Endocr (2007) 31:138–141
DOI 10.1007/s12020-007-0022-y

ORIGINAL PAPER

The G1422A variant of the cannabinoid receptor gene (*CNR1*) is associated with abdominal adiposity in obese men

Armand Peeters · Sigri Beckers · Ilse Mertens · Wim Van Hul · Luc Van Gaal

Received: 15 March 2007 / Accepted: 15 May 2007 / Published online: 12 June 2007

Abstract Since recent data suggest that the endocannabinoid system controls food intake through central, and lipogenesis via peripheral CB1 receptors, we hypothesized that genetic variation at the cannabinoid receptor-1 (*CNR1*) locus could have an effect on adiposity. We investigated, whether a specific *CNR1* G1422A genotype is associated with anthropometric markers of obesity and fat distribution in adult obese individuals. A total of 1,064 obese subjects (BMI ≥ 30 kg/m^2) without diabetes, impaired glucose tolerance or other endocrine diseases and 251 healthy control persons were genotyped for the G1422A variant (rs1049353) with a TaqMan assay. Anthropometric measures as body weight, BMI, waist and waist-to-hip ratio (WHR) were assessed by classical methods. Fat mass (FM) was measured by bio-impedance. The prevalence of the G1422A variant was not significantly different between cases and controls (OR = 1.056; $P = 0.626$). In obese women, no meaningful associations between *CNR1* genotype and anthropometric parameters were found. In obese men, *CNR1* 1422 A/A genotype was significantly associated with higher WHR ($P = 0.009$) and waist circumference ($P = 0.008$) after adjusting for age and BMI. Fat mass percentage showed an association ($P = 0.011$) which disappeared after adjusting for age and BMI. A trend for an association was seen for fat mass (unadjusted $P = 0.099$; adjusted $P = 0.033$). Our data indicate that the G1422A polymorphism in the *CNR1* gene is associated with increased abdominal adiposity in obese men.

Keywords Association · Polymorphism · Cannabinoid receptor · CNR1 · Obesity

A. Peeters (✉) · S. Beckers · W. Van Hul
Department of Medical Genetics, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Antwerp University, Universiteitsplein 1, Wilrijk, Antwerp 2610, Belgium
e-mail: armand.peeters@ua.ac.be

I. Mertens · L. Van Gaal
Department of Diabetology, Metabolism and Clinical Nutrition, Antwerp University Hospital, Antwerp, Belgium

Introduction

The epidemic proportions of obesity have generated a lot of research into the factors underlying this complex disease, as well as to mechanisms linking obesity to its metabolic and cardiovascular complications [1]. Recent data suggest that the endocannabinoid system is a key circuit contributing to central appetitive regulation and to peripheral control of lipogenesis via central and peripheral CB1 receptors [2]. Similarly, Engeli et al. demonstrated that the peripheral endocannabinoid system is activated in obesity and suggest that this activation may contribute to obesity [3].

This functional importance of endocannabinoids in the regulation of adiposity has been confirmed by pharmacological studies, where the cannabinoid receptor (CB1) has emerged as a suitable target for the treatment of obesity [4], evidenced by intervention studies demonstrating that CB1 antagonism (by rimonabant) may lead to weight loss and waist reduction, as well as to improvement of a number of co-morbid risk factors [5]. Interestingly, Sipe et al. recently reported an association between overweight and obesity and a missense variant in the gene for fatty acid amide hydrolase (FAAH), the primary inactivating enzyme of endocannabinoids [6].

In view of the above background, we aimed at evaluating, by means of a genetic association study, whether genetic variation at the cannabinoid receptor locus (*CNR1*)

could have an effect on adiposity and fat distribution. Although it is unlikely to affect CB1 functionality, the synonymous G/A single nucleotide polymorphism (SNP) at position 1422 of the *CNR1* gene was selected, because it resides in the gene's coding region (threonine at position 453), and because it is a common polymorphism with a minor allele frequency of 0.258 (HapMap reference CEU panel) [7].

