β3AR* and *UCP1*, of which the participants were informed, led to good adherence to diet and exercise advice
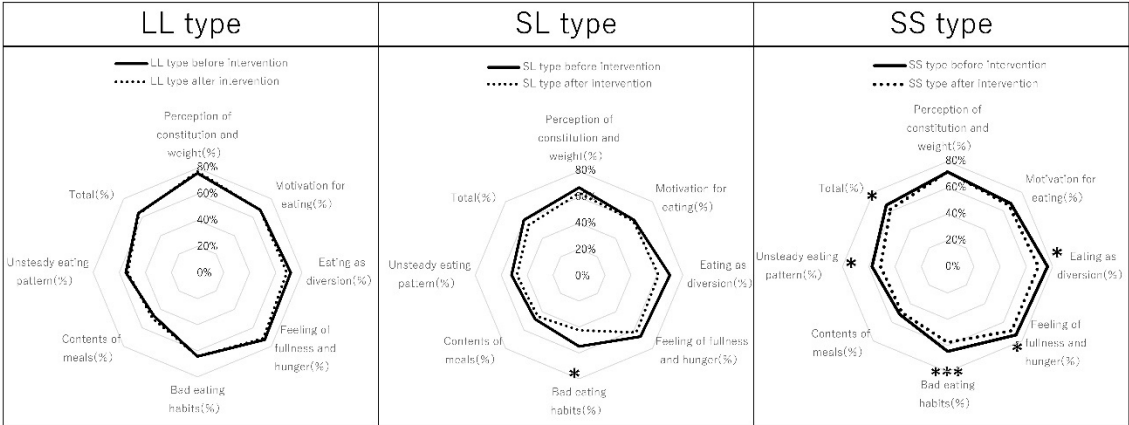
Figure 6. Comparison of the octagon diagrams plotting eating behavior of participants before and after the Healthy Diet Course according to the promoter of serotonin transporter gene polymorphisms of SS, SL, and LL types. The results are comparisons before and after the intervention within each group. The Wilcoxon signed-rank test for paired samples was conducted, indicating a significant difference. * p<0.05, *** p<0.001.
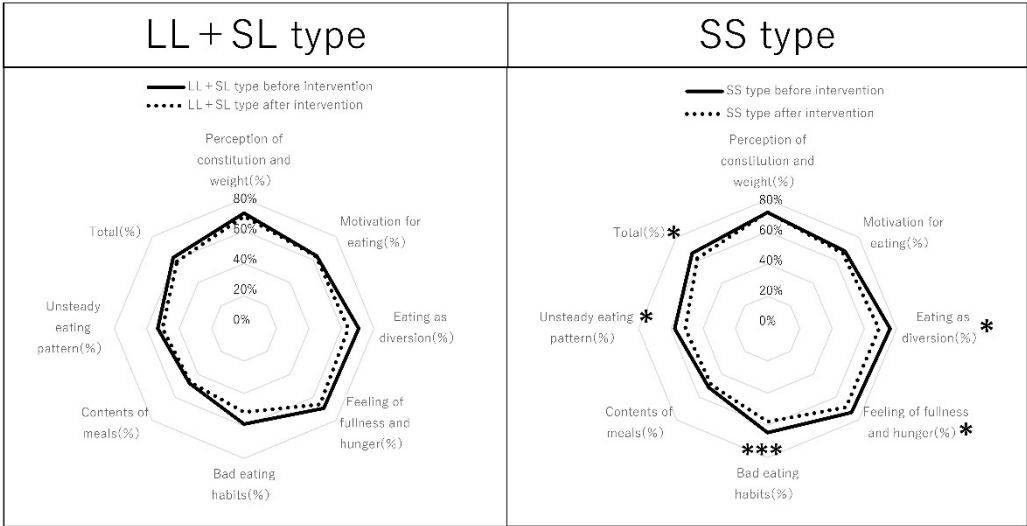

Figure 7. Comparison of the octagon diagrams plotting eating behavior in participants before and after the Healthy Diet Course according to promoter of serotonin transporter gene polymorphisms of the SS and LL+SL types. The results are comparisons before and after the intervention within each group. The Wilcoxon signed-rank test for paired samples was conducted, indicating a significant difference. * p<0.05, *** p<0.001.

