

Original

Relation of C358A polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (faah) with obesity and insulin resistance

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Abstract

Background and aims: Recently, it has been demonstrated that the polymorphism 385 C/A of FAAH (fatty acid amide hydrolase) was associated with overweight and obesity. The aim of our study was to investigate the relationship of missense polymorphism (cDNA 385 C->A) of FAAH gene on obesity anthropometric parameters, cardiovascular risk factors and adipocytokines.

Methods: A population of 279 females with obesity (body mass index >30) was analyzed. An indirect calorimetry, tetrapolar electrical bioimpedance, blood pressure, a serial assessment of nutritional intake with 3 days written food records and biochemical analysis (lipid profile, adipocytokines, insulin, CRP and lipoprotein-a) were performed. The statistical analysis was performed for the combined C385A and A385A as a group and wild type C385C as second group.

Results: One hundred and ninety four patients (69.5%) had the genotype C385C (wild type group) and 76 (27.2%) patients had the genotype C358A or A358A (9 patients, 3.2%) (mutant type group). No differences were detected between groups in anthropometric parameters and dietary intakes. Triglycerides (118.9 ± 59.9 mg/dl vs 107.4 ± 51.3 mg/dl; $p < 0,05$), glucose (100.4 ± 19.9 mg/dl vs 94.8 ± 11.5 mg/dl; $p < 0,05$), HOMA (3.74 ± 2.2 vs 3.39 ± 2.7 ; $p < 0,05$) and interleukine 6 (3.3 ± 1.4 pg/ml vs 1.4 ± 2.1 pg/ml; $p < 0,05$) were higher in wild type group than mutant type group.

Conclusion: The novel finding of this study is the association of the mutant type group A358C and A358A of FAAH with a better cardiovascular profile (triglyceride, glucose, interleukine 6 and HOMA levels) than wild type group.

(Nutr Hosp. 2010;25:993-998)

DOI:10.3305/nh.2010.25.6.4843

Key words: Adipokines. Faah. Insulin resistance. Obesity. Polymorphism. Risk factors.

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Recibido: 8-VI-2010.
1ª Revisión: 12-VIII-2010.
Aceptado: 11-IX-2010.

RELACION DEL POLIMORFISMO C358A DEL ENZIMA DEL SISTEMA ENDOCANABINOIDE (HIDROLASA AMIDA ACIDA) CON LA OBESIDAD Y LA RESISTENCIA A LA INSULINA

Resumen

Antecedentes y objetivos: Recientemente, se ha demostrado que el polimorfismo 385 C/A, de FAAH (hidrolasa amida de ácidos grasos) se asocia con el sobrepeso y la obesidad. El objetivo de nuestro estudio fue investigar la relación de este polimorfismo del gen de FAAH con parámetros antropométricos, factores de riesgo cardiovascular y adipocitoquinas.

Métodos: Una población de 279 mujeres con obesidad (índice de masa corporal > 30) fue analizada. Se realizaron las siguientes determinaciones; calorimetría indirecta, bioimpedancia eléctrica, presión arterial, una evaluación de la ingesta nutricional de 3 días, así como un análisis bioquímico (perfil lipídico, adipocitoquinas, insulina, proteína C reactiva y lipoproteína-(a)). El análisis estadístico se realizó combinando C385A y A385A como grupo mutante y C385C como grupo salvaje.

Resultados: Un total de 194 pacientes (69,5%) tenían el genotipo C385C (genotipo salvaje) y 76 (27,2%) pacientes tenían el genotipo C358A y 9 pacientes (3,2%) el genotipo A358A, formando estos dos el grupo de genotipo mutante. No se detectaron diferencias entre los grupos en los parámetros antropométricos y la ingesta dietética. Sin embargo los pacientes con genotipo salvaje presentaron valores más elevado de triglicéridos ($118,9 \pm 59,9$ mg/dl vs $107,4 \pm 51,3$ mg/dl; $p < 0,05$), glucosa ($100,4 \pm 19,9$ mg/dl vs $94,8 \pm 11,5$ mg/dl; $p < 0,05$), HOMA ($3,74 \pm 2,2$ vs $3,39 \pm 2,7$; $p < 0,05$) y de interleukina-6 ($3,3 \pm 1,4$ pg/ml vs $1,4 \pm 2,1$ pg/ml; $p < 0,05$) fueron mayores en el grupo de tipo salvaje que el grupo de tipo mutante.

