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Common variants in genes related to lipid and energy metabolism are associated with weight loss after an intervention in overweight/obese adolescents

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Abstract

Background: **Some SNPs related to lipid and energy metabolism may be implicated not only in the development of obesity and associated comorbidities, but also in the weight loss response after a nutritional intervention.**

Objective: **In this context, the present study analyzed four SNPs located within four genes known to be associated with obesity and other obesity-related complications, and their putative role in a weight-loss intervention in overweight/obese adolescents.**

Methods: **The study population consisted of 199 overweight/obese adolescents (13-16 yr old) undergoing 10 weeks of a weight loss multidisciplinary intervention: the EVASYON programme (www.estudioevasyon.org). Adolescents were genotyped for 4 SNPs, and anthropometric measurements and biochemical markers were analyzed at the beginning and after the intervention.**

Results: **Interestingly, APOA5(rs662799) was associated with the baseline anthropometric and biochemical outcomes, whereas FTO (rs9939609) seemed to be related with the change of these values after the 10-week intervention. The other two SNPs, located in the CETP (rs1800777) and the APOA1 (rs670) genes, showed important relationships with adiposity markers. Specifically, a combined model including both SNPs turned up to explain up to 24% of BMI-SDS change after 10 weeks of the multidisciplinary intervention, which may contribute to understand the weight loss response.**

Conclusion: **Common variants in genes related to lipid and energy metabolism may influence not only biochemical outcomes but also weight loss response after a multidisciplinary intervention carried out in obese/overweight adolescents..**

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ASOCIACIÓN ENTRE VARIANTES GENÉTICAS RELACIONADAS CON EL METABOLISMO LIPÍDICO Y ENERGÉTICO Y LA PÉRDIDA DE PESO TRAS UNA INTERVENCIÓN EN ADOLESCENTES CON SOBREPESO U OBESIDAD

Resumen

Antecedentes: **Algunas variantes genéticas relacionadas con el metabolismo lipídico y energético pueden estar implicadas en la respuesta a una intervención nutricional además de estar asociadas con el desarrollo de obesidad y comorbilidades asociadas.**

Objetivo: **En este sentido, este artículo analiza cuatro polimorfismos situados en cuatro genes que han sido previamente asociados con la obesidad u otras complicaciones asociadas a la misma, así como su posible papel en la respuesta a una intervención para la pérdida de peso en adolescentes con sobrepeso u obesidad.**

Métodos: **La población en estudio está formada por 199 adolescentes con sobrepeso u obesidad (13-16 años) llevando a cabo una intervención multidisciplinar de 10 semanas para la pérdida de peso: programa EVASYON (www.estudioevasyon.org). Los adolescentes fueron genotipados para los 4 SNPs y tanto al comienzo como al final de la intervención se analizaron marcadores bioquímicos y se tomaron medidas antropométricas.**

Resultados: **Rs662799 del gen APOA5 se asoció al inicio con parámetros antropométricos y bioquímicos, mientras que el rs9939609 del gen FTO parecía estar asociado con el cambio de estas variables tras 10 semanas de intervención. Las variantes rs1800777 del gen CETP y rs670 del gen APOA1 mostraron una importante asociación con marcadores de adiposidad. Concretamente, un modelo combinado incluyendo los dos polimorfismos logró explicar hasta un 24% del cambio en el IMC-SDS tras 10 semanas de intervención.**

Conclusión: **Variantes genéticas previamente relacionadas con el metabolismo lipídico y energético, pueden repercutir no solamente en valores bioquímicos sino también en la respuesta a una intervención multidisciplinar para la pérdida de peso en adolescentes con sobrepeso u obesidad.**

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Palabras clave: *APOA1. CETP. EVASYON. FTO. APOA5.*

Introduction

Overweight and obesity during childhood and adolescence has became a growing public health problem throughout the world[1-2] to the extent that, according to the European Association for the Study of Obesity (EASO), about 16-22% of European adolescents between 14 and 17 years old are overweight or obese, with an annual increase of the prevalence of around 2% in the 1990s and 2000s[3]. These rates in childhood and adolescent obesity appear to be associated with important comorbidities in adulthood, such as type 2 diabetes, coronary artery disease or atherosclerosis, accompanied by elevated costs for public health systems[4,5]. Besides, the imbalance between an increased energy intake and a decreased energy expenditure due to inadequate dietary habits and physical activity patterns, genetic factors, as well as gene x gene and gene x environment interactions, may be also involved in obesity aetiology accounting for 40-70% of obesity phenotypes[6].

