

The gene variant rs2419621 of *ACYL-CoA synthetase long-chain 5* gene is associated with weight loss and metabolic changes in response to a robotic sleeve gastrectomy in morbid obese subjects

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Abstract. – OBJECTIVE: Genetic mechanisms have been involved in the pathogenesis of obesity and weight loss due to bariatric surgery. The aim of our work was to evaluate the effects of rs2419621 genetic variant of ACSL5 gene on weight and metabolic changes after a robotic sleeve gastrectomy.

PATIENTS AND METHODS: 48 patients were enrolled. Comorbidities, biochemical and anthropometric parameters evaluation were registered before and after 3, 6 and 12 months follow up. Genotype of rs2419621 ACSL5 gene was evaluated.

RESULTS: We classified the subjects with a dominant model, in two groups: those carriers T allele (TT+CT, 37.5%) and non-carriers T allele (CC, 62.5%). We reported a statistically significant reduction of body weight, waist circumference, percentage of excess of weight loss (EWL%), blood pressure, glucose, insulin, LDL-cholesterol and triglycerides after surgery. After 12 months, delta of (EWL%; 70.1% vs. 64.2%; $p=0.04$), weight (40.7+4.1 kg vs. 32.5+4.8 kg; $p=0.03$), waist circumference (29.1+3.1 cm vs. 22.2+2.8 kg; $p=0.02$) and triglycerides (51.2+9.1 mg/dl vs. 32.1+8.1; $p=0.02$) were higher in T allele carriers than non-T allele carriers. All comorbidities improved, but the percentage of patients with hypertriglyceridemia diminished early in the 3-month follow-up in the T-allele carriers, and at 12 months, no patient with the T allele had hypertriglyceridemia.

CONCLUSIONS: Our data showed that the genetic variant (rs2419621) of ACSL5 gene are associated with better improvement of adiposity and triglyceride levels in subjects with T allele, after a robotic sleeve gastrectomy.

Key Words:

ACSL5, Robotic sleeve gastrectomy, Rs2419621, Triglyceride, Weight loss.

Introduction

Obesity and overweight are two major risk factors for a variety of chronic diseases including hypertension, diabetes mellitus type 2, hyperlipidemia, cardiovascular diseases and cancer, reaching pandemic diffusion. For example, the prevalence of obesity in the world was 12%¹, while in Spain it was 22%². The cornerstone of all treatments options for obese subjects is to follow a reduced calorie diet with physical activity, with the goal of achieving clinically meaningful weight loss of at least 5-10%. However, these dietary approaches have proven limited effectiveness; conversely, bariatric surgery produces an important weight change, with an interesting effect in reducing comorbidities³.

On the other hand, genetic and epigenetic mechanisms have been implicated in the pathogenesis of obesity and the weight change secondary to the treatments^{4,5}. Genes related to fatty acid (FA) partitioning are strong candidates for weight improvement and metabolic changes. Fatty acyl-CoA molecules are known to be implicated in the energy synthesis by Beta-Oxidation, lipid component of the cell and as energy storage through lipid production. The Acyl-CoA synthetases long-chain (ACSL) induces intracellular free long-chain fatty acids by transforming them to fatty acyl-CoA molecules. Due to the position in the inner mitochondrial membrane in liver, the ACSL5 isoform is involved in producing acyl-CoA destined for mitochondrial oxidation⁶. Besides, the possibility to improve or maintain FA transport capacity may be the main factor in the success of

weight decrease and ACSL5 has been reported to increase with food deprivation in rodent models⁷. Previous studies^{8,9} reported that the rs2419621 genetic variant of *ACSL5* gene (C to T transition) is related with an improved rate of weight and fat loss in obese patients who received a hypocaloric diet. As far as we know, there are no investigations in the literature that have studied the role of this polymorphism in weight loss and metabolic changes after bariatric surgery.