Conclusión: El principal hallazgo de este trabajo es la asociación del genotipo mutante (A358C y A358A) de FAAH con un mejor perfil cardiovascular (triglicéridos, glucosa, interleucina 6 y HOMA) que los pacientes portadores del genotipo salvaje.

(Nutr Hosp. 2010;25:993-998)

DOI:10.3305/nh.2010.25.6.4843

Palabras clave: Adipocitoquinas. FAAH. Resistencia a insulina. Obesidad. Polimorfismo. Factores de riesgo.

Introduction

According to recent surveys, approximately two thirds of the population of Spain is overweight¹. The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation and immunity².

Herbal *Cannabis sativa* (marijuana) has been known to have many psychoactive effects in humans including robust increases in appetite and body weight³. Nevertheless, the mechanism underlying cannabinoid neurobiological effects have been recently revealed⁴. The endogenous cannabinoid system mediates and it is positioned both functionally and anatomically⁵ to be an important modulator of normal human brain behavior. 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 endocannabinoids⁶. The pharmacological effects of some endocannabinoids appear to be regulated by FAAH activity⁷, 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 aminoacid position 129 (P129T) has been described and is significantly associated with drug abuse⁸. Recently, Sipe et al⁹ have been demonstrated that the homozygous FAAH 385 A/A genotype was associated with overweight and obesity. Monteleone et al¹⁰ confirmed previously published significant over-representations of the FAAH 385A allele in overweight/obese subjects. However, in a large study sample (5801 subjects), Jensen et al¹¹ were unable to find association of this polymorphism with overweight or obesity. Considering the evidence that endogenous cannabinoid system plays a role in metabolic aspects of body weight and feeding behavior¹², we decided to investigate the association of this FAAH missense polymorphism with obesity and adipocytokines.

The aim of our study was to investigate the relationship of missense polymorphism (cDNA 385 C->A) of FAAH gene on obesity anthropometric parameters, cardiovascular risk factors and adipocytokines.

Subjects and methods

Subjects

A population of 279 females with obesity (body mass index >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 36 months, total cholesterol > 300

mg/dl, triglycerides > 400 mg/dl, blood pressure > 140/90 mmHg, fasting plasma glucose >110 mg/dl, as well as the use of sulphonilurea, thiazolidinedions, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors and psychoactive medications. Local ethical committee approved the protocol.

Procedure

All patients with a 2 weeks weight-stabilization period before recruitment were enrolled. Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, insulin resistance (HOMA), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood and adipocytokines (leptin, adiponectin, resistin, TNF alpha, and interleukin 6) levels were measured at basal time. A tetrapolar bioimpedance, an indirect calorimetry and a prospective serial assessment of nutritional intake with 3 days written food records were realized. Genotype of FAAH gene polymorphism was studied.

Genotyping of FAAH gene polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, LA, CA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward, 5'-CTA TCT GGC TGA CTG TGA GAC TC-3'; primer reverse, 5'-GAG GCA GAG CAT ACC TTG TAG G-3), and 0.25 uL of each probes (wild probe: 5'-Fam-CTG TCT CAG GCC CCA AGG CAG G-BHQ-1-3') and (mutant probe: 5'-Hex- CTG TCT CAG GCC ACA AGG CAG G-BHQ-1-3') in a 25 uL final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA). DNA was denaturated at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3° for 45 s). The PCR were run in a 25 uL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA) 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, California). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5mUI/L (normal range 0.5-30 mUI/L)¹³ and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values¹⁴. CRP was measured by immunoturbimetry (Roche Diagnostcis GmbH,

Mannheim, Germany), with a normal range of (0-7 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, Calif., USA).

