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Influence of G1359A polymorphism of the cannabinoid receptor gene on anthropometric parameters and insulin resistance in women with obesity

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Abstract

A silent polymorphism (1359 G/A) of the cannabinoid receptor gene was reported as a common polymorphism in white populations. The aim of our study was to investigate the influence of this polymorphism (G1359A) of cannabinoid receptor gene on obesity, insulin resistance, and adipocytokines in women with obesity. A population of 290 women was analyzed. Indirect calorimetry, tetrapolar electrical bioimpedance, blood pressure measurement, serial assessment of nutritional intake with 3-day written food records, and biochemical analysis were performed. One hundred fifty-nine patients (54.8%) had the genotype G1359G (wild-type group), and 131 (45.2%) patients had G1359A (116 patients, 40.0%) or A1359A (15 patients, 5.2%) (mutant-type group). Triglycerides (122.3 ± 65.9 vs 107.2 ± 44.8 mg/dL, $P < .05$), insulin (15.8 ± 9.4 vs 13.6 ± 6.9 mUI/L, $P < .05$), and homeostasis model assessment values (3.85 ± 2.2 vs 3.33 ± 1.9, $P < .05$) were higher in the wild-type group than the mutant-type group. High-density lipoprotein cholesterol levels (56.8 ± 24.1 vs 58.3 ± 13.9 mg/dL, $P < .05$) were higher in the mutant-type group than the wild-type group. The novel finding of this study is the association of the mutant-type group G1359A and A1359A with a better cardiovascular profile (triglyceride, high-density lipoprotein cholesterol, insulin, and homeostasis model assessment levels) than the wild-type group.

1. Introduction

The current view of adipose tissue is that of an active secretor organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation, and immunity [1].

In this scenario, 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 stimulates lipogenesis and fat accumulation. Herbal *Cannabis sativa* (marijuana) has been known to have many psychoactive effects in humans including robust increases in appetite and body weight [2]. Nevertheless, the mechanisms underlying cannabinoid neurobiological effects have been recently revealed [3]. The endogenous cannabinoid system mediates and is positioned both functionally and anatomically [4] to be an important modulator of normal human brain behavior. This system consists of endogenous ligands 2-arachidonoylglycerol and anandamide and 2 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 cannabinoid type-2 receptor (CB2), present in the immune system [5]. A greater insight into the endocannabinoid system has been derived from studies in animals with a genetic deletion of the CB1 receptor, which have a lean phenotype and are resistant to diet-induced obesity and the associated insulin resistance induced by a highly palatable high-fat diet [6]. A silent intragenic biallelic 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 German population [7], reaching frequencies of 24% to 32% for the rarer allele (A).

Considering the evidence that endogenous cannabinoid system plays a role in metabolic aspects of body weight and feeding behavior [8], we decided to investigate the association of this CB1 receptor polymorphism with obesity and adipocytokines.

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The aim of our study was to investigate the influence of the missense polymorphism (G1359A) of CB1 receptor gene on obesity anthropometric parameters, insulin resistance, and adipocytokines in women with obesity.

2. Subjects and methods

2.1. Subjects

A population of 290 female obese (body mass index [BMI] ≥30) nondiabetic outpatients 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 36 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 110 mg/dL, as well as the use of sulfonylurea, thiazolidinediones, insulin, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and psychoactive medications. A local ethical committee approved the protocol.

2.2. Procedure

All patients with a 2-week weight stabilization period before recruitment were enrolled. Weight, blood pressure, basal glucose, C-reactive protein, insulin, insulin resistance (homeostasis model assessment [HOMA]), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, blood, and adipocytokines (leptin, adiponectin, resistin, tumor necrosis factor [TNF]–α, and interleukin [IL]-6) levels were measured at basal time. A tetrapolar bioimpedance, an indirect calorimetry, and a prospective serial assessment of nutritional intake with 3-day written food records were performed. Genotype of cannabinoid receptor gene (*CNR1*) polymorphism was studied.

2.2.1. Genotyping of CNR1 gene polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International, 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'-TTC ACA GGG CCG CAG AAA G-3' and 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' and mutant probe: 5'-Texas red-ATC AAG AGC ACA GTC AAG ATT GCC-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°C 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.

2.3. 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 (Diagnostic Products, Los Angeles, CA) with a sensitivity of 0.5mUI/L (reference range, 0.5-30 mUI/L) [8], and the HOMA for insulin sensitivity was calculated using these values [9]. C-reactive protein was measured by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany), with a reference range of 0 to 7 mg/dL and analytical sensitivity 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.

