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Effects of C358A missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase on weight loss after a hypocaloric diet

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Abstract

The Pro129THr, C385A, polymorphism of FAAH gene (rs324420C>A) has been associated with overweight and obesity. We investigate the role of this polymorphism on anthropometric and metabolic responses to a weight loss program. Obese individuals (n = 122) were assessed at baseline and after 3 months of a hypocaloric diet. There were 76.2% (n = 93) homozygotes for the C allele, 23.8% (n = 27) AC heterozygotes, and 1.6% (n = 2) homozygotes for the A allele. After the dietary intervention, all individuals decreased their body weight (in kilograms), body mass index (in kilograms per square meter), fat mass (in kilograms), waist circumference (in centimeters), and systolic blood pressure (in millimeters of mercury). In mutant-type group, the decrease in weight was 3.5 ± 3.6 kg (decrease in wild-type group, 2.4 ± 3.8 kg); and the decrease in waist circumference was 5.4 ± 6.4 cm (decrease in wild-type group, 2.6 ± 4.8 cm). Individuals with the A/C or AA genotype had a significant reduction ($P < .05$) in glucose (96.5 ± 12.5 vs 92.3 ± 10.5 mg/dL; difference, 2.68 ± 1.81 mg/dL), total cholesterol (215.3 ± 49 vs 193.3 ± 27.6 mg/dL; difference, 14.31 ± 7.21 mg/dL), and low-density lipoprotein cholesterol (133.6 ± 53 vs 106.7 ± 39.2 mg/dL; difference, 15.87 ± 9.61 mg/dL) levels. The A allele at rs324420 in the FAAH gene was associated with larger improvements in glucose, total cholesterol, low-density lipoprotein cholesterol, body mass, and waist circumference after a dietary intervention.

1. Introduction

According to recent surveys, overweight incidence is a growing worldwide problem [1]. Weight reduction is known to be an effective treatment for overweight-obese patients with risk factors of metabolic syndrome and adipocytokines [2].

Herbal *Cannabis sativa* (marijuana) has been known to have many psychoactive effects in humans including increases in appetite and body weight [3]. The main inactivating enzyme of endogenous cannabinoid receptor ligands is fatty acid amide hydrolase (FAAH), which has been identified as the catabolic enzyme capable of inactivating most of the endocannabinoids [4-6]. The pharmacologic effects of some endocannabinoids appear to be regulated by FAAH activity [7], suggesting that FAAH has the role to be a modulating enzyme for human behavior.

A missense polymorphism (cDNA 385 C->A) that predicts a substitution of threonine for a conserved proline residue at amino acid position 129 (P129T) (rs324420) has been described and is significantly associated with drug abuse [8]. Recently, some authors [9,10] have demonstrated that the homozygous FAAH 385 A/A genotype was associated with overweight and obesity. However, in a large study sample (5801 subjects), Jensen et al [11] were unable to find association of this polymorphism with overweight or obesity. There is only one interventional study (Aberle et al [12]) that has shown that carriers of the Pro129Thr mutation had a significantly greater improvement in lipid profile compared with wild type when following a low-fat diet. Considering the evidence that endogenous cannabinoid system plays a role in metabolic aspects of body

DA de Luis designed the study and wrote the article. R Aller recruited patients and made dietary evaluation. M Gonzalez Sagrado performed laboratory test. R Conde performed laboratory test. O Izaola recruited patients and made dietary evaluation.

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weight and feeding behavior, we investigate the role of this polymorphism on anthropometric and metabolic responses to a weight loss program.

2. Subjects and methods

2.1. Subjects

A population of 122 patients with obesity (body mass index [BMI] >30) was analyzed in a prospective way. These patients were recruited in a Nutrition Clinic Unit and signed an informed consent. Exclusion criteria included history of cardiovascular disease or stroke during the previous 24 months, total cholesterol greater than 300 mg/dL, triglycerides greater than 400 mg/dL, blood pressure greater than 140/90 mm Hg, fasting plasma glucose greater than 10 mg/dL, as well as the use of sulfonylurea, thiazolidinediones, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and psychoactive medications. The local ethical committee approved the protocol, and the patients approved the use of their genetic material for this study.

2.2. Procedure

All patients with a 2-week weight stabilization period before recruitment were enrolled. The lifestyle modification program consisted of a hypocaloric diet (1520 kcal; 52% of carbohydrates, 25% of lipids, and 23% of proteins). The exercise program consisted of aerobic exercise for at least 4 times per week (60 minutes each). Weight, blood pressure, basal glucose, C-reactive protein (CRP), insulin, insulin resistance (homeostasis model assessment for insulin sensitivity [HOMA]), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides blood, and adipocytokines (leptin, adiponectin, resistin, tumor necrosis factor–α, and interleukin-6) levels were measured at basal time and at 3 months after treatment. A tetrapolar bioimpedance, an indirect calorimetry, and a prospective serial assessment of nutritional intake with 3-day written food records were realized at both times. Genotype of FAAH gene polymorphism was studied.