In this study we investigated, for the first time, whether variation in the *CNR1* gene has an effect on endophenotypic parameters of fat distribution in adult obese individuals.

Results

We analysed the *CNR1* G1422A polymorphism in 251 control subjects and 1064 healthy obese individuals. Allele frequencies of controls (73.3% wildtype; 26.7% variant) and obese (72.2% wildtype; 27.8% variant) were not significantly different (OR = 1.056; 95% CI = 0.848–1.315; $P = 0.626$). The genotype distribution of the polymorphism is in Hardy–Weinberg equilibrium (controls $P = 0.495$; obese $P = 0.095$). In the patient group, error plots of mean anthropometric parameters by G1422A genotype revealed no difference between wild-type and heterozygotes, whereas A/A homozygotes had higher-mean values, which suggests a recessive effect of the G1422A variant. Obese subjects were thus stratified according to a recessive model (i.e. genotype G/G + G/A vs. A/A). Means of studied anthropometric parameters according to genotype group under a recessive model are presented in Table 1.

Within the group of obese women, no significant associations between genotype group and anthropometric parameters could be detected by Wilcoxon rank sum test under a recessive model. In obese men, homozygosity for the variant A-allele was significantly associated with highest WHR, both unadjusted ($P = 0.007$) and adjusted for age and BMI ($P = 0.009$). Fat mass percentage was also elevated in A/A homozygotes ($P = 0.011$), but this association disappeared after adjusting for age and BMI ($P = 0.218$). Homozygous variant A/A genotype was only associated with increased waist circumference ($P = 0.008$) after the same adjustments. A trend for an association with increased fat mass was also observed (unadjusted $P = 0.099$; adjusted $P = 0.033$).

Discussion

A case–control association study of the *CNR1* G1422A variant showed no difference in prevalence of the SNP in patients and controls, suggesting that this mutation does not contribute to the pathogenesis of obesity in our study population. However, comparative analysis of anthropometric measurements in adult obese patients stratified by gender revealed meaningful differences between obese

Table 1 Associations under a recessive model between G1422A genotypes and anthropometric phenotypes in obese subjects

Phenotype	Genotype	Mean ± SEM	*n*	*P* (unadjusted)	*P* (adjusted)
Women					
Waist (cm)	G/G + G/A	110.1 ± 0.6	514		
	A/A	110.4 ± 1.7	54	0.734	0.199
Waist-to-hip ratio	G/G + G/A	0.92 ± 0.006	506		
	A/A	0.91 ± 0.014	53	0.923	0.165
Fat mass (kg)	G/G + G/A	53.0 ± 0.6	510		
	A/A	54.3 ± 1.8	54	0.299	0.703
Fat mass %	G/G + G/A	51.2 ± 0.2	510		
	A/A	51.5 ± 0.9	54	0.427	0.651
Men					
Waist (cm)	G/G + G/A	123.3 ± 0.6	422		
	A/A	127.3 ± 2.2	33	0.078	**0.008**
Waist-to-hip ratio	G/G + G/A	1.09 ± 0.006	420		
	A/A	1.15 ± 0.022	32	**0.007**	**0.009**
Fat mass (kg)	G/G + G/A	52.2 ± 0.8	432		
	A/A	57.5 ± 3.2	36	0.099	0.033
Fat mass %	G/G + G/A	42.1 ± 0.3	432		
	A/A	44.7 ± 1.0	36	**0.011**	0.218

