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ORIGINAL ARTICLE

Association between cannabinoid type-1 receptor polymorphism and body mass index in a southern Italian population

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Context: Endocannabinoids control food intake via both central and peripheral mechanisms, and cannabinoid type-1 receptor (CB1) modulates lipogenesis in primary adipocyte cell cultures and in animal models of obesity.
Objectives: We aimed to evaluate, at the population level, the frequency of a genetic polymorphism of CB1 and to study its correlation with body mass index.
Design, setting and participants: Healthy subjects from a population survey carried out in southern Italy examined in 1992–1993 and older than 65 years ($n = 419$, M = 237, F = 182) were divided into quintiles by body mass index (BMI). Two hundred and ten subjects were randomly sampled from the first, third and fifth quintile of BMI (BMI, respectively: 16.2–23.8 = normal, 26.7–28.4 = overweight, 31.6–49.7 = obese) to reach a total of 70 per quintile. Their serum and white cells from the biological bank were used to measure the genotype and the blood variables for the study.
Measurements: Anthropometric parameters, blood pressure, serum glucose and lipid levels were measured with standard methods; genotyping for the CB1 1359G/A polymorphism was performed using multiplex PCR. Statistical methods included χ^2 for trend, binomial and multinomial multiple logistic regression to model BMI on the genotype, controlling for potential confounders.
Results: We found a clear trend of increasing relative frequency of the CB1 wild-type genotype with the increase of BMI ($P = 0.03$) and, using a multiple logistic regression model, wild-type genotype, female gender, age, glycaemia and triglycerides were directly associated with both overweight (third quintile of BMI) and obesity (fifth quintile of BMI).
Conclusions: Although performed in a limited number of subjects, our results show that the presence of the CB1 polymorphic allele was significantly associated with a lower BMI.
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Keywords: endocannabinoids; cannabinoid type-1 receptor; BMI; CB1-polymorphism; glycaemia; lipid profile

Introduction

Obesity is becoming truly pandemic in large parts of the world.[1] By severely and profoundly influencing the development of serious diseases, obesity qualifies as one of the most serious dangers threatening the developed world.[2] Efforts to prevent obesity can be focused on recognition and counselling of susceptible subjects. The maintenance of body weight is under genetic control but, rarely, mutations in single genes can result in severe obesity; in common obesity and in the tendency to put on weight, multiple genes, environmental factors and gene–environment interactions play a crucial role. A better knowledge of the genetic variations that influence energy metabolism or predispose to weight gain, together with a clearer understanding of how all the different elements work together, is a public health priority. Only in this way will it be possible to address risk factor assessment and to define long-term responsiveness to therapeutic interventions or to weight management programmes.

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

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lipogenesis and fat accumulation.[3] This system consists of endogenous ligands (anandamide (AEA) and 2-arachidonoylglycerol (2-AG)) 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] The endocannabinoid system is overactivated in genetic animal models of obesity and in response to exogenous stimuli such as excessive food intake. Preclinical studies implicate the endocannabinoid system in the modulation of food intake and adipogenesis, through peripheral mechanisms, and the system might therefore provide a possible treatment target for high-risk overweight or obese patients. 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 highly palatable high-fat diet.[5,6] Further evidence has been provided by an investigation of the selective CB1 receptor blocker rimonabant, which causes weight loss and ameliorates metabolic abnormalities in obese animals.[7] Recently, a large multicentre, multinational randomized, placebo-controlled trial – the RIO (Rimonabant In Obesity) Europe trial – has shown the efficacy and safety of rimonabant in reducing body weight and improving cardiovascular risk factors in overweight or obese patients. These findings suggest that a hyperactive endocannabinoid system is a factor contributing to obesity and related disorders. A recent study has shown that overweight and obesity are associated with a potential genetic malfunction of one of the endocannabinoid degrading enzymes, further substantiating the hypothesis of a hyperactive endocannabinoid system as a possible cause of obesity.[8]

A silent intragenic biallelic polymorphism (1359G/A) of the CB1 gene (CNR1, X54937 GenBank accession number), resulting in the substitution of G to A at nucleotide position 1359 in codon 435 (Thr), was reported as a common polymorphism in the German population,[9] reaching frequencies of 24–32% for the rarer allele (A).