2.3. Genotyping of FAAH gene polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft, Los Angeles, CA). The polymerase chain reaction was carried out with 50 ng of genomic DNA, 0.5 μL of each oligonucleotide primer (primer forward: 5′-ATG TTG CTG GTT ACC CCT CCT C-3′; primer reverse: 5′-CAG GGA CGC CAT AGA GCT G-3′), and 0.25 μL of each probe (wild probe: 5′-Fam-CTG TCT CAG GCC CCA AGG CAG G-BHQ-1-3′ and mutant probe: 5′-Hex-CTG TCT CAG GCC *A*CA AGG CAG G -BHQ-1-3′) in a 25-μL final volume (Termociclador iCycler IQ; Bio-Rad, Hercules, CA). DNA was denaturated at 95°C for 3 minutes; this was followed by 50 cycles of denaturation at 95°C for 15 seconds and annealing at 59.3° for 45 seconds. The polymerase chain reaction was run in a 25-μL final volume containing 12.5 μL of IQTM Supermix (Bio-Rad) with Hot Start Taq DNA polymerase. Hardy-Weinberg equilibrium was assessed.

2.4. Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay (RIA Diagnostic, Los Angeles, CA) with a sensitivity of 0.5 mUI/L (reference range, 0.5-30 mUI/L) [13], and the HOMA was calculated using these values [14]. C-reactive protein was measured by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany) with a reference range of 0 to 7 mg/dL and analytical sensitivity of 0.5 mg/dL. Lipoprotein (a) was determined by immunonephelometry with the aid of a Beckman array analyzer (Beckman Instruments).

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, New York, NY), whereas HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. Low-density lipoprotein cholesterol was calculated using the Friedewald formula.

2.5. Indirect calorimetry

For the measurement of resting energy expenditure, subjects were admitted to a metabolic ward. After a 12-hour overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20-minute period with an open-circuit indirect calorimetry system (standardized for temperature, pressure, and moisture) fitted with a face mask (MedGem; Health Tech, Golden, CO), with a coefficient of variation of 5%. Resting metabolic rate (in kilocalories per day) and oxygen consumption (in milliliters per minute) were calculated [15].

2.6. Anthropometric measurements

Body weight was measured to an accuracy of 0.1 kg, and BMI was computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist to hip ratio were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 50 g [16,17]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Model 310e; Biodynamics, Seattle, WA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat, and fat-free mass.

Blood pressure was measured twice after a 10-minute rest with a random zero mercury sphygmomanometer and a large cuff size and was averaged.

2.7. Dietary intake and habits

Patients received prospective serial assessment of nutritional intake with 3-day written food records. All enrolled subjects received instruction to record their daily dietary intake for 3 days including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference [17]. Regular aerobic physical activity (walking was allowed; no other exercises) was maintained during the period study for at least 4 times per week (60 minutes each).

2.8. Statistical analysis

Sample size was calculated to detect differences greater than 3 kg in body weight with 90% power and 5% significance (n = 90). The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables were analyzed with a 2-way analysis of variance (ANOVA) model with genotype as the intergroup factor and intervention as the intragroup intervention. Qualitative variables were analyzed with the χ^2 test, with Yates correction as necessary, and Fisher test. The statistical analysis was performed for the combined C385A and A385A as a group and wild-type C385C as second group (dominant model). A P value < .05 was considered statistically significant.

3. Results

One hundred twenty-two patients gave informed consent and were enrolled in the study. The mean age was 45.9 ± 14.3 years and the mean BMI was 36.1 ± 6.2, with 33 men (27%) and 89 women (73%).

All subjects were weight stable during the 2-week period preceding the study (body weight change, 0.26 ± 0.1 kg). All patients completed the 3-month follow-up period. Ninety-three patients (76.2%) had the genotype *C358C* (wild group), and 29 (23.8%) patients had the genotype *C358A* (27 patients, 22.2%) or *A358A* (2 patients, 1.6%) (mutant group). Age was similar in both groups (wild type, 45.5 ± 16.8 years vs mutant group, 46.6 ± 16.0 years; not significant). Sex distribution was similar in both groups: men (25.8% vs 31.0%) and women (74.2% vs 69.0%).

Table 1 shows the differences in anthropometric variables. In wild- and mutant-type groups, weight, BMI, fat mass, waist circumference, and systolic blood pressure decreased. After dietary treatment and in mutant-type group, weight and waist circumference were lower than wild-type group. In mutant-type group, the decrease in weight was 3.5 ± 3.6 kg (decrease in wild-type group, 2.4 ± 3.8 kg); and in waist circumference, the decrease was 5.4 ± 6.4 cm (decrease in wild-type group, 2.6 ± 4.8 cm). Both decreases were higher in mutant-type group than wild-type group. No differences were detected among other variables.

