G1359A Polymorphism of the Cannabinoid Receptor 1 Is Not Associated with Overweight and Dyslipidemia in Young Northeastern Mexicans

Ismael Rodríguez-Rodríguez 1, Karla Fernández-Quiroga 2, Pedro Araujo-Moreno 2, Isaías Balderas-Rentería 3, Omar Gonzalez-Santiago 4

1. Chemical Science, Jagiellonian University, Kraków, POL. 2. Chemical Science, Universidad Autónoma De Nuevo León, San Nicolas de los Garza, MEX. 3. Chemical Science, Universidad Autónoma De Nuevo León, Monterrey, MEX. 4. Chemical Science, Universidad Autonoma De Nuevo Leon, San Nicolas de los Garza, MEX

© Copyright 2019 Rodríguez-Rodríguez et al. This is an open access article distributed under the terms of the Creative Commons Attribution License CC-BY 3.0., which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

There is extensive evidence to believe that the endocannabinoid system plays an important role in energy homeostasis through a variety of mechanisms. This study aimed to analyze the association between polymorphism rs12720071 of the cannabinoid type 1 receptor (CNR1) gene with dyslipidemia and overweight in young, healthy Mexicans. The association was analyzed with a logistic regression model and expressed as odds ratio (OR). A total of 148 individuals agreed to participate. Overall, the serum concentrations of lipids were found to be in the normal range. However, females presented higher levels of cholesterol and low-density lipoprotein (LDL) than males [probability value (p) = <0.05]. In addition, females presented higher risk of being overweight (BMI: >25) [OR = 3.57; 95% confidence interval (CI): 1.05-12.20; p = 0.04], than males. Our results suggest that this polymorphism could influence BMI in young females.

Categories: Endocrinology/Diabetes/Metabolism, Public Health, Genetics
Keywords: gene polymorphism, cannabis receptor, serum lipids, mexico, young

Introduction

Mexican diet has been changing constantly over the last few years [1]. This shift in diet habits, along with a sedentary culture and increased food intake, has caused Mexicans to develop conditions such as metabolic syndrome, obesity, and overweight, which are risk factors for a variety of chronic diseases like diabetes mellitus, dyslipidemia, and cardiovascular diseases [2,3].

Overweight and obesity have become an important public health problem in Mexico, and they affect both genders and all age groups [4]. It is estimated that by 2050, the percentage of people of normal weight in México will be only 12 % and 9 % of the population for males and females, respectively. It is projected that the obesity and the overweight epidemic would burden the country by $US 1.7 billion in healthcare-related costs by 2050 [5]. The number of individuals who are obese and overweight worldwide is on the rise despite massive efforts to control this hazardous epidemic [6].
The existing evidence suggests that the endocannabinoid system (ECS) is a critical factor in obesity and metabolism, and has been proposed as an appropriate molecular target for efficient anti-obesity treatment [7,8]. The ECS is comprised of the G-coupled cannabinoid receptor types 1 and 2 (CB1 and CB2), the endogenous endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), and the responsible enzymes for their synthesis and degradation [9]. At the central level, cannabis receptors activate both homeostatic and hedonic food-intake pathways in diverse areas of the central nervous system [10]. It also induces the release of various orexigenic and anorexigenic neuropeptides that regulate food-seeking behavior [11,12].

The cannabinoid type 1 receptor (CNR1) gene that encodes the CB1 receptor is located at 6q14-q15 loci, and it contains 4 exons and 5 introns. The region that encodes the CB1 receptor is located at the 5’ end of the exon 4, and the complete gene is 26.1 kb [13]. Reported genetic variants of this gene have proved to be of great relevance due to their association with anthropometric measures of obesity, metabolic disorders, and dyslipidemia, as recently reported in the European [14,15], Caucasian [16,17], Asian [18,19], and southern Brazilian [20] populations.

The main objective of this study was to evaluate the association of rs12720071 polymorphism with serum concentrations of lipids (cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and very-low-density lipoprotein (VLDL)) and BMI in a sample of young individuals of the northeast of Mexico.