Table 4. Percentage of the total possible scores in the seven categories and total of eating behavior before and after the intervention by serotonin transporter genotype.

	LL+SL type (n=17)			SS type (n=39)		
	Before	After	p value	Before	After	p value
	mean	mean		mean	mean	
Perception of constitution and weight (%)	71.1	69.1	0.599	72.0	72.2	0.859
Motivation for eating (%)	63.2	62.7	0.975	67.9	66.0	0.355
Eating as diversion (%)	70.6	64.2	0.078	76.1	69.1	0.040

Feeling of fullness and hunger (%)	69.4	66.2	0.180	73.6	69.0	0.010
Bad eating habits (%)	58.8	51.5	0.115	64.5	57.7	<0.001
Contents of meals (%)	47.6	46.8	0.788	51.5	49.4	0.319
Unsteady eating pattern (%)	53.2	50.3	0.248	57.6	51.0	0.001
Total (%)	61.8	58.4	0.169	66.1	61.6	0.001

Statistical significance before and after the intervention was tested using the Wilcoxon signed-rank test.

4. Discussion

It has been shown that adherence to instructions required for the prevention of chronic diseases is influenced by genetic polymorphisms that affect individual personality. In this study, genetic regulation of adherence to advice on a healthy eating course by the serotonin pathway of the *5-HTTLPR* in response to dietary behavior was demonstrated. It was found that female carriers of the SS type of the *5-HTTLPR* had significant reductions in weight (Figures 2 and 3), BMI (Table 3), body fat percentage (Table 3), waist circumference (Figures 4 and 5), systolic and diastolic blood pressures, and HbA1c (Table 3). In contrast, both LL and SL carriers showed significantly lower decreases in the above measures throughout the program (Table 3). The improvement in clinical data for SS participants may be due to better behavioral improvement in SS participants than in SL and LL participants (Table 4, Figures 6, 7). That is, SS participants showed better adherence in both clinical data (Table 3, Figures 2, 3) and feeding behavior (Table 4, Figures 6, 7), whereas SL and LL participants did not. In a preliminary study report by one of the authors (Y.K.) that did not include diabetic patients, after advice on a healthy diet course [6], an association with a decrease in fasting blood glucose levels was observed only in the SS group, but data on clinical data other than blood glucose levels and on eating behavior were lacking. In the present study that included diabetic patients, no significant decrease in fasting blood glucose levels was observed except in the SL group, due to the large fluctuations in blood glucose levels in diabetic patients (Table 3). However, the long-term average of blood glucose reflected by HbA1c was significantly decreased in the SS type (Table 3). This study is unique in that the background of medication adherence in Japanese people is different from other ethnicities (7-12) in terms of genetic (18), nutritional (2, 19), anthropometric (6), and psychological (5, 15, 40) aspects. Significant ethnic differences in genetics were observed between Japanese and Caucasian populations in the distribution of the SS genotype of *5-HTTLPR*, 61.9% in Japanese and 20.3% in Caucasian populations [18]. The distribution of the GG genotype of UCP1 was exceptionally high between SL and LL, 60% and 57%, respectively, possibly due to natural selection due to high energy expenditure caused by serotonin-induced UCP1 activity [21]. In terms of nutrition, there are large differences in nutritional intake [19], and as a result, there are large ethnic differences in the obesity rate (BMI >30 kg/m², 3.7% in Japanese women and 37.0% in American women) [19]. In terms of anthropometric aspects, the SS type of the *5-HTTLPR* polymorphism has been reported as a risk gene for obesity in Caucasians [22] and African descent [8], but in the Japanese participants of this study, there were no significant differences in weight, BMI, or waist circumference between the polymorphisms. In the cohort of South American origin studied, the BMI (30.0 ± 0.2 kg/m2) (8) was higher than that of the Japanese participants in the present study (27.2 ± 5.6kg/m²) (Table 1). In terms of psychological aspects, the NEO-five factor inventory scores of Japanese people [15] differed from those of Americans [40]. In terms of metabolic aspects, the energy expenditure of adults with the SS genotype was lower than that of adults with the SL and LL genotypes, and therefore the BMI of adults with the SS genotype was higher than that of adults with the SL and LL genotypes (p<0.0002) [22]. However, the fact that SS people are more likely to follow medical advice does not mean that they are in better health, because there were significantly more centenarians with the LL genotype than with the SS genotype [15]. In fact, persons with the SS type have the tendency to overeat called disinhibition [41]. The S carriers showed an impairment to lose weight, had a lower inhibition

