Role of Genetic Variation in the Cannabinoid Receptor Gene (*CNR1*) (G1359A Polymorphism) on Weight Loss and Cardiovascular Risk Factors After Liraglutide Treatment in Obese Patients With Diabetes Mellitus Type 2

Daniel Antonio de Luis, MD, Hilda F. Ovalle, PhD, Gonzalo Diaz Soto, PhD, Olatz Izaola, PhD, Beatriz de la Fuente, PhD, and Enrique Romero, MD

Background: A polymorphism (1359 G/A) of the cannabinoid receptor 1 (*CNR1*) gene was reported as a common polymorphism (rs1049353) with potential implications in weight loss. We decide to investigate the role of this polymorphism on metabolic changes and weight loss secondary to treatment with liraglutide.

Methods: A population of 86 patients with diabetes mellitus type 2 and obesity, unable to achieve glycemic control (hemoglobine glycate A1c >7%) with metformin alone or associated to sulfonylurea, who require initiation of liraglutide treatment in progressive dose to 1.8 mg/d subcutaneously, was analyzed.

Results: Fifty-one patients (59.3%) had the genotype *G1359G*, and 35 patients (40.7%) had *G1359A* (28 patients, 32.6%) or *A1359A* (7 patients, 8.1%) (A allele carriers). In patients with both genotypes, basal glucose, HbA_{1c}, body mass index, weight, fat mass, waist circumference, and systolic blood pressures decreased. In patients with G1359G genotype, total cholesterol and low-density lipoprotein cholesterol decreased, and in patients with A allele, homeostasis model assessment for insulin resistance decreased, too.

Conclusions: There is an association of the A allele with an improvement of insulin resistance secondary to weight loss after liraglutide treatment in obese patients with diabetes mellitus type 2. Noncarriers of A allele showed an improvement in cholesterol levels after weight loss.

Key Words: cannabinoid receptor gene, liraglutide, metabolic parameters, obesity

(*J Investig Med* 2014;62: 324–327)

Obesity and overweight are major public health problems and have been linked as risk factors for many common diseases such as diabetes mellitus type 2. Hypocaloric diets are known to be an effective treatment for overweight and obese subjects,[1] and the weight reduction also improves parameters of type 2 diabetes mellitus. However, several pharmacological agents have been developed to treat patients with diabetes mellitus type 2 in last years, as glucagon-like peptide 1 (GLP-1)–based therapies.[2]

Moreover, obesity has multiple causes and is determined by the interaction between genetic and environmental factors. For example, the endocannabinoid system is involved in the control of food intake and body weight.[3] This endocannabinoid system is composed of a number of proteins and has been demonstrated to play a role in appetite and body weight. This system consists of endogenous ligand anandamide and 2-arachidonoylglycerol and G protein–coupled cannabinoid receptors such as cannabinoid type 1 receptor (*CNR1*), located in several brain areas and in a variety of peripheral tissues including adipose tissue.[4] A polymorphism (1359 G/A) (rs1049353) of the *CNR1* gene was reported as a common polymorphism in white population,[5] reaching frequencies of 24% to 32% and 44.2% in the Spanish population[6] for the allele (A). At present, there are few intervention studies; Aberle et al.[7] have shown that carriers of the A allele lost more weight with an improvement of metabolic values. De Luis et al.[8] have shown with a conventional hypocaloric diet that carriers of the A allele were associated with larger improvements in adipokine levels. Nevertheless, in other study,[9] the A allele of *CNR1* was associated with a lack of improvement on metabolic parameters after 2 different hypocaloric diets. However, as far as we know, the effect of this polymorphism on weight loss and metabolic improvement after treatment with diabetic drugs has not been explored. Liraglutide is a once-daily human GLP-1 analog. Liraglutide clinical studies have demonstrated blood glucose and weight-reducing effects with a low risk of hypoglycemic events.[10] Furthermore, a potential protective role for liraglutide in cardiovascular events has been suggested from short-term clinical trials.[11]

Considering the evidence that endogenous cannabinoid system plays a role in metabolic aspects of body weight, we decide to investigate the role of polymorphism (G1359A) of *CNR1* receptor gene on metabolic changes and weight loss secondary to treatment with liraglutide in obese diabetic subjects.