Materials And Methods

Subjects

Several young individuals of northeast Mexico were invited to participate. The event took place at the Faculty of Chemical Sciences of Universidad Autonoma de Nuevo Leon (UANL), Nuevo Leon, Mexico from January 2017 through September 2017. Each subject provided a blood sample of 5 ml after 12 hours of nocturnal fasting. The samples were stored at 4° C until their use. Each of the collected blood samples was used for lipid quantification. The inclusion criteria for this study were as follows: (1) subjects should be males or females between the age of 18 and 35; (2) subjects should sign an informed-consent form; (3) subjects should have no prior infectious or any other known diseases. The study had been approved by the institutional board review of the Faculty of Chemical Science (approval number: 04-099604-FAR-11/257).

Measurements

A physical examination was performed in each of the participants. Height (cm) and Weight (kg) were measured and registered. BMI was calculated and serum levels of total cholesterol (TC), HDL, LDL, and VLDL were quantified by spectrophotometry using biochemistry analyzer A25 (BioSystems, Barcelona, Spain).

Genotyping

Genomic DNA was isolated from blood using the phenol-chloroform method, while for genotyping, we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Conditions for both methods had been previously reported by our research team [21].

Statistical analysis

Allelic and genotypic frequencies were determined by direct counting. Hardy-Weinberg equilibrium (HWE) was tested with the χ2 goodness-of-fit test. Comparisons between continuous variables groups were analyzed by Student’s t-test. The association between the
genetic variants of dyslipidemia and overweight (BMI >25) were assessed through logistic regression analyses and expressed as odds ratio (OR) with 95% confidence interval (CI). A probability value (p) of <0.05 was used to assess significance. Statistical analyses were conducted using Minitab version 18 (Minitab, LLC, State College, Pennsylvania).

Results

From the original sample of 200 subjects, 148 agreed to participate; 78 were female and 70 were male. Lipid data of one male individual were not included in the final analysis as he did not meet the fasting requirement adequately. Although the levels of lipids were in the normal range in both genders, females showed higher levels of cholesterol (p = 0.02) and LDL (p = 0.01) than males (Table 1).

|                     | Total  | Male  | Female | Probability value |
|---------------------|--------|-------|--------|-------------------|
| Age (years)         | 19.4   | 19.3  | 19.4   | 0.83              |
| Weight (kg)         | 67.9   | 69.1  | 66.9   | 0.44              |
| BMI (kg/m^2)        | 24.3   | 24.6  | 24     | 0.53              |
| Cholesterol (mg/dL) | 159    | 152.8 | 164.5  | 0.02*             |
| HDL (mg/dL)         | 57.7   | 57.2  | 58.1   | 0.74              |
| LDL (mg/dL)         | 86.1   | 80    | 91.7   | 0.01*             |
| Triglycerides (mg/dL)| 80.3 | 82    | 78.8   | 0.61              |
| VLDL (mg/dL)        | 15.9   | 16.1  | 15.8   | 0.76              |
| Cholesterol/HDL     | 2.9    | 2.8   | 3      | 0.39              |

