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Association of G1359A polymorphism of the cannabinoid receptor gene (CNR1) with macronutrient intakes in obese females

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Abstract

Background: The endogenous cannabinoid system plays a role in metabolic aspects of body weight and feeding behaviour. A polymorphism (1359 G/A) (rs1049353) of the CB1 gene was reported as a common polymorphism in the Caucasian population. The present study aimed to investigate the association of the polymorphism (G1359A) of the CB1 receptor gene on macronutrient intake in females with obesity.
Methods: A sample of 896 females was analysed. A bioimpedance measurement, a blood pressure measurement, a serial assessment of nutritional intake with 3 days of written food records, and a biochemical analysis were all performed. The genotype of the CNR1 receptor gene polymorphism (rs1049353) was studied.
Results: Five hundred and sixteen patients (57.6%) had the genotype G1359G (non-A carriers) and 380 (42.4%) patients had G1359A (328 patients, 36.6%) or A1359A (52 patients, 5.8%) (A carriers). Triglycerides and high-density lipoprotein (HDL) cholesterol levels were higher in A non-A allele carriers than non-A allele carriers. The intakes of dietary cholesterol and saturated fat for the upper tertile (T3) compared to the baseline tertile were inversely associated with the CB1-R 1359 G/A polymorphism [odds ratio (OR) = 0.59; 95% confidence interval (CI) = 0.30–0.92 and OR = 0.66; 95% CI = 0.39–0.91, respectively]. These data were observed in the second tertile (T2) (OR = 0.61; 95% CI = 0.29–0.94 and OR = 0.58; 95% CI = 0.31–0.90, respectively).
Conclusions: The present study reports an association of the A allele with a better lipid profile (triglycerides and HDL cholesterol) than non-A allele carriers. In addition, this polymorphism is associated with a specific macronutrient intake, as well as with low cholesterol and fat saturated intakes.

Introduction

The important role played by the endocannabinoid system is emerging: it controls food intake, energy balance, and lipid and glucose metabolism through both central and peripheral effects, and also stimulates lipogenesis and fat accumulation. Herbal *Cannabis sativa* (marijuana) is known to have many psychoactive effects in humans, including robust increases in appetite and body weight[1]. Nevertheless, the mechanisms underlying cannabinoid neurobiological effects were recently revealed[2]. The endogenous cannabinoid system mediates and is positioned both functionally and anatomically [3] to be an important modulator of normal human brain behaviour. This system consists of endogenous ligands 2-arachidonoylglycerol and anandamide and two types of

G-protein-coupled cannabinoid receptors: cannabinoid type-1 receptor (CB1), located in several brain areas and in a variety of peripheral tissues including adipose tissue, and CB2, present in the immune system[4]. A greater insight into the endocannabinoid system has been derived from studies in animals with a genetic deletion of the CB1 receptor, that have a lean phenotype and are resistant to diet-induced obesity and the associated insulin resistance induced by a high palatable high-fat diet[5]. A intragenic polymorphism (1359 G/A) (rs1049353) of the CB1 gene resulting in the substitution of the G to A at nucleotide position 1359 in codon 435 (Thr) was reported as a common polymorphism in the Caucasian population[6–8]. In Spain, frequencies were 42.4% in morbid obese patients[6], 42.7%[7] in diabetic patients and 45.4% [8] in obese female Caucasian subjects. Recently, in an unselected elderly population, this polymorphism has been linked with a specific macronutrient intake[9]. This is an interesting study and it is the first in the literature to analyse this relationship in a small sample of subjects (n = 118).

Considering the evidence that the endogenous cannabinoid system plays a role in metabolic aspects of body weight and feeding behaviour[4], and the few studies [9] that have been designed in this area, we decided to investigate the association of this CB1 receptor polymorphism with dietary intake. The present study aimed to investigate the association of the polymorphism (G1359A) of the CB1 receptor gene on macronutrient intake in females with obesity.

Materials and methods

Subjects

A sample of 896 obese females [body mass index (BMI) >30 kg m^{-2}] nondiabetic outpatients was analysed in a prospective way. These patients were recruited in a Nutrition Clinic Unit in Valladolid (Spain) and provided their written informed consent. Exclusion criteria included a history of cardiovascular disease or stroke during the previous 36 months, total cholesterol >300 mg dL^{-1}, triglycerides >400 mg dL^{-1}, blood pressure >140/90 mmHg, fasting plasma glucose >110 mg dL^{-1}, as well as the use of sulphonilurea, thiazolidinedions, insulin, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors and psychoactive medications. A local ethical committee approved the protocol.