capacity, and showed more failure (1.6 times) to control the amount of food eaten than the LL type (p < 0.05) [41]. Associations between Five-Factor Model personality traits [12–14], dietary patterns, and BMI have been reported [42]. High scores on the health aware diet dimension were associated with high conscientiousness [42], as supported by genetic analysis of the 5th chromosome [13]. To elucidate these effects, detailed analyses of brain physiology and genetics are needed. Positron emission tomography with an 18F-labelled serotonin analogue in midbrain raphe nuclei showed that a proportionate reduction in serotonin transporter density may be associated with adherence to taking antidepressants [43].

Strength and Limitations of This Study:

One strength of this study is its detailed analysis of adherence based on the polymorphism of the serotonin transporter gene, incorporating biochemical, anthropometric, and behavioral data collected before and after precise instructions were given. However, this study was limited to Japanese women, whose genetic background differs significantly from that of other ethnic groups [18,44] due to the long evolutionary history of adaptation to distinct environments [45]. Though adherence is partly influenced by serotonin transporter polymorphisms in the brain, this study lacked brain imaging data to support this connection [16,17,43]. The serotonergic nuclei, located in the midbrain raphe nucleus, project to a wide area of the brain, regulating the sympathetic nervous system and influencing feeding behavior [46]. Another limitation of this study is that all participants were female. Notably, sex differences exist in the association between serotonin transporter gene polymorphisms and personality traits [47]. This difference may be attributed to the higher methylation of the CpG island of the *5-HTTLPR* gene in S-type females compared with males (P < 0.0008), leading to lower mRNA production (P < 0.0001) [48]. This epigenetic effect is specific to SS-type females, which may contribute to increased variability in clinical and behavioral data. Nevertheless, SS-type individuals still exhibit the highest adherence to instructions.

5. Conclusions

Despite being at risk for obesity, SS-type *5-HTTLPR* participants demonstrated better adherence to instructions than SL-type and LL-type *5-HTTLPR* participants. As a result, they exhibited significant improvements in eating behavior, blood biochemistry, and anthropometric measurements. In the future, it will be essential to refine instructional methods to enhance adherence in LL-type participants and to investigate the genetic and epigenetic mechanisms underlying adherence.

Author Contributions: All authors contributed to preparation of the paper. Y.K. contributed to designing and implementing the study as the President of Institute of Nutritional Sciences and doctor of internal medicine. M.Y. is the primary investigator of this study, and as a registered dietician collecting and analyzing the data statistically. K.K is the main dietitian of the Nutrition Clinic and professor to instruct diet and exercise of the participants. Ku.S. participated in the gene analysis of the participants. M.F. is a registered dietitian and contributed to the analysis of dietary behavior. Ka.S. is a registered dietitian and analyses nutritional intake and biochemical data. O.I. is the head of the Nutrition Clinic and a gynecologist to evaluate health status of the female participants.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