Considering the evidence that the endocannabinoid system plays a significant role in both hedonic and metabolic aspects of feeding behaviour and body weight, we decided to investigate whether homozygosis or heterozygosis for this CB1 receptor gene polymorphism is associated with body mass index (BMI).

Materials and methods

Subjects

Participants in this study were recruited from among individuals included in a population survey on gallstones carried out in a small town in southern Italy (Castellana, Province of Bari, in the Apulia region), with 18 000 inhabitants at the 1991 census. Between May 1985 and June 1986, 3500 individuals (2000 men and 1500 women aged 30–69 years) were randomly selected from the electoral register of the town (the register includes all residents of the town over 18 years of age, and therefore old enough to vote). Of these, 2472 subjects (70.6%, 1429 men and 1043 women) participated in the survey. Between May 1992 and June 1993, 2175 of these subjects were re-examined (88% respondents). As part of both examinations, respondents completed a questionnaire on sociodemographic status, medical history, dietary habits and physical activity in the previous year, and underwent standardized measurement of height, weight and blood pressure. A blood sample was also taken at the examination in the morning, with the subjects fasting for at least 12 h and was kept in a biological bank at -80°C. Subjects examined in 1992–1993 and older than 65 years ($n = 419$, M = 237, F = 182) were divided into quintiles of BMI and 210 subjects were randomly sampled from the first, third and fifth quintile of BMI (BMI, respectively: 16.2–23.8 = normal, 26.7–28.4 = overweight, 31.6–49.7 = obese) to reach a total of 70 per quintile. Their serum and white cells from the biological bank were used to measure the blood variables and the genotype for the study, respectively. In order to minimize, or at least reduce, the influence of behavioural, physical and diet-induced variables, individuals belonging to a limited age range ($\geqslant 65$ and $\leqslant 75$ years) were selected for the study, in view of their smaller variations in dietary and physical activity habits in comparison with younger subjects, as assessed by recurrent interviews. Written informed consent was obtained from all subjects following a complete and extensive description of the study. Sufficient serum and blood cells for analyses were obtained for 60 subjects in the first, 59 in the third and 57 in the fifth quintile.

Genotyping

The expression of the polymorphic A-allele in the CNR1 gene coding for the CB1 receptor was determined using multiplex-polymerase chain reaction (PCR) as previously described.[10] We used PCR primers F1-F (5′-AA GACGGTGTTTGCATTCTG-3′) and THAC-R (5′-AAATTCTTTTCCTGTGCTGCCAGGGAG-3′) and allele-specific primers A3-F (5′-AGTGAGAGTTGCATCAAGAGCACA-3′) and T1-R (5′-GACTTGGCAATCTTGACT-3′) or G4-F (5′-AGTGAGAGTTGCATCAAGAGCACG-3′) and C2-R (5′-GACTTGGCAATCTTGACC-3′) to detect the polymorphic A-allele or the G-allele, respectively.

Measurement

Anthropometric measurements were taken by the same trained person, with participants wearing scrub suits and no shoes. Body weight was measured using a calibrated scale (Detecto; model 437). Standing height was measured with a vertical metal ruler. BMI was calculated as weight in

kilograms divided by height squared in meters ($kg\,m^{-2}$). Participants fasted for 12 h before examination. With the subjects seated, fasting blood was drawn from an antecubital vein into vacuum tubes containing ethylenediaminetetraacetic acid (lipids) or a serum separator gel (glucose). Blood serum and plasma aliquots were stored at $-70^{\circ}C$ and transported to central laboratories for analyses. Total cholesterol and total triglycerides were measured by enzymatic methods; high-density lipoprotein (HDL) cholesterol was measured after dextran-magnesium precipitation. Serum glucose was assayed by the hexokinase/glucose-6-phosphate dehydrogenase method. Alanine aminotransferase (ALT) was determined by the enzymatic-colorimetric method. The coefficient of variation when checking the precision of the measurement on the same day was less than 2%.

