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Serotonin transporter polymorphisms affect human blood glucose control ☆

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Abstract

We measured the effect of nutritional intervention on clinical data, including fasting blood glucose (FBG), and their association with polymorphisms of the serotonin transporter-linked polymorphic region (5-HTTLPR) which might affect adherence. Enrolled in the intervention program were 264 Japanese women not on medication for diabetes, hypercholesterolemia or hypertension. The 5-HTTLPR allele (S and L) frequencies among the subjects differed markedly from those of Caucasians: SS ($n = 183$), LS ($n = 69$), and LL ($n = 12$). The decrease in FBG (ΔFBG) from the beginning to the end of the program (11 weeks; short-term study), and ΔFBG from the beginning to a follow-up check performed between 2002 and 2004 (average of 23 years later; long-term study) was calculated. The SS homozygotes of 5-HTTLPR showed larger ΔFBG ($P = 0.01$ and $P < 0.0001$ in the short- and long-term studies, respectively) than ΔFBG with other genotypes.

Keywords: Blood glucose control; Serotonin transporter; Polymorphism; Personality; Long-term intervention; Dopamine receptor; Adherence; Neurotransmitter; Eating disorder; Obesity

The prevention and treatment of obesity-related diseases depends on both the genes and lifestyle of an individual [1]. Adherence to the advice for lifestyle change is influenced by individual personality [2–4] during an intervention [5]. Personality traits are partially inherited [6], as evidenced by twin studies [7,8], and the frequency of the traits differs depending upon neurotransmitter-related polymorphisms [9–12]. Polymorphisms in the serotonin transporter-linked polymorphic region (5-HTTLPR) and the D4 dopamine receptor (DRD4) are associated with a particular personality [9,11–14]. The serotonin transporter is coded for by a gene on chromosome 17q11.2 and the insertion/deletion polymorphism in the promoter region gives rise to two common alleles; the long (L: 16 repeat insertion) and the short (S: 14 repeat deletion). The S allele possesses lower transcriptional activity leading to a relative reduction in serotonin transporter and serotonin uptake [11]. A meta-analysis of 26 studies ($n = 7657$) on the association between 5-HTTLPR and trait anxiety indicated that the polymorphism has a small but reliable influence on personality, particularly when measured with a neuroticism scale [13]. However, personality tests are not so objective, as shown by meta-analyses [13,14] of contradictory results reported, and depend greatly on the inventory

☆ *Abbreviations:* DBP, diastolic blood pressure; DRD4, D4 dopamine receptor; ΔFBG, difference in FBG between the beginning and the end of the studies; FBG, fasting blood glucose; 5-HT, 5-hydroxytryptamine or serotonin; 5-HTT, serotonin transporter; 5-HTTLPR, 5-HTT-linked polymorphic region; L, long allele of 5-HTTLPR; LL, homozygote of long alleles of 5-HTTLPR; LS, 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, serum total cholesterol.

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used [14]. The neurophysiological amygdala activity measured by functional MRI [15,16] differs depending on the genotypes of the 5-HTTLPR polymorphism.

DRD4 polymorphism and 5-HTTLPR are also associated with eating disorders [17–21]. Serotonergic and dopaminergic drugs are used to treat obesity in the United States [22]. The mechanisms of these disorders, drugs may not involve the elusive personality factor, but rather, may be caused by purely biochemical metabolism in tissues. In fact, the effects of polymorphism in 5-HTTLPR are found in the peripheral cells as well as neurons expressing serotonin transporter [11].

In the present study, we examined the association between clinical data and the alleles of two genes, DRD4 and 5-HTT, which may affect adherence. The data included body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), and fasting blood glucose (FBG) concentration. Differences in the clinical data (ΔFBG, for example) taken at the beginning and the end of the 11-week program (short-term study), and between the beginning and a follow-up check carried out between 2002 and 2004 (average of 23 years = long-term study) were calculated. These data were chosen instead of a potentially controversial personality test [13,14] because such clinical data are completely objective. Moreover, whatever the psychological mechanism, clinical data are the essential results required by nutritionists for intervention. To the best of our knowledge, this is the first report of an association between FBG and polymorphisms of neurotransmitter-related genes.

Materials and methods

Subjects. The subjects were 264 Japanese women aged between 19 and 73 years old (47.8 ± 10.6 years old; mean ± SD) who had been enrolled in the lifestyle modification programs at the Nutrition Clinic of the Kagawa Nutrition University, Japan [23,24]. Their daily activity was within normal limits, and they were all able to visit the clinic without assistance, even 23 years after (on average) the course in 2002–2004. The DNA of the subjects (total 399 samples) was collected from a swab containing the subjects' buccal epithelium which was sent back by mail or from their blood on the occasion of health checkup. In order to eliminate the effect of drugs, women on medication for diabetes, hypercholesterolemia, hyperlipidemia or hypertension were excluded from this study [23]. The aim of the program was to prevent and treat obesity and the related risks (elevated SBP, DBP, TC, and FBG). According to the results of an assessment of the individuals' nutritional status and clinical examination, they were advised to be on diet and exercise for over 11 weeks [23,24]. The data were measured at the beginning of the program, just after the end of the program, and in 2002–2004 for the follow-up study. Written informed consent was obtained from every individual, in accordance with the guidelines of the Medical and Genetic Ethical Committee of Kagawa Nutritional University.