Concerning the genetic basis of obesity, several SNPs located in different genes have been found to be associated with adiposity, dietary patterns or weight loss[7,8]. In this context, several studies have shown significant relationships between adiposity, dyslipidemia, hypertension, diabetes or an increased cardiovascular risk and individual SNPs[9-18], including *APOA1, APOA5, FTO* and *CETP,* which are genes involved in the regulation of plasma lipid levels. Four SNPs that have been previously found to influence plasma lipid levels and cardiovascular disease are rs670 (*APOA1*), rs662799 (*APOA5*), rs1800777 (*CETP*) and rs9939609 (*FTO*)[19-23]. Thus, *APOA1* is a gene that encodes for Apolipoprotein A-1, the major protein component of HDL-cholesterol[24]. *APOA5* encodes for Apolipoprotein A-V, a component of several lipoprotein factors as VLDL or HDL and an important determinant of plasma triglyceride levels[25]. As for *FTO* gene, it has been widely associated with obesity[26-28]. Finally, *CETP* encodes for the plasma lipid transfer protein, a plasma protein that facilitates the transport of cholesterol esters and triglycerides between the lipoproteins[29]. However, the effects of the four SNPs after a lifestyle intervention for weight loss are in most cases still scarce. Therefore, our purpose was to evaluate the effect of these SNPs located in *FTO, APOA5, APOA1* and *CETP* genes, which have been previously associated with obesity, dyslipidemia and other obesity-related pathologies, in the metabolic response after a weight-loss intervention in overweight/obese adolescents.

Subjects and methods

The trial recruited 199 overweight or obese adolescents (39% males) undergoing a 10 week intensive lifestyle intervention, the EVASYON study (www.estudioevasyon.org), which is a lifestyle and nutritional educational weight loss program supported by a multidisciplinary team of nutritionists, physiotherapists, psychologists and paediatricians. Data from these adolescents were collected at the beginning and after 10 weeks of treatment and participants were recruited from five Spanish cities (Granada, Madrid, Pamplona, Santander and Zaragoza). The study included only 12 to 16 years old overweight or obese adolescents, according to Cole s criteria[30], which have been raised in Spain and without diagnosed disease associated with obesity or pharmacological treatment.

Written consent to participate was requested from both parents and adolescents. The study protocols were performed in accordance with the ethical standards laid down in the 1961 Declaration of Helsinki (as revised in South Korea in 2008), following the European Economic Community (EEC) Good Clinical Practice guidelines (document 111/3976/88 of July 1990) and current Spanish laws, which regulates clinical research in humans (Royal Decree 561/1993 regarding clinical trials). The study was approved by the five local ethics committees.

Multidisciplinary intervention

According to food intake questionnaires, a personalized balanced diet (30% of energy as fat, 15% as proteins and 55% as carbohydrates) and a physical activity programme was handed in to each adolescent. During the 10 week intensive program period, the adolescents attended weekly group sessions where they received nutritional and physical advice, as well as psychological support. The description of the complete EVASYON study design has been previously published elsewhere[31].