Statistical analysis

Mean and s.d. for continuous variables, and relative frequency and s.d. of the relative frequency for categorical variables, were used as indices of centrality and dispersion of the distribution. χ^2 test for trend was used to test bivariate associations. Binomial and multinomial multiple logistic regression were used to model BMI on the genotype, controlling for potential anthropometric and serum variable confounders. When testing the null hypothesis of no association, the probability level of α error, two tails, was 0.05. All the statistical computations were made using STATA 8.0 Statistical Software (Stata Corporation, College Station, TX, USA).

Results

Table 1 shows the mean and s.d. or frequency of age, gender, BMI and all the laboratory values in the categories of the genotype. There were only three subjects with genotype aa and their mean BMI was similar to that of genotype ag; hence we decided to study these two genotypes together, creating a new category aa + ag. There was a clear trend of increasing relative frequency of genotype gg with the increase of BMI (χ for trend, $P = 0.03$) (Table 2). Binomial multiple logistic regression of the first quintile of BMI versus the third and fifth quintile, on the genotype and the other potential confounders of the relationship, age, gender, serum variables, is shown in Table 3a. Genotype gg, female gender, age and glycaemia are independently and directly associated with BMI. In Table 3b we report the most parsimonious binomial multiple logistic regression model (backward method) of BMI, normal versus overweight plus obesity, on the genotype, gg versus ag plus aa, and the other potential confounders of the relationship. Genotype gg, age, female gender, glycaemia and also triglycerides were directly and independently associated with BMI at less than the established *P*-value of 0.05.

Using the most parsimonious multinomial multiple logistic regression model (backward method), the genotype gg, female gender, age, glycaemia and triglycerides, as shown in Table 3b, were directly associated with both overweight

Table 1 Descriptive statistics (mean and s.d. or absolute frequency) of BMI, demographic and serum variables in the categories of the genotype

Variables	*Genotype*		
	gg (n = 116)	*ag (n = 57)*	*aa (n = 3)*
Gender (M/F)	64/52	29/28	1/2
Age (years)	69.7 ± 3.6	70.1 ± 3.1	68.0 ± 2.6
BMI (kg/m^2)	28.6 ± 5.5	27.1 ± 6.1	26.9 ± 8.1
Cholesterol (mg/dl)	196.2 ± 38.3	196.5 ± 36.8	182.3 ± 50.8
HDL-Cholesterol (mg/dl)	51.6 ± 12.9	48.7 ± 12.3	55.0 ± 14.0
Triglycerides (mg/dl)	115.5 ± 89.5	139.9 ± 126.0	79.3 ± 25.5
Glycaemia (mg/dl)	105.3 ± 28.7	111.4 ± 28.5	102.0 ± 23.6
ALT ($UI\,dl^{-1}$)	16.5 ± 9.4	17.5 ± 13.6	9.3 ± 3.2

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; HDL, high-density lipoprotein.

Table 2 Genotype distribution of the subjects by BMI quintile

	BMI		
	Normal (n = 60)	*Overweight (n = 59)*	*Obese (n = 57)*
Genotype			
gg	33 (55.00%)	41 (69.49%)	42 (73.68%)
ag/aa	27 (45.00%)	18 (30.51%)	15 (26.32%)

Abbreviation: BMI, body mass index. χ^2 for trend *P*-value = 0.03. BMI: normal (first quintile 16.2–23.8), overweight (third quintile 26.7–28.4), obese (fifth quintile 31.6–49.7).

Table 3 (a) Binomial multiple logistic regression model of BMI (normal versus overweight plus obese) on genotype (gg versus ag plus aa) and other potential confounders. (b) Most parsimonious binomial multiple logistic regression model of BMI (normal versus overweight plus obese) on genotype (gg versus ag plus aa) and other potential confounders (backward method).

	OR	*s.e.*	P-*value*	*95% CI*
(a)				
Gender (M = 0, F = 1)	3.19	1.37	0.007	1.38–7.39
Age (years)	0.88	0.05	0.02	0.78–0.98
Genotype (gg = 0/ag,aa = 1)	0.33	0.13	0.006	0.15–0.72
Cholesterol (mg/100 ml)	1.01	0.01	0.35	0.99–1.02
HDL-Chol (mg/100 ml)	0.97	0.02	0.12	0.94–1.01
Triglycerides (mg/100 ml)	1.01	0.005	0.13	0.998–1.02
Glycaemia (mg/100 ml)	1.05	0.01	0.001	1.02–1.08
SGPT ($U\,l^{-1}$)	1.00	0.02	0.97	0.97–1.04
(b)				
Gender (M = 0, F = 1)	2.63	1.01	0.01	1.24–5.58
Age (years)	0.88	0.05	0.02	0.79–0.98
Genotype (gg = 0/ag,aa = 1)	0.35	0.14	0.008	0.16–0.76
Triglycerides (mg/100 ml)	1.01	0.004	0.008	1.003–1.02
Glycaemia (mg/100 ml)	1.05	0.01	<0.001	1.02–1.08

Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; OR, odds ratios; SE, standard error.