The aim of our study was to evaluate the effects of rs2419821 genetic variant of *ACSL5* gene on weight loss and metabolic improvement after robotic sleeve gastrectomy in morbid obese subjects.

Patients and Methods

Forty-eight obese subjects have been consecutively enrolled from the Department of Endocrinology and Nutrition for weight loss therapy. All participants underwent robotic sleeve gastrectomy (SG) (Table I). Robotic Sleeve Gastrectomy was realized using the DaVinci X™ (Intuitive Surgical LTD, Oxford, UK) platform with 5 trocars (four 8-mm robotic trocars and one 12-mm trocar for AirSeal® iFS (CONMED, NY USA). The gastrocolic ligament was transected with robotic Vessel Sealer Extend™ (Intuitive Surgical LTD, Oxford, UK). Greater curvature was transected with Signia™ Stapling System (Medtronic, Minneapolis, MN, USA) and Endo GIA™ with Tri-Staple™ Technology (Medtronic, Minneapolis, MN, USA). A 36 Fr bougie was used for calibration. For the first 14 days, the patients ate a 1000-calorie diet supplemented with a protein module to reach 1.4 g per Kg of ideal weight (Body Mass Index (BMI) 22 kg/m²) and distribution of macronutrient (30% fat and 70% carbohydrates). After 15 days of bariatric procedure, obese subjects followed the same diet based on the intake of 1200-1400 calories, with the next distribution of macronutrients fats (35%, divided into 10% saturated, 20% monounsaturated and 5% polyunsaturated) and carbohydrates (65%), with a contribution of proteins of 1.2 g per Kg of ideal weight.

The exclusion criteria were age above 65 years and the presence of systemic inflammatory diseases, malignancies, coagulopathy, severe liver or chronic renal diseases. The Ethics Committee of the HCUVa de Valladolid Spain (Committee 18/1080). All subjects signed a written informed

consent and all were in accordance with the Declaration of Helsinki.

Study Design

All the next parameters were recorded at the baseline visit prior to surgery and at each postoperative visit at 3, 6 and 12 months. In each following visit and after 10 hours of fasting, all obese subjects underwent clinical examination: biochemical parameters, blood pressure and anthropometric parameters (body weight, waist circumference and percent excess weight loss (EWL%)). We measured; serum lipid profile (total cholesterol, Low-density lipoprotein cholesterol, High-density lipoprotein cholesterol, triglycerides), basal insulin, fasting glucose, (homeostasis model assessment as method to determine insulin resistance) HOMA-IR. We also recorded associated morbidities (percentage of patients with diabetes mellitus type 2, hypertension, hyperlipidemia or hypertriglyceridemia). The genotype of rs2419821 *ACSL5* gene was determined at basal time.

Anthropometric Measurements, Blood Pressure and Comorbidities

Body weight, height and waist circumference (WC) were determined in the morning before breakfast at baseline and subsequently 3, 6 and 12 months. The weight was measured with an accuracy of ± 100 g, using a manual scale (Seca, Birmingham, United Kingdom). The height was measured with the patient in an upright position, using a stadiometer (Seca Birmingham, United Kingdom). Body mass index was calculated as body weight in Kg/(height in m²). WC was measured in the narrowest diameter between xiphoid process and iliac crest with a flexible tape measure (Omron, Fermont, CA, USA). Percent excess weight loss (EWL%) was determined using the equation: (preoperative weight – current weight $\times 100$ /preoperative weight – ideal weight). Ideal weight was calculated with an ideal BMI 22 kg/m². Arterial blood pressure was measured three times and averaged after a 5-minute rest with a random zero mercury sphygmomanometer (Omron, Fermont, CA, USA).

