Applied nutritional investigation

*Taq*IA polymorphism in dopamine D2 receptor gene complicates weight maintenance in younger obese patients

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ARTICLE INFO

ABSTRACT

Objective: The A1 allele of the *Taq*IA polymorphism in the dopamine D2 receptor gene (rs1800497) has been associated with obesity. However, the effect of the polymorphism on the success in weight loss and/or weight maintenance during weight-loss programs has not been evaluated thus far.
Methods: The rs1800497 was genotyped in 202 (135 female, 67 male) severely obese individuals with an initial body mass index of 41.7 ± 0.5 kg/m^2 who participated in a weight-loss program consisting of a weight-loss phase with a formula diet (12 wk) and a weight-maintenance phase (40 wk). Measurements were collected at baseline, after the weight-loss phase, and at the end of the weight-maintenance phase at 1 y.
Results: Genotyping revealed 4 A1A1, 67 A1A2, and 131 A2A2 genotype carriers. Of the 202 subjects in the program, 66.8% completed the program and 33.2% terminated prematurely. Neither the attrition rate ($P = 0.44$) nor the overall weight loss was influenced by the different genotypes ($P = 0.96$). However, younger A1$^+$ participants (A1A1 and A1A2) had a higher body mass index at all time points (baseline, $P = 0.04$; after weight loss, $P = 0.05$; after weight maintenance, $P = 0.02$). They also showed less overall weight loss ($P = 0.05$), which derived mainly from a greater weight regain during the maintenance phase ($P = 0.02$).
Conclusion: In this program, younger A1$^+$ participants exhibited problems in maintaining weight loss during a weight-loss program.

Introduction

Obesity is a serious global health issue because it has become a worldwide epidemic, with at least 1.1 billion adults and 10% of children being overweight or obese [1,2]. Excess body weight can lead to the metabolic syndrome and a decreased life expectancy owing to cardiovascular disease, type 2 diabetes, or certain types of cancer [2,3].

Obesity results mainly from an imbalance between energy intake and energy expenditure, which are influenced by environmental and genetic factors [4]. Polymorphisms in the

dopamine D2 receptor (*DRD2*) and obesity (*ob*) genes together account for about 20% of the variance in the body mass index (BMI), particularly in younger women [5,6]. One such variant is the A1 allele of the *Taq*IA single nucleotide polymorphism in the *DRD2* gene (rs1800497), for which a decreased brain DRD2 density in the striatum has been described [5,7]. This decreased receptor density has been associated with an increased prevalence of obesity [6,8–13] and substance abuse [14–18]. As an underlying cause, A1 allele carriers have been postulated to have an impaired reward circuitry leading to a state of "reward deficiency syndrome" [18,19]. This condition is aggravated by an increased impulsivity [15,20]. Thus, these behavioral changes make A1$^+$ carriers prone to overeating [21–26] and obesity.

However, the effect of the A1 allele on the success of weight-loss programs is largely unknown [11]. The purpose of this study was to analyze whether the A1 allele of rs1800497 decreases the

likelihood of weight loss and/or weight maintenance in severely obese subjects during a 1-y weight-loss program.

Materials and methods

Weight-loss program OPTIFAST52

The present study was a monocentric, longitudinal investigation involving obese individuals who participated in the multidisciplinary, non-surgical weight-loss program (OPTIFAST52; Nestlé, Inc., Vevey, Switzerland). In 1999 the OPTI-FAST52 program was established in Germany with the aim of treating obese people of at least 18 y of age and with a BMI of 30 kg/m^2 or higher. OPTIFAST52 combines the expertise of physicians, dietitians, physical therapists, and psychologists in an outpatient-based, comprehensive, multidisciplinary approach. The program consists of a four phased lifestyle intervention with four modules (psychology, medicine, dietetics, and exercise) and is designed for 52 wk. During the program, closed groups of 10 to 15 people meet for weekly sessions of about 3.5 h. The four program phases include 1) a 1-wk introduction time; (ii) a 12-wk period of a low-calorie formula diet (800 kcal/d; five packets at 160 kcal dissolved in 250–300 mL of water as a meal replacement; OPTIFAST52 800 formula) accompanied by 12 medical examinations, 12 exercise units, 2 lessons of behavior therapy, and 2 sessions of nutrition counseling; 3) a 6-wk refeeding phase in which solid food is reintroduced and the formula diet is gradually replaced by a normal diet with only a small change of total energy intake accompanied by six medical examinations, six exercise units, two lessons of behavior therapy, and six sessions of nutrition counseling; and 4) a 33-wk stabilization phase in which energy intake is increased step by step to an individual level that allows weight stabilization and in which nutritional education and behavior modification are intensified to determine coping strategies and to achieve long-term weight control, accompanied by 9 medical examinations, 17 exercise units, 26 lessons of behavior therapy, and 8 sessions of nutritional counseling.

For the purpose of the present analyses, it was sufficient to divide the program into two parts: a weight-loss phase of 12 wk and a weight-maintenance phase of 40 wk. In this study, data from the beginning (T0), from week 12 after the formula-based weight-loss phase (T1), and from week 52 after the weight-maintenance period of 40 wk (T2) were included.

Study population

The ethics committee at the University of Heidelberg approved the study and written informed consent was given by all participants. A population of 202 obese adults taking part in the OPTIFAST52 program at the University Hospital of Heidelberg from 2005 through 2010 was analyzed. The study sample included 135 female and 67 male subjects 18 to 72 y old. The mean BMI at the beginning of the program was 41.7 ± 0.5 kg/m^2. The 1-y program was completed by 135 participants and discontinued by 67.