Physical Activity, Energy Intake, Metabolic and Anthropometric Data

All the adolescents were asked to fill in a series of validated questionnaires in order to determine their physical activity level and estimate their basal metabolism rates[31,32]. A semi-quantitative food-frequency questionnaire, previously validated in Spain[33], and containing 132 food items, as well as a 72-hour recall was filled in at the beginning of the follow-up. Weight and height were measured with an electronic scale (Type SECA 861, SECA, Birmingham, UK) and a telescopic height measuring instrument (Type SECA 225, SECA, Birmingham, UK) respectively. BMI was calculated as weight (in kg)/height^2 (in m^2). Then, individual BMI values were converted into standard deviation scores (SDS) using age and specific cut-points according to the Spanish children and adolescent growth references[34]. Skinfolds were measured with a skinfold calliper (Caliper Holtain; Holtain Ltd.,

Walles, UK) and waist and hip circumferences with a flexible non-stretchable measuring tape (Type SECA 200, SECA, Birmingham, UK). Pubertal developmental was determined according to Tanner stage[35]. Blood pressure was obtained using the left arm after the adolescent had rested quietly for 15 minutes using a blood pressure monitor Mod. OMRON M6 (OMROM Health Care Co., Kyoto, Japan) by following validated procedures.

Genotyping

Venous blood samples were collected at the beginning of the study. DNA was extracted from the buffy coat fraction using a commercial kit (Master PureTM; Epicentre, Madison, WI, USA) and its quality and quantity were determined with a NanoDrop ND-1000 spectrometer (NanoDrop Technologies, Wilmington, Delaware, USA). All the subjects were genotyped for 4 SNPs located within *APOA1, APOA5, FTO* and *CETP* genes (rs670, rs662799, rs1800777 and rs9939609, respectively) by using the N+S nutrigenetic test of CINFA (Olloki, Spain). Briefly, targeted regions of genomic DNA were amplified in a multiplex PCR reaction using biotinylated dCTP by using an Applied Biosystems gold plated 96-well Geneamp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR products were then hybridized onto oligonucleotide probes attached to microspheres and labeled with streptavidin-conjugated phycoerythrin (Luminex xMAP® Technology). These beads were analyzed by flow cytometry with the Luminex® 100/200TM System (Luminex Corporation, Austin, TX, USA) by following the usual protocol[36]. The presence of specific polymorphisms in the sample material was determined by correlation of the fluorescence signal intrinsic to each microsphere with the presence or absence of a corresponding phycoerythrin signal. Replicate quality control samples were included in every genotyping plate with more than 99% of concordance.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software 15.0 (SPSS INC., Chicago, IL). A χ^2 test was used to evaluate the Hardy-Weinberg equilibrium. The Kolmogorov-Smirnov test was used to determine variable distribution.

The differences in anthropometric, biochemical and energy intake variables between the SNP genotypes were tested with analysis of the covariance (ANCOVA) adjusted for confounders such as age, sex or baseline BMI-SDS (for normally distributed variables), or the Mann Whitney U test. Multivariate regression models were fitted to assess the association between the genotypes and weight loss after adjusting for confounder factors. The level of probability was set at $p < 0.05$ as statistically significant.

Results

The present study analyzed four SNPs located in *APOA1, APOA5, FTO* and *CETP* genes, previously associated with obesity, diabetes, dyslipidemia and other obesity-related pathologies. Allele frequencies of the four studied SNPs were within expected ranges for Caucasian populations and the Hardy Weinberg equilibrium was fulfilled in this population.

Anthropometric, biochemical and physical activity markers and determinants as well as dietary patterns for overweight/obese adolescents at baseline and after 10 weeks of the EVASYON programme, are shown (table I). Adiposity markers, such as weight, BMI-SDS, fat mass and waist circumference, were significantly reduced. In a similar way, the metabolic profile of the adolescents was improved after the intervention. There was a significant decrease in leptin, insulin, total cholesterol, triglycerides and C-reactive protein among other parameters. In regard to physical activity, obese adolescents not only decreased their sedentary behaviour but also improved their physical skills. Finally, after 10 weeks of intervention, the adolescents showed a significant improvement of dietary patterns decreasing total energy, total fat and SFA intake (table I).