Table 2 shows the classic cardiovascular risk factors. In mutant-type group, glucose, total cholesterol, and LDL cholesterol levels decreased significantly. In mutant-type group and after dietary treatment, insulin, HOMA, CRP, and triglyceride levels were lower than wild-type group values. In mutant-type group, the decreases were as follows: insulin, 0.84 ± 3.8 mUI/L (increase in wild-type group, 0.33 ± 7.3 mUI/L); HOMA, 0.31 ± 0.88 (increase in wild-type group, 0.02 ± 2.3); CRP, 0.5 ± 2.3 (decrease in wild-type group, 1.6 ± 5.3); and triglyceride, 15.7 ± 46.3 mg/dL (increase in wild-type group, 9.38 ± 35.3). All changes were higher in mutant-type group than wild-type group. In the mutant-type group and before dietary treatment, insulin, HOMA and CRP levels were lower than wild-type group values.

Table 3 shows nutritional intake with 3-day written food records, with a significant decrease in the total calorie intake and a significant increase in the exercise time. No statistical

Table 1
Changes in anthropometric variables

Characteristics	C385C		C385A or A385A	
	0 time	At 3 mo	0 time	At 3 mo
BMI	34.5 ± 4.9	33.5 ± 4.8*	33.9 ± 5.1	32.5 ± 5.9*
Weight (kg)	90.0 ± 17.3	87.6 ± 16.4*	87.3 ± 15.4	82.6 ± 15.5*,†
Fat-free mass (kg)	37.1 ± 11.8	37.0 ± 13.1	37.6 ± 11.7	36.3 ± 10.7
Fat mass (kg)	49.2 ± 13.1	47.5 ± 13.1*	48.7 ± 11.7	46.1 ± 11.8*
Waist circumference	107.6 ± 15	105.2 ± 15*	107.1 ± 14	101.7 ± 19*,†
Waist to hip ratio	0.93 ± 0.1	0.91 ± 0.09	0.92 ± 0.1	0.89 ± 0.09
Systolic BP (mm Hg)	134.1 ± 17.3	127.1 ± 12.4*	130.4 ± 12	120.8 ± 10.7*
Diastolic BP (mm Hg)	79.9 ± 10.4	82.5 ± 16.7	78.2 ± 10.2	77.3 ± 7.1
RMR (kcal/d)	1867 ± 419	1913 ± 470	1725 ± 474	1740 ± 422

Data presentation: mean ± standard deviation. BP indicates blood pressure; RMR, resting metabolic rate.
Two-way ANOVA model: *significantly different compared with baseline; †significantly different between genotypes, $P < .05$, in each group with basal values; †$P < .05$, between different genotypes.

Table 2
Classic cardiovascular risk factors

Characteristics	C385C		C385A or A385A	
	0 time	At 3 mo	0 time	At 3 mo
Glucose (mg/dL)	101.7 ± 20.2	99.0 ± 16.6	96.5 ± 12.5	92.3 ± 10.5*
Total ch (mg/dL)	211.8 ± 43.5	208.7 ± 37.7	215.3 ± 49	193.3 ± 27.6*
LDL ch (mg/dL)	123.5 ± 55	118.9 ± 76	133.6 ± 53	106.7 ± 39.2*
HDL ch (mg/dL)	55.6 ± 14.3	55.4 ± 15.8	55.2 ± 12.7	53.7 ± 11.3
TG (mg/dL)	121.8 ± 47	131.5 ± 82	113.3 ± 44	97.6 ± 39.4†,‡
Lp (a) (mg/dL)	33.6 ± 36.2	34.8 ± 37.1	28.8 ± 18	22.9 ± 23
Insulin (mUI/L)	14.8 ± 7.1	15.2 ± 9.5	10.3 ± 6.5†	9.4 ± 6.2†,‡
HOMA	3.93 ± 2.4	3.96 ± 2.6	2.4 ± 1.7†	2.1 ± 1.5†,‡
CRP (mg/dL)	7.5 ± 9.3	5.8 ± 7.4	4.4 ± 3.5†	3.9 ± 3.7†,‡

Data presentation: mean ± standard deviation. Ch indicates cholesterol; Lp (a), lipoprotein (a); TG, triglycerides.
Two-way ANOVA model: *significantly different compared with baseline, †significantly different between genotypes, and ‡significant interaction between genotype and intervention ($P < .05$).

differences were detected in calorie, carbohydrate, fat, and protein intakes between both genotypes. Aerobic exercise per week was similar in both groups.