SUBJECTS AND METHODS

Subjects

A population of 86 patients with diabetes mellitus type 2 and obesity (body mass index [BMI] >30 kg/m^2), unable to achieve glycemic control (hemoglobine glycate A1c [HbA_{1c}] >7%) with metformin alone or associated to sulfonylurea, who require initiation of liraglutide treatment in progressive dose to 1.8 mg/d subcutaneously, was analyzed in a prospective way. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the local ethical committee. Written informed consent was obtained from all subjects. Interviews

From the Center of Investigation of endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, Valladolid, Spain.
Received May 20, 2013, and in revised form October 4, 2013.
Accepted for publication October 22, 2013.
Reprints: Daniel Antonio de Luis, MD, Center of Investigation of Endocrinology and Nutrition, Medicine School, Valladolid University, C/Los perales 16, Simancas 47130, Valladolid, Spain. E-mail: dadluis@yahoo.es.
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
The author report no conflicts of interest.
Authors' contributions: D.A.dL. and G.D.S.: designed the study and wrote the article; H.F.O.: laboratory determinations; O.I., E.R., and B.F.: anthropometric and dietetic works.
Copyright © 2014 by The American Federation for Medical Research
ISSN: 1081-5589
DOI: 10.231/JIM.0000000000000032

Copyright © 2014 American Federation for Medical Research. Unauthorized reproduction of this article is prohibited.

J Investig Med: first published as 10.2310/JIM.0000000000000032 on 15 December 2015. Downloaded from file:/ on April 30, 2024 by guest. Protected by copyright.

collected summarily clinical history of the patients. Laboratory and analytical values were also recorded. Privacy and confidentiality were ensured for collected data.

Procedure and Treatment

Weight, body composition, waist circumference, fat mass by bioimpedance, blood pressure, glucose, HbA_{1c}, insulin, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglycerides, and homeostasis model assessment for insulin resistance (HOMA-R) were measured in fasting condition at baseline and 14 weeks after liraglutide treatment. A tetrapolar bioimpedance was realized. Genotype of *CNR1* receptor gene polymorphism was studied. Obese patients with diabetes mellitus type 2 unable to achieve glycemic control (HbA_{1c} >7%) with metformin alone or associated to sulfonylurea received liraglutide treatment in progressive dose to 1.8 mg/d subcutaneously.

Biochemical Assays and Genotyping of (*CNR1*) Gene Polymorphism

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd, 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 Friedewald formula.

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose Analyser 2; Beckman Instruments, Fullerton, CA). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5 mUI/L (reference range, 0.5-30 mUI/L),[12] and the HOMA-R was calculated using these values.[13] HbA_{1c} was measured by turbidimetric inhibition immunoassay standardized to National Glycohemoglobin Standardization Program quality criteria (Roche Diagnostics, Geneva, Switzerland). DNA extraction was realized with a lysate of blood cells (Gene-all; Biorad, Seoul, Korea). Buffy coats of nucleated cells obtained from anticoagulated blood (ACD or EDTA) were resuspended in 15 mL polypropylene centrifugation tubes with 3 mL of nuclei lysis buffer. The cell lysates were digested overnight at 37°C with 0.2 mL of 10% sodium dodecyl sulfate and 0.5 mL of a protease K solution. After digestion was complete, 1 mL of saturated NaCl (approximately 6 M) was added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 revolutions/min for 15 minutes. The precipitated protein pellet was left at the bottom of the tube, and the supernatant containing the DNA was transferred to another 15-mL polypropylene tube. The precipitated DNA strands were removed with a plastic spatula or pipette and transferred to a 1.5-mL microcentrifuge tube containing 100 to 200 pl TE buffer. The DNA was allowed to dissolve 2 hours at 37°C before quantitating. 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 probes (wild probe: 5′-Fam-ATC AAG AGC ACG GTC AAG ATT GCC-BHQ-1-3′) and (polymorphic 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° 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 with a statistical test (χ^2) to compare our expected and observed counts. The 2 variants were in Hardy-Weinberg equilibrium.

Weight and Anthropometric Measurements

Body weight was measured to an accuracy of 0.1 kg and BMI 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 also measured. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g (Biodynamics Model 310e, Seattle, WA).[14] The same investigator measured patients and control subjects. Precautions taken to ensure valid BIA measurements were as follows: no alcohol within 24 hours of taking the test and no exercise or food for 4 hours before taking the test.[15]

Blood pressure was measured twice after a 10-minute rest with a mercury sphygmomanometer, and averaged.