Procedures

Basal glucose, insulin, insulin resistance (Homeostatic Model Assessment; HOMA), total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and blood triglycerides were measured. Weight, BMI, waist circumference, fat mass and blood pressure were measured. A prospective serial assessment of nutritional intake with 3 days of written food records was carried out. The genotype of the CNR1 receptor gene polymorphism was studied.

Genotyping of the CNR1 gene polymorphism

Oligonucleotide primers and probes were designed with BEACON DESIGNER, version 4.0 (Premier Biosoft International, Los Angeles, CA, USA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 μL of each oligonucleotide primer (forward: 5′-TTC ACA GGG CCG CAG AAA G-3′; reverse 5′-GAG GCA TCA GGC TCA CAG AG-3′) and 0.25 μL of each probe (wild probe: 5′-Fam-ATC AAG AGC ACG GTC AAG ATT GCC-BHQ-1-3′; mutant probe: 5′-Texas red-ATC AAG AGC ACA GTC AAG ATT GCC -BHQ-1-3′) in a 25-μL final volume (Termocycler iCycler IQ (Bio-Rad, Hercules, CA, USA). DNA was denaturated at 95 °C for 3 min, followed by 50 cycles of denaturation at 95 °C for 15 s and annealing at 59.3 °C for 45 s). PCRs were 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.

Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2; Beckman Instruments, Fullerton, CA, USA). Insulin was measured by radioimmunoassay (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI L^{-1} (normal range 0.5–30 mUI L^{-1})[10] and the HOMA for insulin sensitivity was calculated using these values.[11]

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd, New York, NY, USA), whereas HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL-cholesterol was calculated using the Friedewald formula [LDL-cholesterol = total cholesterol – HDL-cholesterol − (trygliceride/5)].

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 were also measured to derive the waist-to hip ratio (WHR). Tetrapolar body electrical bioimpedance was used to determine body composition to an accuracy of 5 g[12]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Model 310e; Biodynamics Corp., Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on the 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-min rest with a random zero mercury sphygmomanometer and averaged.

Dietary intake and habits

Patients received prospective serial assessment of nutritional intake with 3 days of written food records. All subjects who were enrolled received instructions to record their daily dietary intake for 3 days, including a weekend day. Dietary data were handled via a personal computer, incorporating the use of food scales and models to enhance the accuracy of portion sizes. Records were reviewed by a dietitian and analysed with a computer-based data evaluation system (Dietosource, Geneve, SWIS). National composition food tables were used as a reference[13].

Statistical analysis

The results were expressed as the mean (SD). The distribution of variables was analysed with the Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analysed with a two-tailed Student's *t*-test. Nonparametric variables were analysed with the Mann–Whitney *U*-test. Qualitative variables were analysed with the chi-squared test, with Yates correction as necessary, and Fisher's test. Multiple logistic regression was used to investigate the association between the genotypes and the macronutrient intake, corrected by calories and controlling for age and BMI. The amount of macronutrient was divided into tertiles of intake, with the lowest tertile considered as baseline towards the upper tertiles. The statistical analysis was performed for the combined G1359A and A1359A as a group and wild-type G1359G as second group, with a dominant model. $P < 0.05$ was considered statistically significant.

Results

Eight hundred and ninety-six females provided their informed consent and were enrolled in the present study. The mean (SD) age was 45.8 (11.3) years and the mean BMI (SD) was 36.9 (6.1) kg m^{-2}.

Five hundred and sixteen patients (57.6%) had the genotype G1359G (non-A carriers) and 380 (42.4%) patients were G1359A (328 patients, 36.6%) or A1359A (52 patients, 5.8%) (A carriers). Age was similar in both groups [non-A-carriers: 46.4 (15.6) years versus A-carriers: 46.8 (11.8) years: not significant]. Table 1 shows the anthropometric variables. No differences were detected between groups.

Table 1 shows the classic cardiovascular risk factors. Triglycerides values were lower in non-A carriers than A-carriers [120.3 (48.9) mg dL^{-1} versus 106.3 (43.8) mg dL^{-1}, $P = 0.004$]. HDL cholesterol levels were higher in A-carriers than non-A carriers [55.0 (17.1) mg dL^{-1} versus 56.3 (10.9) mg dL^{-1}; $P = 0.003$].

Table 2 shows nutritional intake with 3 days of written food records. Cholesterol and saturated fat intakes were lower in A carriers than non-A carriers. No statistical differences were detected in other dietary intakes.