β3AR	beta 3 adrenergic receptor
BMI	body mass index
DBP	diastolic blood pressure
FBG	fasting blood glucose
HbA1c	hemoglobin A1c
5-HT	5-hydroxytryptamine or serotonin
5-HTT	serotonin transporter
5-HTTLPR	5-HTT-linked polymorphic region
L	long allele of *5-HTTLPR*
LDL-C	low density cholesterol
LL	homozygote of long allele of *5-HTTLPR*
SL	heterozygote of L and S alleles of *5-HTTLPR*
S	short allele of *5-HTTLPR*
SBP	systolic blood pressure
SS	homozygote of S alleles of *5-HTTLPR*
TC	total cholesterol
UCP1	uncoupling protein 1

References

1. Desroches, S.; Lapointe, A.; Ratté, S.; Gravel, K.; Légaré, F.; Turcotte, S. Interventions to enhance adherence to dietary advice for preventing and managing chronic diseases in adults. Cochrane Database Syst Rev. 2013; (2):CD008722. [Cross Ref] [PubMed]

2. Kurotani, K.; Akter, S.; Kashino, I.; Goto, A.; Mizoue, T.; Noda, M.; Sasazuki, S.; Sawada, N.; Tsugane, S.; Japan Public Health Center based Prospective Study Group. Quality of diet and mortality among Japanese men and women: Japan Public Health Center based Prospective Study. BMJ. 2016; 352: i1209. [Cross Ref] [PubMed]

3. Kalantzi, V.; Kalafati, I.P.; Belitsi, V.; Tsiampalis, T.; Koutsonasios, I.; Androutsos, O.; Bonoti, F.; Panagiotakos, D.B.; Kosti, R.I. Cardiometabolic patient-related factors influencing the adherence to lifestyle changes and overall treatment: a review of the recent literature. Life (Basel). 2023;13(5):1153. [Cross Ref] [PubMed]

4. Esposito, C.M.; Ceresa, A.; Buoli, M. The association between personality traits and dietary choices: A systematic review. Adv Nutr 2021; 12, 1149-1159. [Cross Ref] [PubMed]

5. Serretti, A.; Calati, R.; Mandelli, L.; De Ronchi, D. Serotonin transporter gene variants and behavior: a comprehensive review. Curr Drug Targets 2006; 7(12):1659-1669. [Cross Ref] [PubMed]

6. Yamakawa, M.; Fukushima, A.; Sakuma, K.; Yanagisawa, Y.; Kagawa, Y. Serotonin transporter polymorphisms affect human blood glucose control. Biochem. Biophys. Res. Commun. 2005, 334, 1165-1171. [Cross Ref] [PubMed]

7. Lesch, K.P.; Balling, U.; Gross. J.; Strauss. K.; Wolozin, B.L.; Murphy, D.L.; Riederer. P. Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 1994; 95,157–162. [Cross Ref] [PubMed]

8. Meng, Y.; Groth, S.W.; Hodgkinson, C.A.; Mariani, T.J. Serotonin system genes contribute to the susceptibility to obesity in Black adolescents. Obes Sci Pract. 2021; 7(4):441-449. [Cross Ref] [PubMed]

9. Schinka, J.A.; Busch, R.M.; Robichaux-Keene, N. A meta-analysis of the association between the serotonin transporter gene polymorphism (*5-HTTLPR*) and trait anxiety, Mol Psychiatry 2004; 9, 197-202. [Cross Ref] [PubMed]

10. Cloninger, C.R.; Svrakic, D.M.; Przybeck, T.R. A psychobiological model of temperament and character. Arch. Gen. Psychiat. 1993; 50, 975–990. [Cross Ref] [PubMed]

11. Gillespiea, N.A.; Cloninger, C.R.; Heathc, A.C.; Martina, N.G. The genetic and environmental relationship between Cloninger's dimensions of temperament and character. Pers. Individ. Dif. 2003; 35(8): 1931–1946. [Cross Ref] [PubMed]