Comorbidities were defined as hypertriglyceridemia (triglycerides > 150 mg/dl), hypertension (systolic and diastolic blood pressures higher than 130 and 85 mmHg, respectively), elevated LDL cholesterol (>100 mg/dl) or patients who were taking medication for these pathologies. To determine diabetes mellitus, any of the following criteria were necessary (fasting blood glucose >

126 mg/dl or HBA1c > 6.5% or blood glucose after two-hour oral glucose overload test greater than 200 mg/dl or patients who were taking drugs for hyperglycemia.

Assays

Lipid profile (total cholesterol, HDL-Cholesterol and triglycerides) were measured using the COBAS INTEGRA 400 analyser (Roche Diagnostic, Montreal, fCanada) LDL cholesterol was calculated using Friedewald formula (LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5)⁹.

Glucose levels were determined by an automated hexokinase oxidase method (COBAS INTEGRA 400 analyser (Roche Diagnostic, Montreal, Canada). Insulin was determined by electrochemiluminescence assay (COBAS INTEGRA 400 analyser (Roche Diagnostic, Montreal, Canada). The homeostasis model assessment for insulin resistance (HOMA-IR) was evaluated using these values (glucose insulin/22.5)¹⁰⁻¹¹.

Genomic DNA from each obese subject was purified from cells of the oral mucosa using the next kit extraction (Quantum prep, Bio-Rad, Hercules, CA, USA). Genotyping (rs241926) was performed by using TaqMan[®] OpenArray[™] Genotyping platform (Thermo Fisher Scientific, Pittsburg, PA, USA). Samples were loaded using the AccuFill[™] system, and amplification was performed on the QuantStudio 12K Flex Real-Time qPCR instrument (Thermo Fisher Scientific, Pittsburg, PA, USA). A total volume of 5 µl with 2.5 µl TaqMan OpenArray Master Mix (Applied Biosystems, Foster City, CA, USA) and 2.5 µl human DNA samples were loaded and amplified on arrays. Genotype calling and sample clustering were performed in TaqMan Genotyper (Life Technologies, Carlsbad, CA, USA). Hardy Weinberg equilibrium was determined with a statistical test (Chi-square). The variant of *ACSL5* gene was in Hardy Weinberg equilibrium ($p=0.36$).

Statistical Analysis

SPSS Statistics version 23.0 (IBM, Armonk, NY, USA) was used for statistical evaluation. Power analysis reported at least 45 subjects with the change in weight of 40% EWL% using T-allele frequency (15%) in morbid obese subjects, with a type I error of 0.05 and type II error of 0.10 (power=0.9). The statistical analysis was realized with a dominant model with the combined TT and CT as a group and CC genotype as second group. The results were reported as average +/- standard

deviation. The normal distribution of variables was evaluated with the Kolmogorov-Smirnov test. Parameters with normal distribution were analyzed with a two-tailed Student's *t*-test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary. The statistical analysis to evaluate the gene-surgery interaction was univariate ANCOVA with anthropometric and biochemical parameters at 3, 6 and 12 months, taking to account the genotype with a post hoc test Tukey. Correction for multiple hypothesis testing was performed. A *p*-value under 0.05 was considered statistically significant.

Results

A total of 48 morbid obese subjects were enrolled. Table I shows the main parameters of the patients included in the study. Of this group, 38 were females and 10 were males with an average age of 47.3±3.1 years. The allelic frequency was the following 0.85 C and 0.15 T alleles. The genotypic frequency was as follows: 62.5% (30 patients) in CC genotype, 22.9% (11 patients) in CT genotype and 14.6% (7 patients) in TT genotype. We classified the subjects in two groups: those carriers T allele (TT+CT, 37.5%) and non-carriers T allele (CC, 62.5%). The gender distribution was similar in both genotype groups (CC; 16.7% (n=5) males and 83.3% (n=25) females), (CT+CT; 22.2% (n=4) males and 77.8% (n=14) females). Average age was also similar in both genotype groups (CC: 45.2±6.1 years vs. CT+TT 46.8±7.1 years: ns).