2.4. Adipocytokines

Resistin was measured by enzyme-linked immunosorbent assay (ELISA) (Biovendor Laboratory, Brno, Czech Republic) with a sensitivity of 0.2 ng/mL and a reference range of 4 to 12 ng/mL [10]. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Webster, TX) with a sensitivity of 0.05 ng/mL and a reference range of 10 to 100 ng/mL [11]. Adiponectin was measured by ELISA (R&D Systems, Minneapolis, MN) with a sensitivity of 0.246 ng/mL and a reference range of 8.65 to 21.43 ng/mL [12]. Interleukin-6 and TNF-α were measured by ELISA (R&D Systems) with a sensitivity of 0.7 and 0.5 pg/mL, respectively. Normal values of IL-6 were 1.12 to 12.5 pg/mL, and those of TNF-α were 0.5 to 15.6 pg/mL [13,14].

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 0.5 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 5 g [16]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, 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 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 a reference [17].

2.8. Statistical analysis

Sample size was calculated to detect differences over 2 kg in body weight with 90% power and 5% significance (n = 120, in each group). The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a 2-tailed Student *t* test. Nonparametric variables were analyzed with the Mann-Whitney *U* test. 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 G1359A and A1359A as a group and wild-type G1359G as the second group, with a dominant model. A P value < .05 was considered statistically significant.

3. Results

Two hundred ninety women gave informed consent and were enrolled in the study. The mean age was 45.1 ± 17.8 years, and the mean BMI was 36.1 ± 6.2. All subjects were weight stable during the 2-week period preceding the study (body weight change, 0.25 ± 0.2 kg).

One hundred fifty-nine patients (54.8%) had the genotype G1359G (wild-type group), and 131 (45.2%) patients had G1359A (116 patients, 40.0%) or A1359A (15 patients, 5.2%) (mutant-type group). Age was similar in both groups (wild-type group, 42.95 ± 16.6 years vs mutant group, 45.1 ± 16.8 years; without statistical differences). Table 1 shows the anthropometric variables. No differences were detected between groups.

Table 1
Anthropometric variables

Characteristics	G1359G (n = 159)	(G1359A or A1359A) (n = 131)
BMI	35.9 ± 6.9	35.4 ± 6.1
Weight (kg)	91.5 ± 18.2	89.6 ± 15.8
Fat-free mass (kg)	45.4 ± 6.8	45.4 ± 6.8
Fat mass (kg)	45.2 ± 14.1	43.4 ± 12.9
WC (cm)	106.8 ± 14.2	106.6 ± 14.5
Waist-to-hip ratio	0.88 ± 0.1	0.89 ± 0.09
Systolic BP (mm Hg)	128.8 ± 15.1	127.8 ± 18.5
Diastolic BP (mm Hg)	85.6 ± 10.9	82.2 ± 13.4
RMR (kcal/d)	2003 ± 617	2036 ± 603

No statistical differences between groups. WC indicates waist circumference; BP, blood pressure; RMR, resting metabolic rate.

Table 2 shows the classic cardiovascular risk factors. Triglycerides, insulin, and HOMA values were higher in the wild-type group than the mutant-type group. The HDL cholesterol levels were higher in the mutant-type group than the wild-type group.

Table 3 shows nutritional intake with 3-day written food records. No statistical differences were detected in caloric, carbohydrate, fat, and protein intakes. Aerobic exercise per week was similar in both groups.

Table 4 shows levels of adipocytokines. No differences were detected between both groups in serum adipocytokine levels.

4. Discussion

The finding of this study is the association of the G1359A and A1359A *CNR1* phenotypes with lower levels of triglycerides, insulin, and HOMA. Our study shows that HDL cholesterol levels were higher in the G1359A and A1359A CB1 phenotypes than the wild-type group.

We do not know how the G1359A or A1359A polymorphism may exert an influence on lipid profile and

Table 2
Classic cardiovascular risk factors

Characteristics	G1359G (n = 159)	(G1359A or A1359A) (n = 131)
Glucose (mg/dL)	98.8 ± 16.5	98.2 ± 19.3
Total Ch (mg/dL)	203.8 ± 37.1	206.5 ± 46.3
LDL Ch (mg/dL)	122.1 ± 37.9	127.4 ± 45.2
HDL Ch (mg/dL)	56.8 ± 24.1	58.3 ± 13.9*
TG (mg/dL)	122.3 ± 65.9	107.2 ± 44.8*
Insulin (mUI/L)	15.8 ± 9.4	13.6 ± 6.9*
HOMA	3.85 ± 2.2	3.33 ± 1.9*
CRP (mg/dL)	6.2 ± 6.4	6.9 ± 5.4
Lipoprotein (a) (mg/dL)	28.6 ± 27.6	32.8 ± 44.1

Ch indicates cholesterol; TG, triglycerides.

* P < .05, in each group with basal values.