12. Goldberg, L.R. The structure of phenotypic personality traits. Am Psychol. 1993; 48:26–34. [Cross Ref] [PubMed]

13. Park, C.S.; Choi, J.; Kwak, S.; Lee, S-P.; Kim, H-K.; Kim, Y-J.; Kwak, S.H.; Park, J-B. Association between personality, lifestyle behaviors, and cardiovascular diseases in type 2 diabetes mellitus: a population-based cohort study of UK Biobank data. BMJ. Open Diabetes Res. Care. 2024;12(4): e004244. [Cross Ref] [PubMed]

14. Lo, M-T.; Hinds, D.A.; Tung, J.Y.; Franz, C.; Chun-Chieh Fan, C-C.; Wang, Y.C.; Smeland, O.B.; Schork, A.; Holland, D.; Kauppi, K.; Sanyal1, N.; Escott-Price, V.; Smith, D.J.; O'Donovan, M.; Stefansson, H.; Bjornsdottir, G.; Thorgeirsson, T.E.; Stefansson, K.; McEvoy, L.K; Anders M. Dale, A.M.; Andreassen, O.A.; Chen. C-H. Genome-wide analyses for personality traits identify six genomic loci and show correlations with psychiatric disorders. Nat Genet. 2017; 49(1): 152–156. [Cross Ref] [PubMed]

15. Gondo, Y.; Hirose, N.; Arai, Y.; Yamamura, K.; Shimizu, K.; Michiyo Takayama, M.; Yoshinori Ebihara, Y.; Nakazawa, S.; Inagaki, H.; Masui, Y.; Kitagawa, K. Contribution of an affect-associated gene to human longevity: Prevalence of the long-allele genotype of the serotonin transporter-linked gene in Japanese centenarians. Mech Ageing Dev. 2005;126(11): 1178-1184. [Cross Ref] [PubMed]

16. Kim, J-H.; Kim, H-K.; Sang-Wha Lee, S-W.; Son. Y-D.; Kim, J-H. The relationship between character traits and in vivo cerebral serotonin transporter availability in healthy subjects: a high-resolution PET study with C-11 DASB. Pharmaceuticals. 2023;16(5):759. [Cross Ref] [PubMed]

17. Hariri, A.R.; Weinberger, D.R. Functional neuroimaging of genetic variation in serotonergic neurotransmission. Genes Brain Behav. 2003; 2, 341-349. [Cross Ref] [PubMed]

18. Nakamura, M.; Ueno, S.; Sano, A.; Tanabe, H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. Molecular Psychiatry 2000; 5: 32-38. [Cross Ref] [PubMed]

19. Kagawa, Y. Influence of nutritional intakes in Japan and the United States on COVID-19 infection. Nutrients 2022;14(3): 633 [Cross Ref] [PubMed]

20. Tecott, L.H. Serotonin and the orchestration of energy balance. Cell Metab. 2007; 6(5):352-361. [Cross Ref] [PubMed]

21. McGlashon, J.M.; Gorecki, M.C.; Kozlowski, A.E.; et al. Central serotonergic neurons activate and recruit thermogenic brown and beige fat and regulate glucose and lipid homeostasis. Cell Metab. 2015;21(5):692-705. [Cross Ref] [PubMed]

22. Sookoian, S; Gianotti, T.F.; Gemma, C.; Burgueño, A.; Pirola, C.J. Contribution of the functional 5-HTTLPR variant of the SLC6A4 gene to obesity risk in male adults. Obesity. 2008;16(2):488-491. [Cross Ref] [PubMed]

23. Fujisawa, T.; Ikegami, H.; Kawaguchi, Y.; Ogihara, T. J. Meta-analysis of the association of Trp64Arg polymorphism of beta 3-adrenergic receptor gene with body mass index. Clin. Endocrinol. Metabol. 1998;2441-2444 [Cross Ref] [PubMed]

24. Zhu, L.Y.; Hu, L.Y.; Li, X.L.; Wang, G.Y.; Shan, W.; Ma, L.C.; Wang, X.H. Relationship between Trp64Arg mutation in the beta3-adrenergic receptor gene and metabolic syndrome: a seven-year follow-up study. Chin Med J (Engl). 2010;123(17):2375-2378. [Cross Ref] [PubMed]