Table II reports adiposity parameters and blood pressure before surgery and during the 12 months of duration of the study. We did not report statistical differences in adiposity parameters and blood pressure at basal time in both genotypes. When the evolution of adiposity parameters over time was revised, we detected a statistically significant reduction of body weight, waist circumference

Table I. Preoperative characteristics of the patients.

Parameters	Basal time
Morbid obese	44
Super Obese	4
Gender (female/male)	39/9
Age (years)	47.3±3.1
BMI (kg/m ²)	46.5±5.3

Morbid: BMI > 40 kg/m² and < 50 kg/m². Superobese > 50 kg/m².

Table II. Changes in anthropometric variables rs2419621 (mean±SD).

Characteristics	rs2419621 CC (n=30)				rs2419621 CT or TT (n=18)			
	0 time	At 3 months	At 6 months	At 12 months	0 time	At 3 months	At 6 months	At 12 months
	BMI	46.6±5.1	40.8±5.3*	33.6±5.9*	32.8±5.1*	46.3±4.0	40.5±3.1*	33.2±4.1*
Weight (kg)	122.6±21.6	105.9±11.9*	99.1±6.9*	90.1±5.3*	128.8±10.2	102.1±6.2*	91.1±3.1*	88.1±3.9*
WC (cm)	125.2±7.1	120.1±5.1*	102.1±5.0*	103.1±4.0*	124.2±4.0	110.1±4.0*	105.1±3.3*	95.1±4.2*
EWL%	-	52.9	62.4	64.2	-	58.0	66.5	70.1
SBP (mmHg)	145.0±7.0	134.2±4.0*	128.0±7.2*	121.1±6.0*	144.1±5.2	135.0±4.1*	130.1±4.2	127.1±3.0*
DBP (mmHg)	88.1±4.2	87.0±4.2*	84.0±6.2*	83.8±5.1*	91.1±3.2	85.5±3.1*	84.2±3.4	81.9±2.1*

DBP: Diastolic blood pressure. SBP: Systolic blood pressure. WC: Waist circumference. EWL%: Percent excess weight loss (*) $p < 0.05$, in each genotype group with basal values. There are no statistical differences in demographic, anthropometric and metabolic characteristics between the two-genotype groups.

Table III. Biochemical parameters rs2419621 (mean±SD).

Characteristics	rs2419621 CC (n=30)				rs2419621 CT or TT (n=18)			
	0 time	At 3 months	At 6 months	At 12 months	0 time	At 3 months	At 6 months	At 12 months
	Glucose (mg/dl)	115.1±5.1	93.9±4.1*	90.1±4.1*	91.1±4.2*	111.5±4.9	100.9±5.0*	93.1±4.1*
Total ch. (mg/dl)	194.3±11.2	160.4±15.1*	167.1±10.1*	170.2±9.0*	201.9±10.1	167.2±10.3*	168.9±9.0*	161.1±8.0*
LDL-ch. (mg/dl)	131.2±10.1	103.5±9.1*	101.7±7.1*	99.1±7.2*	139.9±12.0	108.0±7.1*	106.1±6.1*	100.8±9.2*
HDL-ch. (mg/dl)	47.1±8.0	45.1±7.4	44.3±9.1	45.1±8.3	48.9±5.2*	46.8±5.2	45.1±6.1	45.2±6.0
TG (mg/dl)	143.9±16.1	123.1±20.2	112.9±13.6	100.1±18.1*	157.1±12.1	115.2±18.9*	117.1±11.8*	106.7±10.2*
Insulin (mUI/L)	22.2±4.4	14.2±5.0*	9.9±4.8*	11.7±4.0*	26.9±2.1	14.1±3.0*	11.2±3.3*	12.0±3.9*
HOMA-IR	5.2±1.8	2.7±1.1*	2.0±1.2*	1.6±0.4*	5.7±0.8	2.9±1.0*	2.5±0.9*	2.1±0.9*