TABLE 1: Lipid profile in the sample of young Mexicans individuals

HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein

*p = <0.05

The sample was studied in Hardy-Weinberg equilibrium (p = 0.91) with a frequency distribution for various genotypes as follows: AA = 0.85, AG = 0.14, GG = 0.01. Because the GG genotype was only present in one subject, analyses were carried out according to the G dominant model (AA vs AG+GG). In the overall sample, there was no significant difference between AA and AG+GG in lipid profile or BMI. Female carriers of G allele showed higher BMI and lower levels of HDL than those of AA genotype; however, the HDL levels were within the normal range in both genders (Table 2).
| Variable       | Total    | Male         | Female        |
|---------------|----------|--------------|---------------|
|               | AA (N=125) | AG+GG (N=22) | Probability value | AA (N=61) | AG+GG (N=9) | Probability value | AA (N=64) | AG+GG (N=13) | Probability value |
| Age (years)   | 19.4     | 19.1         | 0.37          | 19.3       | 19.3       | 0.98           | 19.5       | 19           | 0.28             |
| Weight (kg)   | 67.1     | 73           | 0.16          | 69         | 69.9       | 0.9            | 65.2       | 75.1         | 0.06             |
| BMI (kg/m²)   | 24       | 26.2         | 0.09          | 24.7       | 24         | 0.75           | 23.3       | 27.7         | <0.01*           |
| Cholesterol (mg/dL) | 160.4   | 150.7        | 0.17          | 153.8      | 146.6      | 0.47           | 166.8      | 153.5        | 0.19             |
| HDL (mg/dL)   | 58.5     | 53.2         | 0.15          | 57.1       | 57.9       | 0.9            | 59.8       | 49.9         | 0.03*            |
| LDL (mg/dL)   | 86.6     | 83.7         | 0.67          | 80.5       | 76.3       | 0.65           | 92.3       | 88.9         | 0.72             |
| Triglycerides (mg/dL) | 82.3   | 69           | 0.12          | 84.9       | 62         | 0.13           | 79.8       | 73.8         | 0.54             |
| VLDL (mg/dL)  | 16.3     | 13.8         | 0.14          | 16.7       | 12.3       | 0.15           | 16         | 14.8         | 0.55             |
| Cholesterol/HDL | 2.9     | 2.9          | 0.94          | 2.9        | 2.5        | 0.32           | 2.9        | 3.1          | 0.45             |

**TABLE 2: Anthropometric variables and serum lipid levels according to genotypes**

HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein; AA, AG, AG: genotypes

*p = <0.05

According to the results of the logistic regression analyses, the rs12720071 polymorphism is not associated with dyslipidemia [OR = 0.22 (95% CI: 0.02-1.74)] nor overweight [OR = 1.49 (95% CI: 0.59-3.77)]. However, a significant association was found between this polymorphism and a higher risk of being overweight in women carrying the G allele [OR = 3.57 (95% CI: 1.04-12.19)] (BMI = 25 kg / m² as the cutoff point).

**Discussion**

Obesity is a major health issue caused by genetic and environmental factors [22]. Since some studies have associated the ECS with different obesity-related conditions, it has become a potential target for new genotype-phenotype association studies, offering a new perspective on how genetics plays an important role in people acquiring this condition [23].

In this study, we quantified serum lipid levels in a sample of young Mexicans who had previously been genotyped in the CNR1 gene. We believe this is the first study about the association between cannabis receptor polymorphism and lipid levels in a sample of young individuals (18-25 years old). The calculated allelic frequency of the genotype was consistent with those reported for a Mexican-ancestry population from Los Angeles, California [24], as well as with those reported in a Caucasian population [25].

Overall, there was no significant difference between AA and AG+GG genotypes of rs12720071 polymorphism in the lipid profile and BMI. This finding suggests that this polymorphism probably has no impact on the lipid metabolism of young Mexican individuals. However,
previous studies in adults of other countries indicate that carriers of the G allele present obesity traits such as a higher android fat deposit [14], increased subscapular skinfold thickness and waist circumference [26]. In fact, the literature regarding this genetic variant appears to be inconsistent since other studies have reported no association between it and any adiposity-related trait [20,27], such as nonalcoholic fatty liver disease in women with polycystic ovary syndrome [28]. In addition, a study has shown a lower prevalence of the metabolic syndrome in those subjects carrying the G allele (OR: 0.598; p = 0.003), pointing it out as an independent predictive factor for the lack of this syndrome [27]. These inconsistencies could be explained by a wide number of environmental factors such as lifestyle, diet, gender, age, and genetic background.

An interesting finding of this study was the significant difference between AA and AG+GG genotypes of females. Carriers of allele G showed higher BMI and lower levels of HDL than the AA genotype. This finding suggests that, unlike men, the polymorphism studied may affect BMI and lipid profile of women. However, more studies are needed since other factors such as sex hormones may have greater effect than polymorphism studied. In this sense, it should be remembered that the adverse effects of cannabis consumption are more pronounced in women than men [29].