Table 3 shows the odds ratio (OR) and 95% confidence interval (CI) of the OR for the allele A variant for tertiles of each dietary variable compared to the lowest tertile, corrected for calories and controlling for age and BMI. There were no differences in proteins, lipids, carbohydrates, fibre, and monounsaturated and polyunsaturated fats. The intake of dietary cholesterol for the upper tertile (T3) compared to the baseline tertile was inversely associated with the CB1-R 1359 G/A polymorphism (OR = 0.59; 95% CI = 0.30–0.92) and this was also the case in the second tertile (T2) (OR = 0.61; 95% CI = 0.29–0.94).

Table 1 Anthropometric and biochemical variables

Characteristics	G1359G ($n = 454$)	(G1359A or A1359A) ($n = 342$)
BMI	37.1 (6.6)	36.7 (6.6)
Weight (kg)	92.5 (17.2)	91.1 (16.8)
Fat mass (kg)	42.9 (12.9)	42.1 (11.9)
WC (cm)	109.8 (14.1)	109.1 (13.5)
Waist to hip ratio	0.90 (0.1)	0.89 (0.09)
Systolic BP (mmHg)	137.1 (12.1)	137.8 (13.5)
Diastolic BP (mmHg)	84.5 (11.9)	82.8 (13.4)
Glucose (mg dL^{-1})	99.6 (19.5)	99.9 (17.3)
Total cholesterol (mg dL^{-1})	202.8 (39.1)	206.2 (41.3)
LDL-cholesterol (mg dL^{-1})	122.1 (37.9)	127.4 (45.2)
HDL-cholesterol (mg dL^{-1})	55.0 (17.1)	56.3 (10.9)*
TG (mg dL^{-1})	120.3 (48.9)	106.3 (43.8)*
Insulin (mUI L^{-1})	13.7 (8.4)	13.1 (6.6)
HOMA	3.40 (2.3)	3.14 (1.9)

*$P < 0.05$, in each group with basal values.
No statistical differences between groups.
BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; LDL, low-density lipoprotein; TG, triglycerides; WC, waist circumference. Data are the mean (SD).

Table 2 Dietary intake

Characteristics	G1359G (n = 454)	(G1359A or A1359A) (n = 342)
Energy (kcal day^{-1})	1849.3 (589)	1708.6 (358)
CH (g day^{-1})	193.1 (72.1)	187.5 (68.9)
Fat (g day^{-1})	79.6 (34.9)	73.5 (33.2)
S-fat (g day^{-1})	21.3 (11.1)	13.5 (10.5)*
M-fat (g day^{-1})	35.9 (15.3)	34.7 (15.5)
P-fat (g day^{-1})	9.3 (6.1)	8.5 (5.4)
Protein (g day^{-1})	85.6 (24.1)	84.7 (22.1)
Cholesterol (mg day^{-1})	387.9 (200.1)	332.8 (178.6)*
Dietary fibre	14.37 (5.5)	14.24 (3.8)

*$P < 0.05$, in each group with basal values.
CH, carbohydrate; M-fat, monounsaturated fat; P-fat, polyunsaturated fat; S-fat, saturated fat. Data are the mean (SD).

The intake of saturated fat for the upper tertile (T3) compared to the baseline tertile was inversely associated with the CB1-R 1359 G/A polymorphism (OR = 0.66; 95% CI = 0.39–0.91) and this was also the case in the second tertile (OR = 0.58; 95% CI = 0.31–0.90). The test for trend was significant for dietary cholesterol ($P = 0.02$) and saturated fat ($P = 0.02$).

Discussion

The present study reports an association of the G1359A and A1359A CNR1 genotypes with lower levels of triglycerides and higher levels of HDL-cholesterol. We show that the 1359 G/A CB1-R polymorphism was associated with a specific macronutrient intake. The intake of cholesterol and saturated fats was inversely associated with this polymorphism.

We do not know how the G1359A or A1359A polymorphism may exert an influence on lipid profile without changes in BMI. However, the literature supports the notion that the endocannabinoid system is positioned for regulation of endocannabinoid levels that could influence craving and reward behaviours through the relevant neuronal circuitry and metabolic parameters[14]. Also, the CB1 receptor is expressed in some peripheral human tissues studied in relation to the pathogenesis of obesity and obesity-associated metabolic disorders and a marked down-regulation of the fatty acid amide hydrolase gene expression was found in the adipose tissue of obese women, suggesting that adipose tissue may be an important contributor to endocannabinoid inactivation[15]. In the present study, triglyceride levels were higher in obese patients carrying the wild-type CB1 allele than in A allele carriers as shown by Gazzerro *et al.*[16]. This metabolic relationship between the polymorphism and lipid profile has been detected by Alberle *et al.*[17], where it was shown that carriers of at least one A allele in CNR1 lost more weight and showed reduced LDL cholesterol compared to wild-type patients. Recently, another study [18] has demonstrated that genetic