Concerning plasma lipid levels, the rs662799 SNP of the *APOA5* gene was associated with higher levels of HDL-cholesterol at the beginning of the intervention ($B = 7.22$; $TEM = 2.80$; $p = 0.011$) but no differences were found after the intervention. Meanwhile, rs9939609 SNP of the *FTO* gene was associated with a higher decrease of HDL-cholesterol after the intervention ($B = -4.00$; $TEM = 1.39$; $p = 0.005$), as well as with a higher decrease of apolipoprotein A1 after 10 weeks of the EVASYON intervention ($B = -7.88$; $TEM = 3.10$; $p = 0.013$). On the other hand, rs670 SNP of the *APOA1* gene showed an association with apolipoprotein B levels; G allele carriers presented higher baseline apolipoprotein B plasma concentrations and, after 10 weeks of a multidisciplinary intervention, they achieved a greater decrease of the circulating levels of this apolipoprotein.

The four SNPs studied (rs670 of *APOA1* gene, rs662799 of *APOA5* gene, rs9939609 of *FTO* gene and rs1800777 of *CETP* gene), showed a strong association with adiposity indicators, both at the beginning and after 10 weeks of the EVASYON treatment (table II). In particular, SNPs in *APOA1* and *CETP* genes evidenced a significant association with weight and BMI-SDS loss after the intervention (Figure 1A and 1B). Regression analyses studying these effects are showed (table III). Concerning BMI-SDS reduction, both SNPs showed a significant effect (figs. 2A and 2B). Particularly, rs670 of *APOA1* gene seemed to

Table I
Anthropometric, biochemical, physical activity and dietary data concerning the participant adolescents (n = 199) at the beginning and after 10 weeks of intervention

	At the beginning		*After 10 weeks*		
	Mean	*TEM*	*Mean*	*TEM*	*p*
Gender (% boys)	39.2				
Age (yr)	14.5	0.08	80.2	1.1	< 0.001
Weight	83.8	1.2	80.2	1.1	< 0.001
BMI-SDS	4.5	0.2	3.8	0.1	< 0.001
Waist circunference (cm)	103.5	0.8	101.6	0.8	< 0.001
Hip circunference (cm)	90.8	2.1	88.1	2	< 0.001
Waist/Hip ratio	1.26	0.03	1.27	0.03	0.08
Waist/Height ratio	0.63	0	0.61	0	< 0.001
SBP (mm Hg)	111	1.5	106.2	1.5	<0.001
DBP (mm Hg)	74.4	0.8	70.2	0.8	< 0.001
Fat mass (kg)	37.3	0.9	32.8	0.8	<0.001
Fat mass (%)	43.7	0.6	40.1	0.5	<0.001
Σ 2 Skinfolds	57.5	0.9	51.7	0.9	< 0.001
Σ 4 Skinfolds	108.5	1.6	99.2	1.6	< 0.001
Σ 7 Skinfolds	179.7	2.2	165.2	2.4	< 0.001
Leptin (pg/ml)	5,158.6	532.3	2,999.9	304.8	< 0.001
Insulin (microU/ml)	18.6	1.9	14.2	1.1	0.005
PYY (pg/ml)	66.4	4.5	63.8	4.8	0.57
Adiponectin (pg/ml)	5,445.8	724.2	5,535.5	722.1	0.702
Glucose (mg/dL)	84.3	0.7	81.5	0.8	< 0.001
Total cholesterol (mg/dL)	155.7	2.5	143	2.5	<0.001
Tryglicerides (mg/dL)	92.9	4.1	83.3	3.9	0.002
HDL-cholesterol (mg/dL)	46.4	1	44.5	1	0.006
LDL-cholesterol (mg/dL)	90.1	2.2	82	2.2	0.009
CRP (mg/dL)	2.8	0.3	2	0.2	0.006
Sedentary activity (min/week)	543.8	6.4	521.8	6.8	0.002
Physical activity (counts)	377	13.9	408.6	13.2	0.011
Total energy (kcal)	2,188.8	68.1	1,685	56.8	< 0.001
Protein (gr)	100.9	3.6	94.7	3.3	0.047
Carbohydrates (g)	208.8	7.5	172.4	6.6	< 0.001
Lipids (g)	104.9	3.7	68	2.8	<0.001
SFA (g)	34	1.2	22.3	0.9	< 0.001
MUFA (g)	44.2	1.8	28.8	1.4	< 0.001
PUFA (g)	17	0.8	10	0.5	< 0.001