Table 4 Most parsimonious multinomial multiple logistic regression model of BMI (normal versus overweight and normal versus obese) on genotype (gg versus ag plus aa) and other potential confounders (backward method)

	OR	*s.e.*	P-*value*	*95% CI*
Overweight				
Gender (M = 0, F = 1)	2.01	0.85	0.10	0.88–4.60
Age (years)	0.92	0.05	0.17	0.82–1.04
Genotype (gg = 0/ag,aa = 1)	0.41	0.18	0.04	0.17–0.96
Triglycerides (mg 100 ml^{-1})	1.01	0.004	0.02	1.002–1.02
Glycaemia (mg 100 ml^{-1})	1.05	0.01	<0.001	1.02–1.08
Obese				
Gender (M = 0, F = 1)	3.65	1.62	0.004	1.53–8.70
Age (years)	0.83	0.05	0.006	0.74–0.95
Genotype (gg = 0/ag, aa = 1)	0.29	0.14	0.009	0.11–0.73
Triglycerides (mg 100 ml^{-1})	1.01	0.004	0.005	1.004–1.02
Glycaemia (mg 100 ml^{-1})	1.05	0.01	0.001	1.02–1.08

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratios; s.e., standard error.

(third quintile of BMI) and obesity (fifth quintile of BMI) (Table 4).

Discussion

Our findings show that the polymorphic CB1 receptor A allele was significantly associated with a lower BMI.

We do not know how the 1359G/A or A/A polymorphism may exert an influence on BMI. CB1 receptor levels seem to be a fundamental element for cannabinoid-mediated biological effects and CB1 receptor expression has been found to be higher in adipocytes from obese animals compared to lean controls;[11] this finding seems to confirm the notion that hyperactivity of the endocannabinoid system is associated with obesity.

The CB-1 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 (FAAH) gene expression was found in the adipose tissue of obese women, suggesting that increased endocannabinoid levels may be secondary to decreased enzymatic degradation and that adipose tissue may be an important contributor to endocannabinoid inactivation.[12] Moreover, a recent study has shown that overweight and obesity are associated with a potential genetic malfunction of the fatty acid amide hydrolase (FAAH), one of the endocannabinoid degrading enzymes, further substantiating the hypothesis of a hyperactive endocannabinoid system as a possible cause of obesity.[8]

To date we have no evidence of mRNA CB1-receptor stability or protein expression levels in A-allele carriers but the significant association of the presence of the polymorphic variant to a lower BMI is an intriguing issue. Moreover, Ravinet *et al* [5] 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 serum samples, glucose and triglyceride levels were higher in overweight and obese subjects carrying the wild type G/G CB1 allele than in heterozygous subjects (G/A and A/A) assigned to the same BMI categories (data not shown).

Cannabinoids modulate the expression of several cellular target genes via the CB1 receptor dependent pathway. In brown adipose tissue, treatment with SR141716 may favour thermogenesis: microarray experiments suggest that cannabinoid antagonist treatment is able to stimulate the expression of genes favoring energy dissipation through mitochondrial heat production.[13] Finally, the increased expression of adiponectin induced by CB1 antagonists, *in vitro*, in 3T3 F442A adipocytes[11] and *in vivo* in obese mice[7] suggests a close relationship between CB1 receptor blockade and the production of this adipocyte-derived protein.

In summary, although performed in a limited number of subjects, our results show that polymorphism of the CNR1 gene is associated with BMI. Thus, CB1 receptor gene variants could be important in screening subjects predisposed to obesity and may contribute to specific differences in responsiveness to endocannabinoids or to anti- obesity drugs.

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