4. Discussion

A common C358A single nucleotide polymorphism of the FAAH results in a mutation producing a FAAH with defective expression [18,19]. Studies of the C358A variant and obesity from various authors have yielded conflicting results [8-11].

In our design, we investigated the effect of FAAH genetic variation on weight and also on metabolic parameters after a hypocaloric diet and exercise. The novel finding of this study is the association of the allele A358 of FAAH with higher decreases of weight and waist circumference than mutant-type group. This better anthropometric improvement was associated with a significant decrease in glucose, total cholesterol, and LDL cholesterol levels. Before and after treatment, patients with genotypes C358A and A358A of FAAH had lower HOMA, insulin, and CRP levels than patients with C358C genotype.

The lack of association between this polymorphism and anthropometric parameters has been described by other authors. The results of our study agree with those of Jensen et al [11] or Papaglou et al [19] and contrast with those of Sipe et al [9]. It is noteworthy that when BMI was evaluated as a continuous variable in subjects, the median BMI was significantly higher in subjects with the A385A genotype compared with the median BMI of the other subjects [9]. The mesolimbic addition and reward/craving circuit including the medial forebrain bundle projections to the *nucleus accumbens* shows a high correlation of FAAH enzyme expression and CB1 receptor density [20,21]. However, the inconsistencies between association studies may reflect the complex interactions between multiple population-specific genetic and environmental factors. Perhaps, these different results could be explained by dietary intakes of subjects in previous studies of the literature. These previous studies would require composition analysis of the diet to determine whether dietary components could be responsible for the anthropometric and biochemical variations. In our study, dietary intake was controlled; and it was an interventional variable with different response in both genotype groups.

Some evidence indicates that the endogenous cannabinoid system is an essential homeostatic regulator of energy balance and weight via central appetite-stimulating mechanisms as well as peripheral lipogenesis [21,22]. The anatomical convergence of CB1 receptors and FAAH enzyme activity capable of regulating endocannabinoid tone supports the notion that FAAH is positioned for regulation of endocannabinoid levels that could influence craving and reward behaviors through the relevant neuronal circuitry.

An association between this polymorphism and metabolic profile has been described. Interestingly, Aberle et al [12] have shown that carriers of the A allele had a significantly greater decrease in the total cholesterol and triglycerides as compared with wild type when following a low-fat diet. Our results show a significant decrease in glucose, total cholesterol, and LDL cholesterol levels in mutant-type group after dietary treatment. According to other studies [9], the A allele FAAH encodes a functionally deficient protein, expressing about half the enzymatic activity as the wild type. Because FAAH is the major anandamide degrading protein, a functionally less active protein may lead to an increased hepatic and central endocannabinoid

Table 3
Dietary intake

Characteristics	C385C		C385A or A385A	
	0 time	At 3 mo	0 time	At 3 mo
Energy (kcal/d)	1753 ± 478	1556 ± 379*	1746 ± 344	1588 ± 775*
CH (g/d)	168.2 ± 71	166.1 ± 53	174.3 ± 51	155.7 ± 78
Fat (g/d)	70.3 ± 23.3	63.7 ± 18.7	79.8 ± 21	76.1 ± 48
Protein (g/d)	83.6 ± 23	79.3 ± 21	85 ± 20	79.8 ± 21.3
Exercise (h/wk)	0.79 ± 1.5	2.5 ± 2.7*	0.85 ± 2.3	2.7 ± 2.7*

Data presentation: mean ± standard deviation. CH indicates carbohydrate.
Two-way ANOVA model: *significantly different compared with baseline ($P < .05$).

concentration resulting in an up-regulation of energy storage and to a rise of triglyceride storage. Under a hypocaloric diet, this induction decreases in a higher way than patients with wild-type genotype. In a state of a genetically overactivated endocannabinoid system, the down-regulation of hepatic endocannabinoid tone would be above average and thereby could result in the observed improvement in lipid profile.

In our study, the lower levels of CRP, insulin, and HOMA in mutant-type group could be related with a decreased postprandial response of fatty acids absorption (not measured in our design). Decreased free fatty acids decrease the accumulation of triglycerides in the adipocyte, and it is related with imbalance of lipoprotein lipase activity and underproduction of proinflammatory markers. The lack of association of this anti-inflammatory state with fat mass or BMI in mutant-type group could indicate the existence of complex unmeasured gene-gene or gene-environment interactions that may enhance metabolic abnormalities in obese patients.

In conclusion, the allele A358 of FAAH was associated with higher decreases of weight and waist circumference than mutant-type group. This better anthropometric improvement was associated with a significant decrease in glucose, total cholesterol, and LDL cholesterol levels. Further investigations are needed to clarify these results; metabolic modifications secondary to weight loss after altering dietary advice in obese patients could be influenced by this polymorphism.

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