Anthropometric and laboratory measurements

During the 52-wk program, weight and blood pressure were monitored regularly. Body weight was determined on a calibrated scale (model 764, Seca, Hamburg, Germany) after an 8-h fast. The BMI was calculated as weight in kilograms divided by the square of the height in meters. To control laboratory values, blood samples were collected at five different time points during the program after a fasting time of at least 8 h. The laboratory parameters were measured in the central laboratory of the University Hospital of Heidelberg. In this study, concentrations of blood glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triacylglycerols from T0, T1, and T2 were included. Blood collection tubes contained sodium fluoride (Sarstedt S-Monovette, no. 04.1903, Nümbrecht, Germany) for the assessment of glucose levels and lithium heparin (Sarstedt S-Monovette, no. 01.1634) for the assessment of lipid profiles. Samples were directed to the central laboratory without delay, and plasma was separated out and analyzed on a ADVIA 2400 chemistry analyzer (Siemens, Eschborn, Germany). Concentrations of glucose, total cholesterol, HDL, and triacylglycerols were determined using the appropriate Siemens test kits (nos. B01-4597-01, 04993681, 08058065, and B01-4133-01, respectively) according to the manufacturer's instructions. Further, concentrations of LDL were calculated according to the Friedewald equation.

Questionnaire on body weight and lifestyle

As part of the diagnostic procedure, all participants in the OPTIFAST52 program completed a non-standardized psychological questionnaire provided by the manufacturer (Nestlé, Inc.) at the beginning of the program. The questionnaire includes information about the development of body weight, previous diets, previous weight loss, causes of overweight, eating behavior, physical activity, life crises, self-assessment, smoking and alcohol consumption, education and profession, current life situation, and stress factors.

Genotyping

The DRD2 TaqIA polymorphism was assayed by polymerase chain reaction (PCR) restriction fragment length polymorphism analyses [27,28]. Genomic DNA was collected and isolated from 250 µL of whole blood anticoagulated with ethylenediaminetetraacetic acid using the peqGOLD Blood DNA Mini Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany).

PCR conditions

Genomic DNA, 1× Green GoTaq Flexi Buffer, 37.5 nmol of magnesium chloride solution (Promega GmbH, Mannheim, Germany), deoxynucleotide triphosphates (Fermentas GmbH, St. Leon-Rot, Germany), forward (5′-CCGTCGACGGCTGGC CAAGTTGTCTA-3′) and reverse (5′-CCGTCGACCCTTCCTGAGTGTCATCA-3′) oligonucleotide primers (Eurofins MWG Synthesis GmbH, Ebersberg, Germany), and 1 U of GoTaq Flexi DNA Polymerase (Promega GmbH) in a total volume of 25 µL was processed at 94°C for 4 min followed by 40 cycles at 94°C for 30 s, 68°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 3 min. Digestion of 10 µL of the PCR products was accomplished at 65°C for 50 min with 4 U of the TaqαI restriction enzyme (New England BioLabs, Ipswich, MA, USA). The final products were resolved on an ethidium bromide–stained 2% agarose gel. After incubation of the 310-bp PCR product, the A1 allele remained intact, whereas the A2 allele resulted in two pieces of 180 and 130 bp. On each gel, a negative control (water) and one positive control were added. During gel electrophoresis, the GeneRuler 100-bp Plus DNA Ladder (Fermentas GmbH) was used as a DNA size marker.

Statistical analysis

SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analyses. Participants who carried the A1A1 or A1A2 genotype were considered A1$^+$ and participants with the A2A2 genotype were considered A1$^-$ [11]. A per-protocol analysis was chosen to analyze the influence of the A1$^+$ and A1$^-$ genotypes on weight loss and weight maintenance during the 1-y program. The Wilcoxon–Mann–Whitney test (U test) was used to assess differences between the A$^+$ and A1$^-$ genotype groups. To determine whether the A1$^+$ and A1$^-$ genotypes were in Hardy–Weinberg equilibrium, a chi-square test was performed. $P \leq 0.05$ was considered statistically significant. Variables are expressed as mean ± SEM or percentage.

Results

This study was performed as a monocentric longitudinal study in which 202 unrelated Caucasian subjects with severe obesity were analyzed for the rs1800497 polymorphism in the DRD2 gene.

Across all subjects, genotyping showed 4 A1A1 (2%), 67 A1A2 (33.2%), and 131 A2A2 (64.8%) genotype carriers. The minor allele frequency of the A1 allele was 18.6% and allele frequencies were in Hardy–Weinberg equilibrium ($P = 0.17$). Of the 202 subjects, 135 (66.8%) completed the program and 67 (33.2%) terminated prematurely. The A1 allele did not influence the dropout rate of the program (A1$^+$ versus A1$^-$, $P = 0.44$). In accordance with the per-protocol analysis, only completers of the program were included in further analyses (Table 1).

The influence of the single nucleotide polymorphism rs1800497 on BMI was analyzed (Fig. 1). Based on previous findings in young adults, the group was separated according to age and gender [6]. Although no difference between A1$^+$ and A1$^-$ was observed for the groups of all completers (men and women), the group of younger completers (21–40 y old) showed significant differences in the BMI at T0 (A1$^+$, 43.6 ± 1.4 kg/m^2; A1$^-$, 40.4 ± 1.2 kg/m^2; $P = 0.039$), at T1 (A1$^+$, 36.9 ± 1.3 kg/m^2; A1$^-$, 33.8 ± 1.2 kg/m^2; $P = 0.045$), and at T2 (A1$^+$, 38.7 ± 1.8 kg/m^2; A1$^-$, 33.4 ± 1.1 kg/m^2; $P = 0.015$; Fig. 1D). However, when younger completers were separated according to gender, this effect was almost lost because of the smaller sample (Fig. 1E,F). No effect was observed for the group of older completers (41–60 y old; Fig. 1G–I).