25. Jia, J.J.; Tian, Y.B.; Cao, Z.H.; Tao, L.L.; Zhang, X.; Gao, S.Z.; Ge, C.R.; Lin, Q.Y.; Jois, M. The polymorphisms of UCP1 genes associated with fat metabolism, obesity and diabetes. Mol. Biol. Rep. 2010; 37(3):1513-1522. [Cross Ref] [PubMed]

26. Chathoth, S.; Ismai, M.H.; Vatte, C.; Cyrus, C.; Ali, Z.A.; Ahmed, K.H.; Sadananda Acharya, S.; Barqi, A.M.A.; Ali, A.A. Association of Uncoupling Protein 1 (UCP1) gene polymorphism with obesity: a case-control study. BMC. Med. Genet. 2018;19(1):203. [Cross Ref] [PubMed]

27. Carey, G.B. Mechanisms regulating adipocyte lipolysis. Adv. Exp. Med. Biol. 1998;441:157-170. [Cross Ref] [PubMed]

28. Kim, Y.; Yeung, S.L.A.; Sharp, S.J.; Wang, M.; Jang, H.; Luo, S.; Brage, S.; Wijndaele, K. Genetic susceptibility, screen-based sedentary activities and incidence of coronary heart disease. BMC. Med. 2022;20(1):188. [Cross Ref] [PubMed]

29. Cassidy, S.; Chau, J.Y.; Catt, M.; Bauman, A.; Michael I Trenell, M.I. Cross-sectional study of diet, physical activity, television viewing and sleep duration in 233,110 adults from the UK Biobank; the behavioral phenotype of cardiovascular disease and type 2 diabetes. BMJ. Open. 2016; 6(3): e010038. [Cross Ref] [PubMed]

30. Miller, T.A. Health literacy and adherence to medical treatment in chronic and acute illness: A meta-analysis. Patient Educ. Couns. 2016; 99(7):1079-1086. [Cross Ref] [PubMed]

31. Leibowitz, S.F.; Alexander, J.T. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biol. Psychiatry 1998;44:851–864. [Cross Ref] [PubMed]

32. Kagawa, Y.; Kagawa, A. Secondary prevention of cardiovascular diseases of outpatients of the nutrition clinic. In Lovenberg, W. and Yamori, Y. Eds. Nutritional Prevention of Cardiovascular Diseases. Academic Press. 1984; 339-348.

33. Hirai, C., Kagawa, Y. The concentrations of blood sugar and HbA1c are significantly higher in g/g homozygotes of adiponectin t45g polymorphism than in heterozygotes and wild types. Asia Pac. J. Public Health 2008;20:80-86. [PubMed]

34. Obata, K., Segawa, O., Yakabe, M., Ishida, Y., Kuroita, T., Ikeda, K., Kawakami, B., Kawamura, Y., Yohda, M., Matsunaga, T., and Tajima, H. Development of a novel method for operating magnetic particles, Magtration Technology, and its use for automating nucleic acid purification, J. Biosci. Bioeng. 2001;91:500–503[Cross Ref] [PubMed]

35. Logsdon, G.A.; Vollger, M.R.; E Eichler, E.E. Long-read human genome sequencing and its applications. Nat Rev Genet. 2020;21(10):597-614. [Cross Ref] [PubMed]

36. https://www.biorxiv.org/content/10.1101/2022.10.25.513646v1.abstract.

37. Kagawa, Y.; Hiraoka, M.; Miyashita-Hatano, Y.; Shishido-Oki, M.; Yoshida, M.; Kondou, S.; Sugiura, M.; Sawakami-Kobayashi, K.; Takahashi, M.; Tajima, H; Yohda, M. Automated single nucleotide polymorphism typing using bead array in capillary tube. J. Biosci. Bioeng. 2010;110(4):505-508. [Cross Ref] [PubMed]

38. Japan Society for the Study of Obesity. Guidelines for the management of obesity disease 2016 (in Japanese). Life Science Publisher, Tokyo, 2016; pp.40-42.