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PYY: Peptide YY; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

explain more than a 20% of this BMI-SDS change after adjusting for age and sex. Moreover, the analysis of the combined effect of both SNPs turned out to explain more than a 24% of BMI-SDS loss after the intervention, with a decrease of 0.24 points of BMI-SDS for each minor allele present in the genotype. Similar results were obtained for weight loss. A combined regression model of *APOA1* and *CETP* was able to explain more than a 14% of weight loss after the intervention. For each minor allele present in the adolescent genotypes, they showed a decrease of -1.4 kg in body weight.

Discussion

In this study, we analyzed the contribution of four obesity-related SNPs located in the *APOA1, APOA5, FTO* and *CETP* genes to adiposity markers in a Spanish population of overweight and obese adolescents undergoing a multidisciplinary intervention programme for weight loss (EVASYON).

After three months of treatment, the adolescents achieved a significant decrease in adiposity, as well as an improvement in their physical skills and in their metabolic profile. Thus, a significant decrease in leptin, insulin, glucose and C-reactive protein levels, among other parameters, confirmed the effectiveness of the EVASYON project as an overweight/obese adolescent weight loss programme. Similar results were found in a pilot study of the EVASYON programme[37].

ApoA1 and ApoA2 are the major protein constituents of HDL-cholesterol. Concerning rs670 SNP of the *APOA1* gene, a strong association with weight and BMI-SDS loss after the 10-weeks interven-

Table II
Anthropometric data according to the SNPs rs670 (APOA1 gener) and rs1800777 (CETP gene). n = 199 obese/overweight adolescents

	APOA1 rs670			*CETP rs1800777*		
	XX (n = 102)	*XY/YY (n = 76)*	*p*	*XX (n = 172)*	*XY/YY (n = 6)*	*p*
Weight (kg)	-2.9 ± 0.3	-4.5 ± 0.3	< 0.001	-3.4 ± 0.2	-8.0 ± 2.1	< 0.001
BMI-SDS	-0.57 ± 0.04	-0.82 ± 0.05	< 0.001	-0.66 ± 0.03	-1.30 ± 0.22	0.001
Weight circumference (cm)	-1.0 ± 0.6	-3.0 ± 0.5	0.017	-1.7 ± 0.4	-5.9 ± 1.0	0.067
Waist/hip ratio	0.02 ± 0.01	0.001 ± 0.01	0.154	0.01 ± 0.01	-0.02 ± 0.01	0.279
Waist/height ratio	-0.008 ± 0.003	-0.020 ± 0.003	0.022	-0.013 ± 0.002	-0.040 ± 0.004	0.055
Body fat (Σ 7 Skinfolds)	-12.0 ± 1.7	-17.4 ± 2.1	0.044	-14.2 ± 1.4	-22.6 ± 7.5	0.227