A limitation of our study was the small sample size, which could have lead to low statistical power and may explain the lack of any findings related to association with dyslipidemia and overweight in man. Another limitation was the number of analyzed genetic variants in our study. We assessed only one polymorphism of the CNR1 gene, but there are many others that have been recently implicated with adverse metabolic profiles and obesity characteristics.

Conclusions

We found that the G1359A polymorphism of the CNR1 gene is not associated with overweight and dyslipidemia in the young Mexicans we studied. However, female carriers of G allele present higher BMI and lower levels of HDL than those of AA genotype. The ongoing research regarding the association of the CNR1 gene with obesity and dyslipidemia is critical for the understanding of how the endocannabinoid system regulates overall energy expenditure in the human body. We believe further studies with bigger sample sizes are needed in order to confirm our findings.

Additional Information

Disclosures

**Human subjects:** Consent was obtained by all participants in this study. Board Review of Faculty of Chemical Science issued approval 04-099604-FAR-11/257. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors declare that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

This work was supported by the Faculty of Chemical Science of the Universidad Autónoma de Nuevo León.
References

1. Salas R, Bibiloni M del M, Ramos E, Villarreal JZ, Pons A, Tur JA, Sureda A: Metabolic syndrome prevalence among northern Mexican adult population. PLoS ONE. 2014, 9:e105581. Accessed: September 26, 2019: 10.1371/journal.pone.0105581

2. Ama Moor VJ, Ndongo Amougou S, Ombotto S, Ntone F, Wouamba DE, Ngo Nonga B: Dyslipidemia in patients with a cardiovascular risk and disease at the University Teaching Hospital of Yaoundé, Cameroon. Int J Vasc Med. 2017, 10.1155/2017/6061306

3. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM: Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007, 49:405-14. 10.1016/j.jacc.2006.09.052

4. Hernández-Cordero S, Cuevas-Nasu L, Morales-Ruán MC, Humarán IM, Ávila-Arcos MA, Rivera-Dommarco JA: Overweight and obesity in Mexican children and adolescents during the last 25 years. Nutr Diabetes. 2017, 7:e280. Accessed: September 26, 2019: 10.1038/nutd.2017.29

5. Rtveladze K, Marsh T, Barquera S, et al.: Obesity prevalence in Mexico: impact on health and economic burden. Public Health Nutr. 2014, 17:233-9. 10.1017/S1368980015000086

6. NCD Risk Factor Collaboration (NCD-RisC): Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet. 2017, 390:2627-42. 10.1016/S0140-6736(17)32129-3

7. Matias I, Gatta-Cherifi B, Cota D: Obesity and the endocannabinoid system: circulating endocannabinoids and obesity. Curr Obes Rep. 2012, 1:229-35. Accessed: September 26, 2019: 10.1007/s13679-012-0027-6

8. Pacher P, Bátkai S, Kunos G: The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev. 2006, 58:389-462. 10.1124/pr.58.3.2

9. Naughton SS, Mathai ML, Hryciw DH, McAinch AJ: Fatty acid modulation of the endocannabinoid system and the effect on food intake and metabolism. Int J Endocrinol. 2013, 10.1155/2013/361895

10. Wenzel JM, Cheer JF: Endocannabinoid regulation of reward and reinforcement through interaction with dopamine and endogenous opioid signaling. Neuropsychopharmacology. 2018, 43:103-15. 10.1038/npp.2017.126

11. Silvestri C, Di Marzo V: The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab. 2013, 17:475-90. 10.1016/j.cmet.2013.03.001

12. Kirkham TC, Williams CM, Fezza F, Di Marzo V: Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol. 2002, 136:550-7. 10.1038/sj.bjp.0704767

13. Laprairie RB, Kelly MEM, Denovan-Wright EM: The dynamic nature of type 1 cannabinoid receptor (CB(1)) gene transcription. Br J Pharmacol. 2012, 167:1583-95. 10.1111/j.1476-5381.2012.02175.x