39. Miwa, T.; Tajirika, S.; Hanai, T.; Imamura, N.; Adachi, M.; Horita, R.; Fukao, T.; Shimizu, M.; Yamamoto, M. Usefulness of a questionnaire for assessing the relationship between eating behavior and steatotic liver disease among Japanese male young adults. Sci. Rep. 2024;14:2194. [Cross Ref] [PubMed]

40. Chapman, B.P.; Franks, P.; Duberstein, P.R.; Jerant, A. Differences between individual and societal health state valuations: any link with personality?. Med. Care. 2009; 47(8) 902-907. [Cross Ref] [PubMed]

41. Bonnet, G.; Gómez-Abellán, P.; Vera, B.; Juan Francisco Sánchez-Romera, J.F.; Hernández-Martínez, A.M.; Sookoian, S.; Pirola, C.J.; Garaulet, M. Serotonin-transporter promoter polymorphism modulates the ability to control food intake: Effect on total weight loss. Mol. Nutr. Food Res. 2017; 61(11)1700494 [Cross Ref] [PubMed]

42. Mõttus, R.; McNeill, G.; Jia, X.; Leone C A Craig, L.C.A.; Starr, J.M.; Deary, I.J. The associations between personality, diet and body mass index in older people. Health Psychol. 2013;32(4):353-560. [Cross Ref] [PubMed]

43. Yeh, Y-W.; Ho, P-S.; Kuo, S-C.; Chen, C-Y.; Liang, C-S.; Yen, C-H.; Huang, C-C.; Ma, K-H.; Shiue, C-Y.; Huang, W-S.; Shyu, J-F.; Wan, F-J.; Ru-Band Lu, R-B.; Huang S-Y. Disproportionate reduction of serotonin transporter may predict the response and adherence to antidepressants in patients with major depressive disorder: a positron emission tomography study with 4-[18F]-ADAM. Int. J. Neuropsychopharmacol. 2015;18(7):pyu120. [Cross Ref] [PubMed]

44. The 1000 Genome Project Consortium. A global reference for human genetic variation. Nature 2015; 526: 68-74. [Cross Ref] [PubMed]

45. Kagawa, Y.; Yanagisawa, Y.; Hasegawa, K.; Suzuki, H.; Yasuda, K.; Kudo, H.; Abe, M.; Matsuda, S.; Ishikawa, Y.; Tsuchiya, N.; Sato, A.; Umetsu, K.; Kagawa, Y. Single nucleotide polymorphisms of thrifty genes for energy metabolism: evolutionary origins and prospects for intervention to prevent obesity-related diseases. Biochem. Biophys. Res. Commun. 2002;295(2):207-222 [Cross Ref] [PubMed]

46. Val-Laillet, D.; Aarts, E.; Weber, B.; Ferrari, M.; Quaresima, V.; Stoeckel, L.E.; Alonso-Alonso, M.; Audette, M.; Malbert, C.H.; Stice, E. Neuroimaging and neuromodulation approaches to study eating behavior and prevent and treat eating disorders and obesity. Neuroimage Clin. 2015;8:1-31. [Cross Ref] [PubMed]

47. Du, I.; Bakish, D.; Hrdina, P.D. Gender differences in association between serotonin transporter gene polymorphism and personality traits. Psychiatr. Genet. 2000;10(4):159-164. [Cross Ref] [PubMed]

48. Philibert, R.A.; Sandhu, H.; Hollenbeck, N.; Gunter, T.; Adams, W.; Madan, A. The relationship of 5HTT (SLC6A4) methylation and genotype on mRNA expression and liability to major depression and alcohol dependence in subjects from the Iowa Adoption Studies. Am. J. Med. Genet. B. Neuropsychiatr, Genet. 2008;0(5):543–549. [Cross Ref] [PubMed]