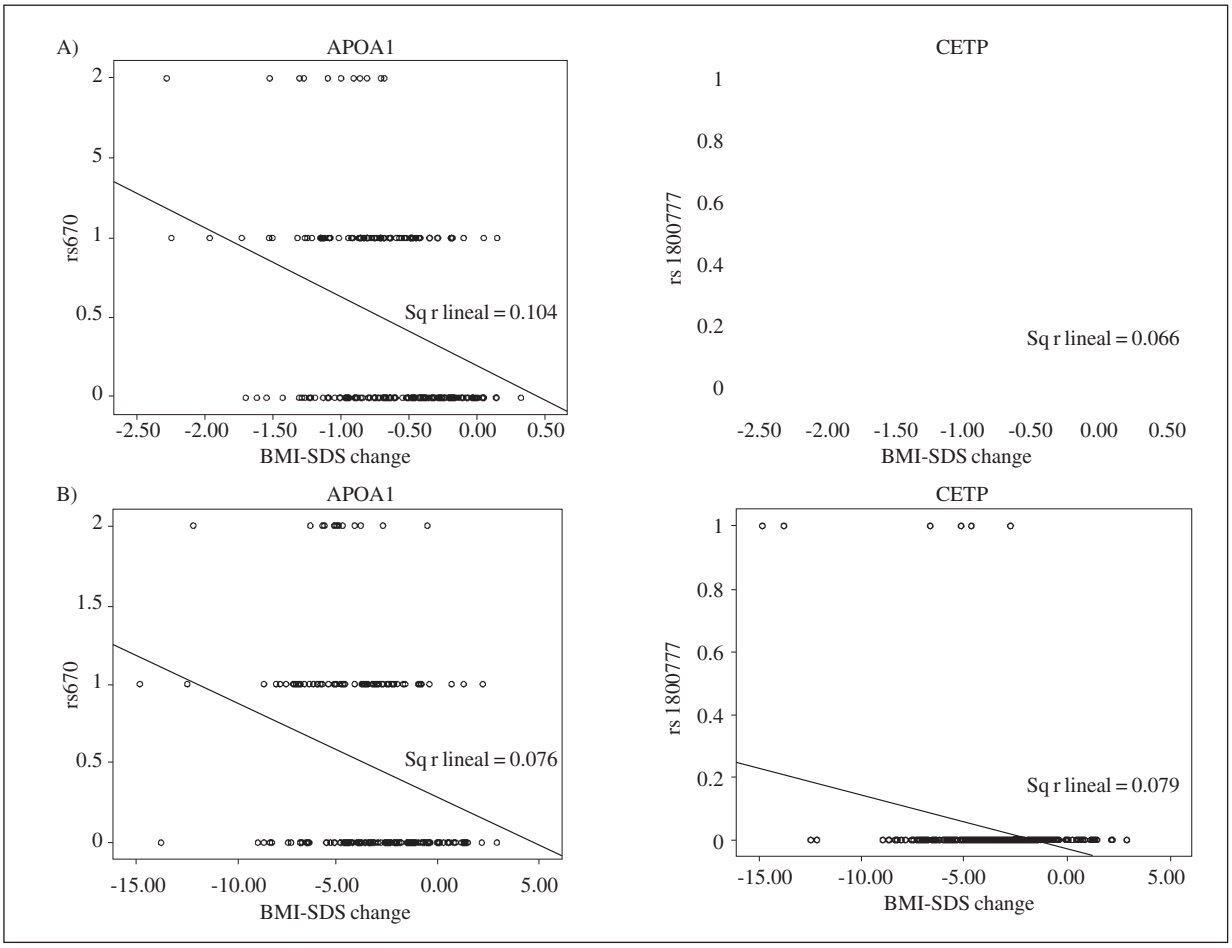

Fig. 1.—Correlations between the rs670 SNP of the APOA1 gene and the rs1800777 SNP of the CETP gene and IMC-SDS (A) and weight (B) loss.

tion was found, but there were no significant associations with plasma lipid profile before or after the intervention. In this sense, a study carried out by Xiao et al.[38] did not found a relationship between the SNP and plasma HDL-cholesterol levels or CVD in a population of controls and patients with proven CVD. On the other hand, other studies have reported an interaction between the SNP and dietary nutrients on plasma lipid levels and the metabolic syndrome. Thus, Phillips et al., found in a study of metabolic syndrome cases and controls that *APOA1* rs670 may influence metabolic syndrome, with G allele homozygotes showing an increased risk of MetS apparently explained by their increased abdominal obesity and impaired insulin sensitivity. Moreover, this association could be modulated by sex and dietary fat composition[15]. In a similar way, a recent study carried out by Rudkowska et al.[39] showed that the rs670 SNP of the *APOA1* gene inter-

Table III

Regression analyses showing the effect of two SNPs (both individually and combined): rs670 of APOA1 *and gene rs 1800777 of* CETP *gene, on weight and IMC-SDS loss after the intervention. n = 199 obese/overweight adolescents*