LDL: Low density lipoprotein. HDL: High density lipoprotein. Chol: Cholesterol. TG: Triglycerides. HOMA-IR (homeostasis model assessment). (*) $p < 0.05$, in each group with basal values. No statistical differences between genotypes.

and percentage of excess of weight loss (EWL%) after surgery at 3, 6 and 12 months. Blood pressures also decreased in both genotypes. Improvements in adiposity parameters were higher in T allele carriers than non T allele carriers. After 12 months, delta of these parameters was higher in T allele carriers; (EWL%; 70.1% vs. 64.2%; $p = 0.04$), weight (40.7±4.1 kg vs. 32.5±4.8 kg; $p = 0.03$) and waist circumference (29.1±3.1 cm vs. 22.2±2.8 kg; $p = 0.02$).

Table III reports changes in all biochemical parameters. No differences in basal values were detected in all of these parameters. As expected, fasting glucose, insulin, HOMA-IR, total cholesterol, LDL-cholesterol and triglyceride levels decreased in both genotype groups after surgery. Although the improvement of triglyceride levels was significant in both genotypes, this change

was earlier in the T allele carriers and as soon as 3 months after surgery, and we only detected the improvement of this parameter at 12 months in non-T allele carriers (Table III). After 12 months, delta of triglyceride levels was higher in T allele carriers than non-T allele carriers; (51.2±9.1 mg/dl vs. 32.1±8.1; $p = 0.02$). The remaining deltas of the biochemical parameters were similar.

Table IV reports the improvement in comorbidities (percentage of hypertriglyceridemia, hypertension, and high-LDL cholesterol levels and diabetes mellitus type 2). These percentages were similar in both genotype groups and all rates decreased in both genotypes. However, the percentage of patients with hypertriglyceridemia diminished early in the 3-month follow-up in the T-allele carriers and at 12 months.

Discussion

The principal finding of this investigation was that individuals with a robotic sleeve gastrectomy and T allele of SNP rs2419621 showed a significantly greater improvement of body weight, fat mass, waist circumference, and triglyceride levels than non-T allele carriers after 12 months.

Genetic and epigenetic mechanisms have been implied in obesity physiology¹². As an important component of these metabolic pathways, fatty acyl-CoA molecules have been related to energy status through lipid synthesis and energy production by Beta-oxidation. The acyl-CoA synthetases long-chain (ACSL) stimulates intracellular free long-chain fatty acids by converting them to fatty acyl-CoA molecules. ACSL subtype 5 is distributed in a wide range of tissues as liver, skeletal muscle and brain¹³, this enzyme plays a role in modulating fatty acid channelling between catabolic Beta-oxidation and anabolic lipid synthesis¹⁴. A total of eight SNPs has been described along the ACSL5 gene, but only rs2419621 have showed a significant association with weight change^{8,9,15}. This polymorphism could play as a cis-acting regulatory variant affecting ACSL5 expression levels. Secondly the effect of the T allele on adiposity and biochemical parameters support that this genetic variant may modulate the amount of weight change by increasing ACSL5 levels and promoting the fatty acid Beta-oxidation.

After a total-meal replacement of 900 calories per day for 6 weeks, Adamo et al⁸ reported a better weight response in T allele carriers. Izaola et al¹⁵ observed, after 3 months of dietary intervention with a partial-meal replacement hypocaloric diet of 1069 calories per day, that T allele carriers dis-

played a significantly greater improvement of adiposity parameters, insulin levels, HOMA-IR and triglyceride levels than non T allele carriers. In the third dietary study of the literature, Rajkumar et al⁹ demonstrated that obese subjects carrying the T allele of the rs2419621 variant are more responsive to two different dietary interventions. The caloric restriction targets for these different^{8,9} oscillated between 500 till 800 calories from participants daily energy needs without meal replacement strategy. In these three studies the weight loss was always higher in the T allele carriers. In two of the previous studies^{8,9}, biochemical parameters were not evaluated. However, in the third study¹⁵, a greater decrease in triglycerides and insulin resistance was detected, similarly to our current study results of triglycerides with bariatric surgery.