Values are represented as mean ± standard error of the mean (SEM). Adjusted *P*-values are derived from Mann-Whitney Rank Sum test of data adjusted for age and BMI. *P*-values significant after Bonferroni correction for multiple testing ($P < 0.0125$) are indicated in bold

men with distinct genotypes of the *CNR1* G1422A variant. Under a recessive model, we found three significant associations in the same direction viz. 1422A/A homozygotes are more abdominally obese, which adds evidence that these are true associations. Our data indicate that the absence of a *CNR1* gene with the G-allele at position 1422 increases the risk for obesity in males. Although we do not know whether it is visceral or subcutaneous fat that is contributing to the enlarged waist and the increase in fat mass in our study group, abdominal adiposity is indeed linked to a higher risk for cardiovascular disease, particularly in men. The fact that the associations were only seen in obese men can potentially be explained by gender differences in eating in general and fat ingestion in particular, as was previously suggested [8]. On the other hand, the observed discrepancies could also be clarified by the existence of obesity susceptibility gene sets that are different for men and women [9]. While the G1422A polymorphism does not lead to an amino acid change, several other synonymous mutations have previously been associated with complex disease (e.g. ref. [10]). It cannot be excluded, however, that the G1422A SNP is in linkage disequilibrium with a variant which does have an effect on CB1 expression or function. In view of the involvement of endocannabinoids in brain reward mechanisms, it is tempting to speculate that genetic variation in the CB1 receptor may contribute to obesity through overfeeding. Unfortunately, no data are available on the use of rimonabant in the patient population, as it would be interesting to see whether the G1422A variant has an effect on the efficiency of treatment with rimonabant. Since CB1 receptors have been found on the adipocyte [11] and the liver as well [12], it cannot be excluded that genetic variation in these peripheral CB1 receptors may also contribute to metabolic pathways involved in fat distribution. Further research into pathways or genes that are involved in the regulation of food intake, as candidates for obesity, and in the regulating of peripheral metabolism could prove rewarding.

In conclusion, our data indicate that the G1422A polymorphism in the cannabinoid receptor-1 gene is associated with increased abdominal adiposity in obese men.

Material & Methods

Subjects

A total of 251 healthy control individuals (69 men; 182 premenopausal women) were enrolled among the university and hospital personnel. A sum of 1,064 Obese subjects (488 men; 576 pre-menopausal women) were recruited from patients consulting the outpatient obesity clinic at the University Hospital. Subjects were included on the basis of a body mass index (BMI) ≥ 30 kg/m^2 as obese and $18.5 \leq$ BMI < 25 kg/m^2 as controls (Table 2). Postmenopausal women as well as patients defined as being diabetic or with impaired glucose tolerance, on the basis of an oral glucose tolerance test (OGTT) and according to the WHO criteria [13, 14], were excluded from the study. All subjects were Caucasian and at enrolment none were involved in an ongoing weight management program. The study protocol—DNA sampling in particular—was approved by the ethics committee of the Antwerp University Hospital and all subjects gave their written informed consent before participation.

Anthropometry

Height was measured to the nearest 0.5 cm, body weight was measured with a digital scale to the nearest 0.1 kg. BMI was calculated as weight (in kg) over height (in m) squared. Waist circumference was measured at mid-level between the lower rib margin and the iliac crest, and hip circumference at the level of the trochanter major and the waist-to-hip ratio (WHR) was calculated. Fat mass (in kg) was assessed by bio-impedance analysis (BIA) and calculated with the formula of Deurenberg et al. [15].

Genotyping of *CNR1* G1422A

G1422A (rs1049353) genotypes were determined by TaqMan SNP genotyping assays (ABI, Foster City, USA) on a LightCycler LC 480 (Roche, Penzberg, Germany) using a standard protocol as described elsewhere. Blank samples and samples with known genotype were included as negative and positive controls, respectively. We achieved 100% concordance in the analysis of duplicate samples (6% of total).