Table 3 Odds ratio (OR) and 95% confidence interval (CI) of AG+AA/GG in tertiles of each dietary variable (upper tertile compared to the lowest tertile) corrected by calories and controlling by age and body mass index, using a conditional multiple logistic regression dietary intake

Dietary variables	Tertiles*	OR	95% CI	*P*-value†	*P*-value§
CH (g day^{-1})	T2	0.78	0.49–1.25	0.48	0.32
	T3	0.83	0.51–1.35	0.51	
Fat (g day^{-1})	T2	1.01	0.55–1.46	0.10	0.39
	T3	0.74	0.44–1.22	0.09	
S-fat (g day^{-1})	T2	0.66	0.39–0.91	0.01	0.02
	T3	0.58	0.31–0.90	0.02	
M-fat (g day^{-1})	T2	0.71	0.44–1.15	0.39	0.19
	T3	0.70	0.41–1.21	0.41	
P-fat (g day^{-1})	T2	0.79	0.48–1.30	0.45	0.72
	T3	0.83	0.47–1.46	0.46	
Protein (g day^{-1})	T2	0.92	0.62–1.14	0.56	0.43
	T3	0.86	0.52–1.44	0.54	
Cholesterol (mg day^{-1})	T2	0.61	0.29–0.94	0.03	0.03
	T3	0.59	0.30–0.92	0.01	
Dietary fibre	T2	1.1	0.65–1.74	0.69	0.69
	T3	0.87	0.52–1.44	0.71	

CH, carbohydrate; M-fat, monounsaturated fat; P-fat, polyunsaturated fat; S-fat, saturated fat.
No statistical differences (AG+AA/GG). Data are the mean (SD). The statistical analysis was performed for the combined G1359A and A1359A as a group and wild-type G1359G as second group, with a dominant model.
*t1 is the reference category;
†p value for association;
§p value for linear trend.

variation in CB1 is associated with derangements in lipid homeostasis, independent of BMI. Perhaps a direct role of the endocannabinoid system in lipid levels could be hypothesised independently of BMI.

In the present study, obese females with the polymorphic variant followed a diet with less saturated fats and dietary cholesterol compared to the diet of homozygous subjects. This finding showed a difference in the choice of foods. Evidence suggests that cannabinoids increase the drive to eat, specifically by enhancing the reward or hedonic properties of food. In the central nervous system, CB1 receptors are expressed particularly in areas such as the hippocampus and entopeduncular nucleus, which are either directly involved in the hedonic aspects of eating and are connected to reward-related brain areas[19]. Some studies [20] have shown that a hedonic stimulus can release anandamide in the limbic forebrain. Two previous studies reported an association in humans between this polymorphism and macronutrient intake. Caruso *et al.* [9] demonstrated that the intake of cholesterol and saturated fat was inversely associated with this polymorphism, in agreement with the results of the present study. Bienertova *et al.* [21] showed that allelic variations and common haplotypes in the CNR1 gene were associated with the daily intake of proteins and fibre. These findings show that genotyping for CB1-R is linked to differences in the choice of foods. It suggests that cannabinoids increase the drive to eat specifically by enhancing the reward of hedonic properties of food. A study in rats has observed that increased activity of the cannabinoid system determines the appetite for palatable food[22]. Moreover, consistent with the ability of delta-9-tetrahydrocannabinol (THC) to increase sucrose palatability is the observation that, under THC pretreatment, sucrose acquires the ability to induce the release of dopamine in the shell of the nucleus accumbens and this property undergoes adaptation after repeated exposure to taste habituation[3]. It is possible that the endogenous cannabinoids generated following ingestion of relatively triglyceride-rich palatable food could over-ride local satiety pathways by binding to gut cannabinoid receptors[23].

A limitation of the present study is the self-reported dietary intake. Recalled data inevitably have a large measurement error. However, we assume that the recall bias was similar for both genotypes and did not affect the study outcome. Future research with different measures are needed to explain these findings.

In conclusion, the present study reports an association of the A allele with a better lipid profile (triglyceride and HDL cholesterol) than non-A allele carriers. In addition, this polymorphism is associated with a specific macronutrient intake, as well as with low cholesterol and fat saturated intakes.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interests.

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DADL wrote the article. OI, RA, JJL and BT carried out the nutritional evaluation. GD and EG performed the biochemical evaluations. ER conducted the statistical analysis.

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