All these studies agree on greater weight loss after the intervention in patients with the T allele, and especially in visceral fat loss. In our present work we have indirectly evaluated it in patients with bariatric surgery by determining the waist circumference. Nevertheless, Rajkumar et al⁹ reported a greater decrease in visceral fat in the carriers of the T allele determined by dual-energy X-ray absorptiometry. This improvement in visceral fat explains the metabolic findings. It is well known that excess accumulation of visceral fat, characterized as fat packed between inner organs is associated with impaired metabolic parameters¹⁶. Specifically, central obesity produces a high lipolysis activity within visceral adipocytes, with increased delivery of free fatty acids into the liver, resulting in a potential insulin resistance called "portal theory"¹⁷.

These reported metabolic effects have a molecular basis, as we are going to indicate below. The

Table IV. Preoperative and postoperative comorbidities of the patients.

Parameters	Baseline	3 months	6 months	12 months
High levels LDL Cholesterol				
CC	33.3%	26.6%	26.6%	16.6%*
CT+TT	33.3%	27.7%	27.7%	16.6%*
High Levels TG				
CC	30.0%	23.3%	23.3%	13.3%*
CT+TT	27.7%	11.1%*	0%*	0%*
Blood Hypertension				
CC	33.3%	23.3%	23.3%	13.3%*
CT+TT	27.7%	22.2%	22.2%	11.1%*
Diabetes mellitus				
CC	10.0%	10.0%	6.7%*	6.7%*
CT+TT	11.1%	5.6%*	5.6%*	5.6%*

(*) $p < 0.05$, in each group with basal values. HyperTG Hypertriglyceridemia (triglycerides > 150 mg/dl), hypertension (systolic and diastolic blood pressures higher than 130 and 85 mmHg respectively), High LDL cholesterol (>100 mg/dl)

presence of T allele produces a new cis-regulating E-box site at the promoter region of ACSL5 in addition to the two wild type E box elements¹⁶. This new E-box produces an increment of MyoD (Myoblast Determination Protein) and it is recruited to the ACSL5 promoter and increases the expression of the downstream gene¹⁶. Since ACSL5 is mitochondrially located, T allele carriers might produce higher fat oxidation levels due to increased level of ACSL5¹⁸. All these data suggest that the rs2419621 genetic variant modulates the rate of weight loss by increasing ACSL5 levels and promoting Beta-oxidation over triglyceride degradation.

The importance of our work is that it is the first in the literature to evaluate the effect of this genetic variant on weight loss secondary to robotic sleeve gastrectomy. Moreover, our work has some limitations. Firstly, we did not measure tissue ACSL5 levels in the study population. Secondly, other unknown non-genetic factors could modulate the relationships in our design (exercise, hormone status, and so on) and even epigenetic factors. Thirdly, the lack of a dietary assessment in the obese subjects might be a bias¹⁹. Finally, the absence of a control group without bariatric surgery might also be a bias.

Conclusions

In summary, our data suggest that the genetic variant (rs2419621) of *ACSL5* gene is associated with better improvement of adiposity and triglyceride levels in subjects with T allele, after a robotic sleeve gastrectomy. Furthermore, future investigations with *in vivo* models are necessary in order to evaluate the role of rs2419261 (T) polymorphism on fatty acid oxidation and weight loss. These investigations allow the design of more personalized medicine in patients undergoing bariatric surgery.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or National Research Committee (HCUVa-Committee-201708) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Informed consent

Informed consent was obtained from all individual participants included in the study.

Authors contribution

Daniel Antonio de Luis designed the study and wrote the article. Olatz Izaola realized nutritional and clinical evaluation. D. Primo realized biochemical evaluation. D Pacheco realized surgery.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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