Statistical Analysis

Sample size was calculated to detect differences over 3 kg in body weight with 90% power and 5% significance (n = 80). The results were expressed as average ± SD. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables were analyzed with a 2-way analysis-of-variance model. Qualitative variables were analyzed with the χ^2 test, with Yates correction as necessary, and Fisher test. A χ^2 test was used

TABLE 1. Changes in Anthropometric Variables

	G1359G		*G1359A* or *A1359A*	
Characteristics	**0 Time**	**At 14 wk**	**0 Time**	**At 14 wk**
BMI	34.1 ± 6.9	32.7 ± 6.5*	31.8 ± 5.1†	30.4 ± 5.7*†
Weight, kg	90.7 ± 19.4	87.6 ± 19.2*	82.9 ± 15.3†	79.9 ± 15.1*†
Fat free mass, kg	55.5 ± 10.4	54.8 ± 11.1	53.1 ± 7.6	51.9 ± 7.6
Fat mass, kg	34.8 ± 14.2	32.3 ± 14.8*	29.9 ± 11.4†	28.1 ± 11.1*†
Waist circumference, cm	108.8 ± 15.7	107.1 ± 16.1*	105.4 ± 12.9†	103.1 ± 11.4*†
Waist-to-hip ratio	1.01 ± 0.09	0.98 ± 0.09*	1.00 ± 0.1	0.96 ± 0.09*
Systolic BP, mm Hg	146.1 ± 25.4	140.4 ± 19.7*	145.1 ± 22.1	142.1 ± 19.5*
Diastolic BP, mm Hg	78.3 ± 10.5	79.1 ± 8.8	80.5 ± 14.2	77.5 ± 11.5

*$P < 0.05$ in each group with basal values.

†$P < 0.05$ comparison in a different genotype group in the same time (basal tome or at 14 weeks).

© 2014 The American Federation for Medical Research

Copyright © 2014 American Federation for Medical Research. Unauthorized reproduction of this article is prohibited.

J Investig Med: first published as 10.2310/JIM.0000000000000032 on 15 December 2015. Downloaded from file:/ on April 30, 2024 by guest. Protected by copyright.

J Investig Med: first published as 10.2310/JIM.0000000000000032 on 15 December 2015. Downloaded from file:/ on April 30, 2024 by guest. Protected by copyright.

to evaluate the Hardy-Weinberg equilibrium. Nonparametric variables were analyzed with the Wilcoxon test. The statistical analysis was performed for the combined *G1359A* and *A1359A* as a group and genotype G1359G as second group, with a dominant model. $P < 0.05$ was considered statistically significant.

RESULTS

Eighty-six obese patients with diabetes mellitus type 2 gave informed consent and were enrolled in the study. The mean age was 60.3 ± 9.6 years, and the mean BMI was 33.2 ± 6.3 kg/m^2, with 44 males (51.2%) and 42 females (48.8%).

Fifty-one patients (59.3%) had the genotype *G1359G*, and 35 (40.7%) patients had *G1359A* (28 patients, 32.6%) or *A1359A* (7 patients, 8.1%) (A allele carriers). Age was similar in both genotype groups (48.9 ± 16.3 vs 47.3 ± 16.4 years; not statistically significant). Sex distribution was similar in both groups, G1359G (49.0% males vs 51.0% females) and A allele carriers (54.3% males vs 45.7% females).

Table 1 shows the differences in anthropometric variables. In patients with both genotypes, BMI, weight, fat mass, waist circumference, and systolic blood pressures decreased. The amount of these decreases was similar in both genotypes. Patients with genotype G1359G lost 3.4% ± 3.7% of weight and patients with A allele (3.2% ± 10.5%). The percentage of no responders (patients without weight loss) was 13.7% (n = 7) in G1359G genotype and 17.1% (n = 6) in carriers of A allele. Before and after treatment, BMI, weight, fat mass, and waist circumference were higher in patients with G1359G genotype than carriers of A allele.

Table 2 shows the biochemical parameters. In patients with G1359G genotype, basal glucose levels, HbA_{1c}, total cholesterol, and LDL cholesterol decreased. In patients with A allele, basal glucose, HbA_{1c}, and insulin resistance (HOMA-R) decreased. The decrease in basal glucose and HbA_{1c} was similar in both genotypes. Before and after treatment, biochemical parameters were similar in both genotypes.

DISCUSSION

The main finding of this study is the association of the A allele with an improvement of insulin resistance secondary to weight loss after liraglutide treatment in obese patients with diabetes mellitus type 2. Noncarriers of A allele showed an improvement in cholesterol levels after weight loss. Weight loss and improvement of glycemic control were similar in patients with both genotypes.