		IMC-SDS loss				*Weight loss*			
SNP	*Gene*	R^2	*B*	*TEM*	*p*	R^2	*B*	*TEM*	*p*
rs670	APOA1	0.213	-0.234	0.05	6.03×10^{-6}	0.116	-1.287	0.323	9.8×10^{-5}
rs1800777	CETP	0.174	-0.618	0.174	4.8×10^{-4}	0.112	-4.426	1.143	1.5×10^{-4}
	APOA1+CETP	0.241	-0.244	0.045	2.3×10^{-7}	0.147	-1.411	0.294	3.3×10^{-6}

B: effect for each minor allele present in the genotype. Linear regression analysis adjusted by age and sex.

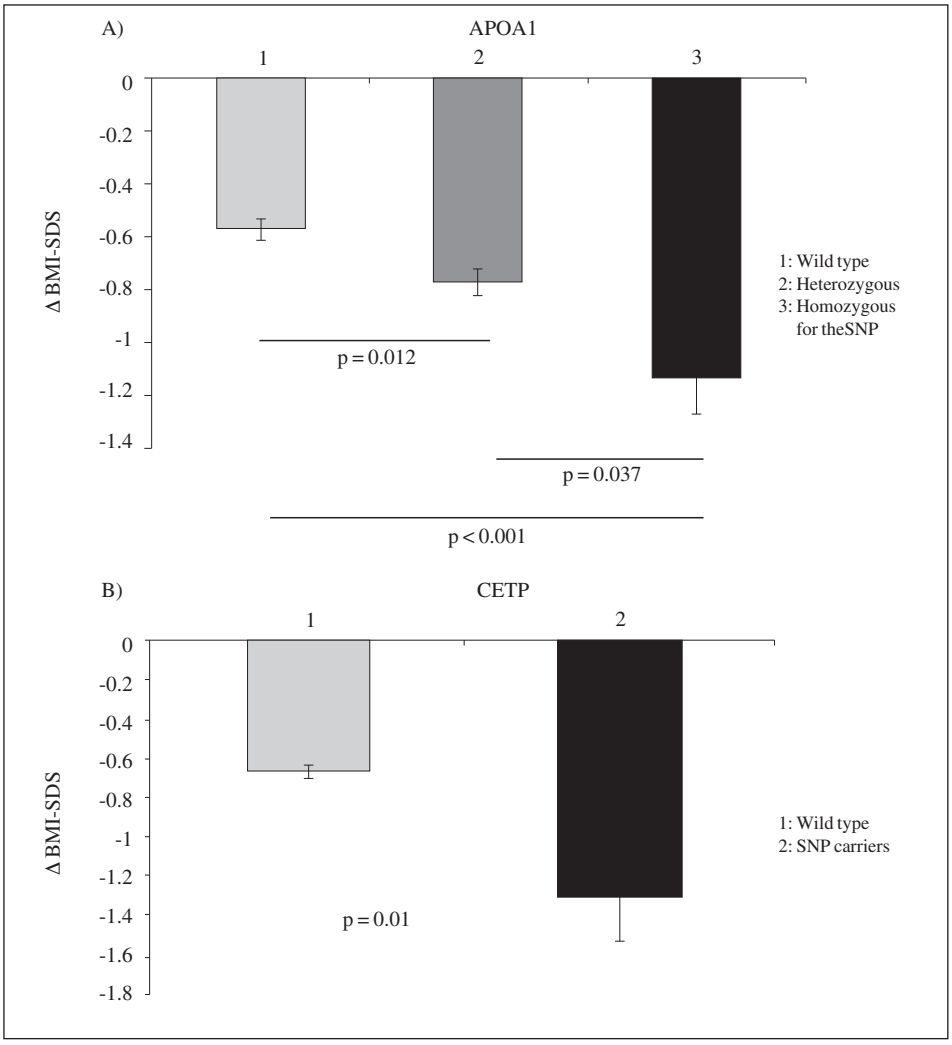

Fig. 1.—BMI-SDS change depending on rs670 SNP of APOA1 *(A) and rs1800777 SNP of* CETP *(B) genotypes.*

acted with dietary saturated fat on total cholesterol levels and with dietary total and saturated fat on LDL-cholesterol levels.