Statistical Analyses

The HWE program of LINKUTIL (http://www.genemapping.cn/linkutil.htm) was applied to check for deviations of genotype frequencies from Hardy–Weinberg equilibrium. A common odds ratio (OR) was determined with the

Table 2 Characteristics of the study population

Parameter	Controls $N = 251$	Obese $N = 1{,}064$
Age (years)	35 ± 8	41 ± 12
Height (m)	1.69 ± 0.10	1.71 ± 0.09
Weight (kg)	64.4 ± 7.7	61.9 ± 0.7
BMI (kg/m^2)	22.2 ± 1.7	38.1 ± 6.1

Values are represented as mean ± standard deviation (SD)

DeFinetti software [16]. Linear regression was used to adjust anthropometric parameters for age and BMI. Differences between means of parameters by wildtype or variant genotype were evaluated by Wilcoxon rank sum test, for men and women separately. To adjust for multiple testing, the significance level was set at $P = 0.0125$ after Bonferroni correction. All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA).

Acknowledgments We thank J. Vertommen, M. Vinckx and P. Aerts for sampling handling. Financial support to this study was provided by the University of Antwerp (NOI-BOF grant) and the Fund for Scientific Research (FWO) Flanders (research project) both to LVG and WVH. SB holds a predoctoral research grant from the Instituut voor de aanmoediging van innovatie door Wetenschap en Technologie (IWT) in Vlaanderen. The authors do not report any conflict of interest in relation to this article.

References

1. L. Van Gaal, I. Mertens, C. De Block, Nature **444**, 875 (2006)
2. D. Cota, G. Marsicano, M. Tschop, Y. Grubler, C. Flachskamm, M. Schubert, D. Auer, A. Yassouridis, C. Thone-Reineke, S. Ortmann, F. Tomassoni, C. Cervino, E. Nisoli, A.C. Linthorst, R. Pasquali, B. Lutz, G.K. Stalla, U. Pagotto, J. Clin. Invest. **112**, 423 (2003)
3. S. Engeli, J. Bohnke, M. Feldpausch, K. Gorzelniak, J. Janke, S. Batkai, P. Pacher, J. Harvey-White, F.C. Luft, A.M. Sharma, J. Jordan, Diabetes **54**, 2838 (2005)
4. U. Pagotto, G. Marsicano, D. Cota, B. Lutz, R. Pasquali, Endocr. Rev. **27**, 73 (2006)
5. L.F. Van Gaal, A.M. Rissanen, A.J. Scheen, O. Ziegler, S. Rossner, Lancet **365**, 1389 (2005)
6. J.C. Sipe, J. Waalen, A. Gerber, E. Beutler, Int. J. Obes. (Lond) **29**, 755 (2005)
7. D. Gadzicki, K. Muller-Vahl, M. Stuhrmann, Mol. Cell. Probes **13**, 321 (1999)
8. N. Geary, Physiol. Behav. **83**, 659 (2004)
9. P. Arner, Int. J. Obes. Relat. Metab. Disord. **24** Suppl 4, S57 (2000)
10. J. Duan, M.S. Wainwright, J.M. Comeron, N. Saitou, A.R. Sanders, J. Gelernter, P.V. Gejman, Hum. Mol. Genet. **12**, 205 (2003)
11. R. Roche, L. Hoareau, S. Bes-Houtmann, M.P. Gonthier, C. Laborde, J.F. Baron, Y. Haffaf, M. Cesari, F. Festy, Histochem. Cell. Biol. **126**, 177 (2006)
12. D. Osei-Hyiaman, M. DePetrillo, P. Pacher, J. Liu, S. Radaeva, S. Batkai, J. Harvey-White, K. Mackie, L. Offertaler, L. Wang, G. Kunos, J. Clin. Invest. **115**, 1298 (2005)
13. K.G. Alberti, P.Z. Zimmet, Diabet. Med. **15**, 539 (1998)
14. R.A. DeFronzo, E. Ferrannini, Diabetes Care **14**, 173 (1991)
15. P. Deurenberg, J.A. Weststrate, J.G. Hautvast, Am. J. Clin. Nutr. **49**, 33 (1989)
16. T.M. Strom, W.F. Wienker, Case control studies: Tests for deviation from Hardy–Weinberg equilibrium and tests for association. Available from http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; accessed 7 May 2007