Our study showed a prevalence of GA genotype (40.7%), similar to others, 43.5%[15] and 33.1%.[16] Moreover, the association between BMI and this polymorphism is in agreement with the results obtained by Gazzerro et al.[16] with SNP G1359A of CB1 receptor, A3813G, A4895G, G1422A, A3813A, and A4895A SNPs of CB1 receptor[17] and with (G1422A) SNP of *CNR1* receptor.[18]

Our present findings show an interaction between biochemical changes secondary to liraglutide and this polymorphism (rs1049353). In the literature, there are no studies that have evaluated the interaction of this polymorphism with the effects of treatment with diabetic drugs. Weight loss was related to an improvement in body composition measured by tetrapolar body electrical bioimpedance, as previously described.[15] Our data show that this polymorphism did not have any effect on weight loss secondary to liraglutide treatment. In obese patients and after hypocaloric diets, de Luis et al.[8,9] did not detect an interaction between rs1049353 and weight loss. Nevertheless, Aberle et al.[7] have shown that carriers of the A allele lost more weight.

The effects of this polymorphism on metabolic changes after weight loss are also a contradictory area. In human models, Aberle et al.[7] have shown that this polymorphism is related with lipid changes after weight loss. This relation was also observed with 2 different hypocaloric diets.[9] Our results showed an improvement of lipid profile after weight loss in patients without A allele.

Homeostasis model assessment evaluation under GLP-1 treatment has been previously studied. Homeostasis model assessment is a widely validated clinical and epidemiological tool for the estimation of insulin resistance (HOMA-IR).[19] In our study, liraglutide treatment showed statistically significant lower insulin resistance compared with baseline in A allele carriers. Previous clinical studies with GLP-1 analogs[11,20] have shown this improvement; however, the genotype of diabetic patients have not been evaluated in previous studies; a potential interaction with genetic background of these patients could be present. Improvement in glucotoxicity and lipotoxicity under liraglutide treatment could explain HOMA amelioration.

Some reasons could explain the contradictory results of the literature with the effect of this polymorphism in weight loss and metabolic changes. First, the different type of intervention to lose weight could influence the metabolic results and their interactions with rs1049353. Second, duration of intervention may influence secondary metabolic responses to weight loss as a function of this polymorphism. The duration of dietary interventions has been around 6 weeks[7] until 12 weeks,[9,10] and it is the first study in the literature to evaluate the effect with a drug. Perhaps the interaction rs1049353 polymorphism and

TABLE 2. Classic Cardiovascular Risk Factors

	G1359G		*G1359A* or *A1359A*	
Characteristics	**0 Time**	**At 14 wk**	**0 Time**	**At 14 wk**
Glucose, mg/dL	185.1 ± 62.4	152.4 ± 56.3*	172.8 ± 61.4	142.8 ± 48.8*
Total cholesterol, mg/dL	195.5 ± 41.9	187.7 ± 34.5*	185.6 ± 39.7	179.6 ± 27.3
LDL cholesterol, mg/dL	115.1 ± 33.8	110.8 ± 31.3*	109.9 ± 33.1	104.5 ± 25.6
HDL cholesterol, mg/dL	47.1 ± 13.1	45.8 ± 12.6	44.6 ± 12.6	45.7 ± 10.8
TG, mg/dL	172.5 ± 96.1	162.9 ± 84.4	160.1 ± 49	147.2 ± 69.5
Insulin, mUI/L	13.5 ± 9.5	14.6 ± 10.7	16.5 ± 15.7	14.4 ± 13.4
HOMA	5.1 ± 4.1	4.1 ± 3.9	7.6 ± 8.8	5.8 ± 7.4*
HbA_{1c}	8.6 ± 1.4	7.4 ± 1.2*	8.6 ± 1.4	7.6 ± 1.1*

*$P < 0.05$ in each group with basal values.

TG indicates triglycerides.

© 2014 The American Federation for Medical Research

Copyright © 2014 American Federation for Medical Research. Unauthorized reproduction of this article is prohibited.

weight loss secondary to diet are modulated during the time. Finally, the amount of weight loss could also have an influence. Recently, a surgical study with bariatric procedure[21] has shown different effects of rs1049353 on metabolic parameters after a massive weight loss.

In conclusion, there is an association of the A allele with an improvement of insulin resistance secondary to weight loss after liraglutide treatment in obese patients with diabetes mellitus type 2. Noncarriers of A allele showed an improvement in cholesterol levels after weight loss. Weight loss and glycemic control were similar in patients with both genotypes. Further studies will be designed to clarify the interaction of this polymorphism with metabolic response secondary to liraglutide.