As for rs662799 SNP of *APOA5* gene, it has been largely associated with the plasma lipid profile[40-42]. In this study, this SNP has been identified as a significant predictor for plasma HDL-cholesterol concentration, with C allele carriers showing significantly higher HDL-cholesterol levels. However, these results did not in agree with works conducted in East Asian populations[41,43]. With regards to HDL-cholesterol levels after a weight loss intervention, data from literature is scarce. There are some studies analyzing the effect of the SNP on HDL-cholesterol plasma levels after fenofibrate therapy. Lai et al.[44] found that, after drug intervention, both carriers and non-carriers of the rs662799 SNP showed no significant differences in HDL-cholesterol plasma levels and similar results were described by

Feitosa et al. shortly after[45]. However, to our knowledge, no studies concerning HDL-cholesterol levels depending on this SNP have been previously conducted after a multidisciplinary weight loss intervention.

The *CETP* gene codifies for the cholesteryl ester transfer protein, which facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins and, therefore, participates in plasma lipid level regulation[22]. Specifically, the rs1800777 SNP of the *CETP* gene is located within the lipid-binding region and may result in the loss of positive charge, altering binding of *CETP* to cholesteryl esters. Results concerning the analysis of this SNP are scarce. A study carried out in 2008 by Lu et al. found that, compared with the ancestral allele the rs1800777 SNP of the *CETP* gene was associated with lower plasma HDL-cholesterol levels[46]. A meta-analysis also corroborated that a dominant model of the rs1800777 SNP was accompanied by lower levels of plasma HDL-cholesterol[22]. Our results evidenced that carriers of the rs1800777 of *CETP* gene showed a strong association with adiposity indexes, both at the beginning and after 10 weeks of the EVASYON treatment, especially with weight and BMI-SDS loss after the intervention. However, to our knowledge, no studies analyzing the effects of this SNP after an intervention have been carried out to date.

Concerning the *FTO* gene, the rs9939609 SNP has been widely associated with obesity and cardiovascular disease risk[47,48], especially during childhood[49-51]. It has been demonstrated that this SNP also influences weight loss after a weight loss intervention, both in adults and children or adolescents[52]. With regards to the putative impact of rs9939609 on plasma lipid levels, our study did not shown baseline differences between the carriers and the non-carriers. On the other hand, after the 10-week intervention, A allele carriers of the SNP underwent a significant decrease of HDL-cholesterol whereas adolescents with a TT genotype presented a slightly increase in this plasma biomarker. A study carried out by Freathy et al. found that each copy of the *FTO* rs9939609 A allele was associated with lower baseline HDL cholesterol levels[53]. So far, there are no evidences in the literature on the impact that a weight loss intervention may have on HDL plasma cholesterol, in spite of the relationship between *FTO* gene and lipid metabolism. Most of the studies carried out after an intervention did not found a direct effect of rs9939609 SNP on plasma lipid levels[54,55].

One of this study's strength is the analysis of a population of adolescents. This ensures the absence of obesity-associated comorbidities or pharmacological treatments that could mask the results. Moreover, obesity treatment during adolescence should be a priority subject of study, since improvements in obesity at this stage have been demonstrated to lead to maintained changes during adulthood that could decrease the risk of developing obesity-related comorbidities, such as metabolic syndrome[56], hypertension[57] or even some types of cancer[58].

In conclusion, in this study two SNPs in the *APOA5* (rs662799) and *FTO* (rs9939609) genes were associated with HDL-cholesterol plasma levels at baseline and after the intervention, respectively. Moreover, two SNPs in the *CETP* (rs1800777) and the *APOA1* (rs670) genes showed important effects on body weight and adiposity. Specifically, a combined model including both SNPs turned up to explain up to 24% of BMI-SDS change after 10 weeks of the multidisciplinary EVASYON intervention.

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