Table 3
Dietary intake

Characteristics	G1359G (n = 159)	(G1359A or A1359A) (n = 131)
Energy (kcal/d)	1776.7 ± 737	1681 ± 568
CH (g/d)	179.7 ± 84	172.3 ± 66
Fat (g/d)	76.5 ± 36	71.7 ± 36
S-fat (g/d)	21.8 ± 14.1	21.5 ± 12.5
M-fat(g/d)	37.2 ± 14.3	34.8 ± 15.5
P-fat (g/d)	7.6 ± 4.4	7.4 ± 3.9
Protein (g/d)	85.9 ± 29	83.4 ± 25
Exercise (h/wk)	1.72 ± 2.9	1.39 ± 2.6
Dietary fiber	14.47 ± 6.5	14.14 ± 7.5

No statistical differences. CH indicates carbohydrate; S-fat, saturated fat; M-fat, monounsaturated fat; P-fat, polyunsaturated fat.

insulin resistance without changes in BMI. However, the literature supports the notion that endocannabinoid system is positioned for regulation of endocannabinoid levels that could influence craving and reward behaviors through the relevant neuronal circuitry and metabolic parameters [18]. This provides a link between the consequences of this polymorphism and the present epidemiologic study indicating that the CB1 receptor G1359A polymorphism may be one risk factor for susceptibility to obesity. Furthermore, the CB1 receptor is expressed in some peripheral human tissues studied in relation to the pathogenesis of obesity and obesity-associated metabolic disorders; and 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 [19].

The percentage of AA genotype (5.2%) was similar with other studies, for example, in obese patients: 1.5% [20], 4.3% [21], and 4.98% [22]. The percentage of GA genotype was (40%) similar with other studies: 43.5% [20], 19.6% [21], and 33.1% [22]. The lack of association between BMI and this polymorphism is in contrast with the association detected by Gazzerro et al [20] with single nucleotide polymorphism (SNP) G1359A of *CNR1* receptor; A3813G, A4895G, G1422A A3813A, and A4895A SNPs of *CNR1* receptor [23]; and with (G1422A) SNP of CB1 receptor [24]. The inconsistencies between similar studies may reflect the complex interactions between multiple population-specific genetic and environmental factors. Perhaps, these different results could be explained by inclusion criteria for subjects in other studies. These previous studies would require composition analysis of the diet to determine whether dietary components could be responsible for the metabolic profiles. In our study, dietary intake did not show statistical differences between groups. In this way, we have controlled for dietary intake; and previous discrepancies could be explain by this uncontrolled factor (dietary intake).

Table 4
Circulating adipocytokines

Characteristics	G1359G (n = 159)	(G1359A or A1359A) (n = 131)
IL-6 (pg/mL)	1.70 ± 1.4	1.74 ± 2.1
TNF-œ (pg/mL)	6.64 ± 4.1	6.15 ± 4.1
Adiponectin (ng/mL)	46.21 ± 26.8	46.41 ± 28.4
Resistin (ng/mL)	3.79 ± 1.8	3.72 ± 1.8
Leptin (ng/mL)	98.4 ± 93.1	112.6 ± 88.1

No statistical differences between groups.

In another study [24], in which the A1422A homozygote patients were more abdominally obese, the absence of a *CNR1* gene with the G allele at position 1422 increased the risk of obesity in men. The association was only seen in obese men and can potentially be explained by sex differences in eating in general and fat ingestion in particular. Carriers of 3813G allele had a higher level of total body fat and central fat deposition [23]; no association was observed with A4895G variant. Benzinou et al [25] showed a positive correlation of the A10908G and T5489C polymorphisms with obesity in 2 obese populations. Our study did not detect associations among a lot of the anthropometric parameters and the G1359A polymorphism of the CB1 receptor.

Ravinet et al [6] found that CB-1 gene-deficient mice were lean and resistant to diet-induced obesity and showed reduced plasma insulin and leptin levels. In our patients, glucose, HOMA, and triglycerides levels were higher in obese patients carrying the wild-type CB1 allele than in heterozygous subjects (G/A and A/A) as shown by Gazzerro et al [20]. This metabolic relationship between the polymorphism and metabolic profile has been detected by another study. Aberle et al [21] have shown that carriers of at least one A allele in *CNR1* lost more weight and reduced LDL cholesterol than wild-type patients.

The theoretical explanation of this association could be due to the adipose tissue. Cannabinoids modulate the expression of several cellular target genes via the *CNR1* receptor-dependent pathway. In brown adipose tissue, cannabinoid antagonist treatment is able to stimulate the expression of genes favoring energy dissipation through mitochondrial heat production [26]. Another way is the increased expression of adiponectin induced by *CNR1* antagonists, in vitro, in 3T3 F442A adipocytes [27] and in vivo obese mice [28], which suggests a close relationship between *CNR1* receptor blockade and the production of this adipocyte-derived protein. However, our data did not support the role of this polymorphism on adipocytokines levels. Recently, another study [29] has demonstrated that genetic variation in cannabinoid receptor 1 is associated with derangements in lipid homeostasis, independent of BMI. Perhaps, a direct role of the endocannabinoid system in lipid and glucose homeostasis could be hypothesized independently of BMI.

In conclusion, the novel finding of this study is the association of the mutant-type group G1359A and A1359A with a better cardiovascular profile (triglyceride, HDL cholesterol, insulin, and HOMA levels) than wild-type group. Further studies are needed to elucidate this complex relationship independent of BMI.

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