14. Milewicz A, Tworowska-Bardzińska U, Jędrzejuk D, Lwow F, Dunajska K, Laczmarski Ł, Pawlak M: Are endocannabinoid type 1 receptor gene (CNR1) polymorphisms associated with obesity and metabolic syndrome in postmenopausal Polish women?. Int J Obes (Lond). 2011, 35:373-7. 10.1038/ijo.2010.145

15. Benzinoiu M, Chèvre JC, Ward KJ, et al.: Endocannabinoid receptor 1 gene variations increase risk for obesity and modulate body mass index in European populations. Hum Mol Genet. 2008, 17:1916-21. 10.1093/hmg/ddn089

16. Baye TM, Zhang Y, Smith E, et al.: Genetic variation in cannabinoid receptor 1 (CNR1) is associated with derangements in lipid homeostasis, independent of body mass index. Pharmacogenomics. 2008, 9:1647-56. 10.2217/1462249.11.1647

17. de Luis DA, González Sagrado M, Aller R, Izaola O, Conde R: Relation of G1359A polymorphism of the cannabinoid receptor (CB1) gene with metabolic syndrome by ATP III classification. Diabetes Metab Res Rev. 2011, 27:506-11. 10.1002/dmr.1200

18. Zhuang M, Yang Y, Cao F, et al.: Associations of variants of CNR1 with obesity and obesity-related traits in Chinese women. Gene. 2012, 495:194-8. 10.1016/j.gene.2011.12.037

19. Mutombo PB, Yamasaki M, Nabika T, Shiwaku K: Cannabinoid receptor 1 (CNR1) 4895 C/T genetic polymorphism was associated with obesity in Japanese men. J Atheroscler Thromb.
20. Jaeger JP, Mattevi VS, Callegari-Jacques SM, Hutz MH: Cannabinoid type-1 receptor gene polymorphisms are associated with central obesity in a southern Brazilian population. Dis Markers. 2008, 25:67-74. 10.1155/2008/841490

21. Rodríguez-Rodríguez IA, Fernandez-Quiroga KA, Morales-San Claudio PD, Balderas-Rentería I, González-Santiago O: No association between G1359A CB1 polymorphisms and pain in young northeastern Mexicans. Pharmacogenomics. 2018, 19:1251-58. 10.2217/pgs-2018-0125

22. Sheikh AB, Nasrullah A, Haq S, et al.: The interplay of genetics and environmental factors in the development of obesity. Cureus. 2017, 9:e1435. Accessed: September 26, 2019: 10.7759/cureus.1435

23. Lyon HN, Hirschhorn JN: Genetics of common forms of obesity: a brief overview. Am J Clin Nutr. 2005, 82:215S-217S. 10.1093/ajcn/82.1.215S

24. Genomes Project Consortium, Auton A, Brooks LD, et al.: A global reference for human genetic variation. Nature. 2015, 526:68-74. 10.1038/nature15393

25. Ho B-C, Wassink TH, Ziebell S, Andreasen NC: Cannabinoid receptor 1 gene polymorphisms and marijuana misuse interactions on white matter and cognitive deficits in schizophrenia. Schizophr Res. 2011, 128:66-75. 10.1016/j.schres.2011.02.021

26. Russo P, Strazzullo P, Cappuccio FP, et al.: Genetic variations at the endocannabinoid type 1 receptor gene (CNR1) are associated with obesity phenotypes in men. J Clin Endocrinol Metab. 2007, 92:2382-6. 10.1210/jc.2006-2523

27. Lieb W, Manning AK, Florez JC, et al.: Variants in the CNR1 and the FAAH genes and adiposity traits in the community. Obesity (Silver Spring). 2009, 17:755-60. 10.1038/oby.2008.608

28. Kuliczewska Plaksej J, Laczmanski L, Milewicz A, et al.: Cannabinoid receptor 1 gene polymorphisms and nonalcoholic fatty liver disease in women with polycystic ovary syndrome and in healthy controls. Int J Endocrinol. 2014, 10.1155/2014/232975

29. Craft RM, Marusich JA, Wiley IL: Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system?. Life Sci. 2015, 92:476-81. 10.1016/j.lfs.2012.06.009