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Adipocytokines

Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml¹⁵. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml¹⁶. Adiponectin was measured by ELISA (R&D systems, Inc., Minneapolis, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml¹⁷. Interleukin 6 and TNF alpha were measured by ELISA (R&D systems, Inc., Minneapolis, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNFalpha (0.5-15.6 pg/ml)¹⁷⁻¹⁹.

Indirect calorimetry

For the measurement of resting energy expenditure, subjects were admitted to a metabolic ward. After a 12 h overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20 min period with an open-circuit indirect calorimetry system (standardized for temperature, pressure and moisture) fitted with a face mask (MedGem; Health Tech, Golden, USA), coefficient of variation 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated²⁰.

Anthropometric measurements

Body weight was measured to an accuracy of 0.5 Kg and body mass index computed as body weight/(height²). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g²¹. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model

310e, Seattle, WA, USA) 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 minutes rest with a random zero mercury sphygmomanometer, and averaged.

Dietary intake and habits

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instruction to record their daily dietary intake for three 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 registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference²².

Statistical analysis

Sample size was calculated to detect differences over 2 kg in body weight with 90% power and 5% significance (n=80, in each group). The results were expressed as average \pm standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed Student's-t test. Non-parametric variables were analyzed with the U-Mann-Whitney test. The statistical analysis was performed for the combined C385A and A385A as a group and wild type C385C as second group. A p-value under 0.05 was considered statistically significant.

Results

Two hundred and seventy nine patients gave informed consent and were enrolled in the study. The mean age was 44.8 \pm 12 years and the mean BMI 35.4 \pm 6.1. All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.28 \pm 0.1 kg).

One hundred and ninety four patients (69.5%) had the genotype C358C (wild type group) and 76 (27.2%) patients had the genotype C358A or A358A (9 patients, 3.2%) (mutant type group). Age was similar in both groups (wild type: 44.70 \pm 15.5 years vs mutant group: 45.5 \pm 15.2 years:ns).

Table I shows the anthropometric variables. No differences were detected between groups.

Table II shows the classic cardiovascular risk factors. Triglycerides, glucose and HOMA values were higher in wild type group than mutant type group.

Table I
Anthropometric variables

Characteristics	C385C (n=194)	(C385A or A385A) (n=85)
BMI	35.8±5.2	35.1±5.8
Weight (kg)	90.6±17.6	89.4±15.8
Fat free mass (kg)	45.5±6.9	44.7±7.3
Fat mass (kg)	44.1±14.1	44.1±13.8
WC (cm)	106.6±15.8	106.1±12.5
Waist to hip ratio	0.88±0.1	0.89±0.09
Systolic BP (mmHg)	128.3±17.3	128.1±14.8
Diastolic BP (mmHg)	86.6±10.9	82.2±9.3
RMR(kcal/day)	2075±671	1969±814

RMR: resting metabolic rate. WC: Waist circumference. No statistical differences between groups.

Table II
Classical cardiovascular risk factors

Characteristics	C385C (n=194)	(C385A or A385A) (n=85)
Glucose (mg/dl)	100.4±19.9	94.8±11.5*
Total ch. (mg/dl)	203.1±39.6	210.6±46.4
LDL-ch. (mg/dl)	121.5±36.6	133.5±46.4
HDL-ch. (mg/dl)	57.9±22.9	56.4±12.1
TG (mg/dl)	118.9±59.9	107.4±51.3*
Insulin (mUI/L)	14.8±7.8	14.6±10.1
HOMA	3.74±2.2	3.39±2.7*
CRP (mg/dl)	6.8±10.5	5.7±4.6
Lipoprotein-a (mg/dl)	27.3±31.6	37.6±50.5

Ch: Cholesterol. TG: Triglycerides CRP: c reactive protein. HOMA: Homeostasis model assessment. (*) p<0.05, between groups.