REFERENCES

1. de Luis DA, Aller R, Izaola O, et al. Effects of lifestyle modification on adipocytokine levels in obese patients. *Eur Rev Med Pharmacol Sci*. 2008;12:33–39.
2. Inzucchi SE, Bergenstal RM, Buse JB, et al. American Diabetes Association (ADA); European Association for the Study of Diabetes (EASD). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*. 2012;35(6):1364–1379.
3. Ameri A. The effects of cannabinnoids on the brain. *Prog Neurobiol*. 1999;58:315–348.
4. Felder CC, Glass M. Cannabinoid receptors and their endogenous agonists. *Ann Rev Pharmacol Toxicol*. 1998;38:179–200.
5. Gadzicki D, Muller-Vahl K, Stuhrmann M. A frequent polymorphism in the coding exon of the human cannabinoid receptor (*CNR1*) gene. *Mol Cell Probes*. 1999;13:321–323.
6. de Luis DA, Gonzalez M, Aller R, et al. Relation of G1359A polymorphism of the cannabinoid receptor gene with metabolic syndrome ATPIII classification. *Diabet Metab Res Rev*. 2011;27:506–511.
7. Aberle J, Fedderwitz I, Klages N, et al. Genetic variation in two proteins of the endocannabinoid system and their influence on body mass index and metabolism under low fat diet. *Horm Metab Res*. 2007;39:395–397.
8. de Luis DA, Gonzalez Sagrado M, Aller R, et al. Role of G1359A polymorphism of the cannabinoid receptor gene (*CNR1*) on weight loss and adipocytokines after a hypocaloric diet. *Nutr Hosp*. 2011;26(2):317–322.
9. de Luis DA, Gonzalez Sagrado M, Aller R, et al. Role of G1359A polymorphism of the cannabinoid receptor gene (*CNR1*) on weight loss and adipocytokines after 2 hypocaloric diets. *Metab Clin Exp*. 2010;59:1387–1392.
10. Vilsbøll T, Zdravkovic M, Le-Thi T, et al. Liraglutide significantly improves glycemic control, and lowers body weight without risk of either major or minor hypoglycaemic episodes in subjects with type 2 diabetes. *Diabetes Care*. 2007;30:1608–1610.
11. Vilsbøll T, Brock B, Perrild H, et al. Liraglutide, a once-daily human GLP-1 analogue improves β-cell function and arginine-stimulated insulin secretion at hyperglycaemia in patients with type 2 diabetes mellitus. *Diabet Med*. 2008;25:152–156.
12. Duart MJ, Arroyo CO, Moreno JL. Validation of a insulin model for the reactions in RIA. *Clin Chem Lab Med*. 2002;40:1161–1167.
13. Mathews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–414.
14. Aberle J, Flitsch J, Alessia N, et al. Genetic variation may influence obesity only under conditions of diet: analysis of three candidate genes. *Mol Genet Metab*. 2008:95:188–191.
15. Lukaski H, Johson PE. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr*. 1985;41(4):810–817.
16. Gazzerro P, Caruso MG, Notarnicola M, et al. Association between cannabinoid type 1 receptor polymorphism and body mass index in a southern Italian population. *Int J Obes*. 2007;31:908–912.
17. Russo P, Strazzullo P, Cappuccio F, et al. Genetic variations at the endocannabinoid type 1 receptor gene (*CRN1*) are associated with obesity phenotypes in men. *J Clin Endocrinol Metab*. 2007;92:2382–2389.
18. Peeters A, Beckers S, Mertens I, et al. The G1422A variant of the endocannabinoid receptor gene is associated with abdominal adiposity in obese men. *Endocr*. 2007:31:138–141.
19. Buse JB, Rosenstock J, Sesti J, et al. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet*. 2009;374:39–47.
20. Vilsbøll T, Christensen M, Junker AE, et al. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *BMJ*. 2012;344:d7771.
21. de Luis DA, Pacheco D, Aller R, et al. G1359A polymorphism of the endocannabinoid receptor gene *CNR1* and clinical results of biliopancreatic diversion. *Eur Rev Med Pharmacol Sci*. 2011;14:197–201.

J Investig Med: first published as 10.2310/JIM.0000000000000032 on 15 December 2015. Downloaded from file:/ on April 30, 2024 by guest. Protected by copyright.

© 2014 The American Federation for Medical Research

Copyright © 2014 American Federation for Medical Research. Unauthorized reproduction of this article is prohibited.