Anthropometric and clinical data. We calculated the difference between the data that were taken at the beginning, the end of the program, and at a time between 2002 and 2004. BMI and right arm blood pressure (SBP and DBP) were measured with precautions, essentially the same as those described in The National Nutrition Survey in 2002 [25]. A blood sample was taken from the cubital vein, and TC and FBG were measured using commercial kits (Wako Pure Chemical Industries, Osaka, Japan, L-type Wako cholesterol, and Sikarikido, respectively [26]), with essentially the same precautions as described in The National Nutrition Survey in 2002 [25].

The short-term change in data was the difference between the beginning and end of the 11-week course. The long-term change in values was the difference between the beginning of the course and the average of 23 years after the course. A total of 291 clients (of which 264 were analyzed for the clinical study) were genotyped for both polymorphisms: a 48-base-pair repeat in the gene and a 44-base-pair insertion or deletion in the 5-HTTLPR.

Laboratory methods for genetic polymorphism. For 5-HTT and DRD4 genotyping, genomic DNA from basal epithelial cell was extracted from the buccal epithelium in a swab (BBL CultureSwab EZ; BD, NJ) using a DNeasy Tissue Kit (Qiagen, CA). The DNA fragment was amplified using polymerase chain reaction (PCR) with primers as described below. The reaction products were electrophoresed through 8% acrylamide gel and visualized by ultraviolet illumination (240 nm) in ethidium bromide.

The 5-HTTLPR was examined by the method of Lesch et al. [11] with minor modifications. DNA was amplified with primers (5′-GG CGTTGCCGCTCTGAATGC-3′; sense) and (5′-GAGGGACTGA GCTGGACAACCAC-3′; antisense) of the 5-HTT gene 5′-flanking regulatory region to generate 486- or 530-bp fragments. The amplification was carried out for 35 cycles (95 °C for 1 min, 61 °C for 2.5 min, and 72 °C for 1 min) in a 20 μL aliquot of the reaction mixture with 20 ng genomic DNA, 10 pmol primers, and 0.85 U polymerase (Ex Taq; TaKaRa Shuzo, Kyoto, Japan) using a programmable thermal cycler (Gene Amp 9700; Applied Biosystems, CA).

The polymorphism in DRD4 was examined by PCR with oligonucleotide primers according to Ono et al. [27]. The amplification was carried out for 35 cycles (95 °C for 1 min, 72 °C for 3 min, and 72 °C for 3 min) in a 20 μL aliquot of the reaction mixture with 20 ng genomic DNA, 5 pmol of primers (5′-AGGTGGCACGTCGCGCC AAGCTGCA-3′; sense, 5′-TCTGCGGTGGAGTCTGGGGTGGG AG-3′; antisense), and 0.5 U polymerase (Ex Taq; TaKaRa Shuzo) using a programmable thermal cycler (Gene Amp 9700; Applied Biosystems, CA).

Nutritional intervention. Subjects were instructed to consume a mixture of variety food items from four major food groups, as follows [23,24]. They were asked to consume 240 kcal from each of the following groups: group 1 (milk, dairy products, and egg), group 2 (meat, fish, and beans), and group 3 (vegetables and fruits) [23,24]. Their total energy intake was adjusted by varying their intake of group 4 (grains, oil, and sugar), to reduce body fat with minimal loss of non-fat body weight [23,24]. The protein–fat–carbohydrate ratio was 16.8:25.7:57.5, while the saturated:monounsaturated:polyunsaturated fatty acid ratio was 2.8:3.5:3.6, in the typical intervention diet. In addition to the diet, the subjects were advised to regularly follow a program of slow exercise (usually 10,000 walking steps/day) [23,24]. In previous studies, the most important feature of this intervention was the reduction of visceral fat (−3.5 ± 3.5 kg), with minimal loss of lean body mass (LBM, −0.51 ± 1.7 kg) during the 11 weeks, estimated by whole body ^{40}K radioactivity counting [24] and dual X-ray absorptiometry after 2000 [23]. Weight reduction was nearly independent ($r = 0.180$) of the change in whole body ^{40}K radioactivity (LBM in kg = 68.1 × total body potassium in mEq) [24]. During 1967–1976, significant decreases ($P < 0.001$, $n = 756$) in BMI, SBP, DBP, TC, triglyceride, and FBG were achieved [1,24].

Statistical analysis. The effects of the program and lifestyle changes were examined using a paired t test. The association of genotypes with the year of intervention, participant age at enrollment, short-term changes in BMI, SBP, DBP, TC, and FBG, and the association of genotypes with year of the intervention, years passed from the intervention, long-term BMI, SBP, DBP, TC, and FBG were examined via