Table III
Dietary intake

Characteristics	C385C (n=194)	(C385A or A385A) (n=85)
Energy (kcal/day)	1746.7±537	1719.9±568
CH (g/day)	175.9±65.1	179.8±66.2
Fat (g/day)	75.2±31.9	72.2±28.2
S-fat (g/day)	21.8±14	21.3±13.5
M-fat(g/day)	35.9±14.3	34.1±16.5
P-fat (g/day)	7.6±4.2	7.4±3.8
Protein (g/day)	85.3±25.3	84.9±24.5
Exercise (hs./week)	1.45±3.4	1.32±2.6
Dietary fiber	14.3±6.2	14.4±6.1

CH: Carbohydrate. S-fat: saturated fat. M-fat: monounsaturated fat. P-fat: polyunsaturated fat. No statistical differences.

Table IV
Circulating adipocytokines

Characteristics	C385C (n=194)	(C385A or A385A) (n=85)
IL 6 (pg/ml)	3.3±1.4	1.4±2.1*
TNF-α (pg/ml)	6.5±4.4	6.3±3.3
Adiponectin (ng/ml)	45.1±65.8	50.9±76.8
Resistin (ng/ml)	3.8±1.9	3.7±1.6
Leptin (ng/ml)	109.1±96.9	90.4±73.8

IL-6: interleukin 6. (*) p<0.05, between groups.

Table III shows nutritional intake with 3 days 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 IV shows levels of adipocytokines. IL-6 levels were higher in wild type group than mutant type group. No differences were detected between both groups in other serum adipocytokine levels.

Discussion

Since genetic influences are thought to play a greater role in obesity, identification of obesity genes may be more easily detected in the former. A common C358A single nucleotide polymorphism (SNP) of the FAAH results in a missense mutation producing a FAAH with defective expression²³. Studies of the C358A variant and obesity from various authors have yielded conflicting results⁸⁻¹¹.

In our study, the percentage of AA genotype (3.2%) was similar than others, for example; 3.7%⁹, 2.3%²⁵ and 1%¹⁰. The percentage of AC genotype (27.2%) was similar than other studies; 28.1%⁹, 24.1%²⁵ and 36.5%¹⁰, too.

In our design, we investigated the effect of FAAH genetic variation on weight, and also on metabolic parameters. The novel finding of this study is the association of the C385C FAAH genotypes with higher levels of IL6, glucose, HOMA and triglycerides than C385A and A385A FAAH genotypes than wild type group, without differences in anthropometric parameters related with obesity.

An association between this polymorphism and metabolic profile has been described. Interestingly, Aberle et al²⁴ have shown that carriers of the A allele had a significantly greater decrease in the total cholesterol and triglycerides as compared to wild type when following a low fat diet.

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 al¹¹ or Papazoglou et al²⁵ and contrast with those of Sipe et al⁹. 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 to the median BMI of the other subjects⁹. The effects of the FAAH A385A could result in the upregulation of natural reward behaviours such as consumption of sweets and palatable food associated with overweight and obesity. Under normal circumstances, brain endocannabinoid signalling may exert tonic control on the levels of certain neurotransmitters. 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 density²⁶. 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 inclusion criteria 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 lipid profile modifications. In our study dietary intake did not show statistical differences between groups, in this way our data have been controlled by dietary intake and previous discrepancies could be explained by this uncontrolled factor (dietary intake).

Accumulating 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 lipogenesis²⁷. 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. Patients with this polymorphism have half the FAAH enzymatic activity and protein expression when compared with wild-type subjects²³. This provides a link between the consequences of this polymorphism and the present epidemiological study indicating that the FAAH A385A polymorphism may play a role in decrease or increase metabolic risk associated to obesity.

In our study, the elevation of inflammation markers (IL-6) in patients with wild type genotype could be related with an altered postprandial response of fatty acids absorption (no measured in our design). Elevated free fatty acids increases the accumulation of triglycerides in the adipocyte, related with imbalance of lipoprotein lipase activity and overproduction of adipokines such as IL-6. The lack of association of this proinflammatory state with fat mass or BMI could indicate the existence of complex unmeasured gene-gene or gene-environment interactions that may enhance metabolic abnormalities in obese patients.

In conclusion, the novel finding of this study is the association of the mutant type group A385C and A385A of FAAH with a better cardiovascular profile (triglyceride, glucose, interleukine 6 and HOMA lev-

els) than wild type group. Further studies are needed to elucidate this complex relationship in an independent way of body mass index.

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