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Associations of the lipid genetic variants Thr54 (*FABP2*) and -493T (*MTTP*) with total cholesterol and low-density lipoprotein cholesterol levels in Mexican subjects

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Abstract

Objective: Mexico has one of the world's highest rates of obesity, which is influenced by lipid-genetic and lifestyle factors. This study aimed to determine whether *FABP2* (Ala54Thr) and *MTTP* (-493 G/T) genetic polymorphisms are associated with metabolic disorders in Mexican subjects.
Methods: A total of 523 subjects participated in a cross-sectional study. Genotyping for FABP2 and MTTP was performed using real-time RT-PCR. Biochemical and anthropometric data were evaluated.
Results: The genetically at-risk group (Thr54/-493T) was associated with significantly higher total and low-density lipoprotein cholesterol levels (difference between genetically at-risk group and wild-type group: 10.6 mg/dL and 8.94 mg/dL, respectively). Carriers within the genetically at-risk group had a significantly higher prevalence rate of hypercholesterolaemia (42.5% vs. 32.0%) and higher LDL-C levels (37.6% vs. 26.4%) than did non-carriers.

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Conclusions: Subjects who are genetically at risk (Thr54/-493T) have higher total cholesterol levels, low-density lipoprotein cholesterol levels, and prevalence rate of hypercholesterolaemia. These findings highlight the importance of basing nutritional intervention strategies for preventing and treating chronic diseases on individual genetic characteristics.

Keywords
Lipid genetic variants, hypercholesterolaemia, polymorphism, *FABP2*, *MTTP*, Mexico

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Introduction

One of Mexico's most serious public health problems is its rate of overweight and obesity (72.5%),[1] which is among the world's highest.[2] Obesity is characterized by the presence of metabolic disorders, such as dyslipidaemia (hypercholesterolaemia, hypertriglyceridaemia, hypoalphalipoproteinaemia), hyperinsulinaemia, insulin resistance, and hypoadiponectinaemia, and it is considered to be a non-communicable chronic disease. High body mass index (BMI), waist circumference, and dyslipidaemia (particularly hypercholesterolaemia and high low-density lipoprotein cholesterol [LDL-C] levels) are strongly associated with increased cardiovascular risk.[3] Importantly, cardiovascular disease is a leading cause of mortality worldwide and in Mexico.[2,4] Obesity and non-communicable chronic disease are influenced by lifestyle factors, such as diet, as well as genetic variants, some of which are related to lipid metabolism.[5]

Protein-coding genes involved in the regulation of lipid metabolism play a critical role in determining genetic risk factors.[6] The fatty acid-binding protein 2 (*FABP2*) gene is involved in the aetiology of some disorders in which lipid metabolism plays an important role, including obesity and type 2 diabetes.[7] The *FABP2* gene encodes intestinal fatty acid binding protein (I-FABP), which is secreted by enterocytes, and its main functions are related to transport of long-chain fatty acids. The *FABP2* gene presents a polymorphism due to substitution of G for A at codon 54 of exon 2. This genomic modification produces a substitution of the amino acid alanine (Ala) for threonine (Thr).[8] Subjects with the Thr54 variant have a double affinity for long-chain fatty acids.[9,10] Consequently, the Thr54 isoform is associated with obesity-related metabolic disorders, such as hypertriglyceridaemia, hyperinsulinaemia, and insulin resistance.[9,11]

The microsomal triglyceride transfer protein *(MTTP)* gene is also involved in lipid metabolism, and this gene encodes MTTP. This protein is involved in the transfer of lipids and in the assembly and secretion of chylomicrons and very low-density lipoprotein (VLDL). MTTP is also associated with obesity, mainly abdominal obesity.[12,13] If there is a polymorphism at position -493 G/T of the *MTTP* gene, this T allele could have a biological effect by increasing activity of the *MTTP* gene. This leads to increased expression and production of MTTP. Consequently, this genetic variant is associated with metabolic disorders, such as hypertriglyceridaemia, hypercholesterolaemia, and high LDL-C levels.[13,14]

This study aimed to determine whether there is an association between metabolic disorders and the genetically at-risk group Thr54/-493T.

Methods

Study population

A total of 523 unrelated adult subjects who had not been previously diagnosed with chronic disease were recruited between April, 2011 and December, 2013 to participate in a cross-sectional study. This study was conducted in Guadalajara, Jalisco, Mexico at the Medical Molecular Biology Service of the "Fray Antonio Alcalde" Civil Hospital of Guadalajara. We excluded subjects with any chronic illness (including cardiovascular, liver, kidney, or pancreatic diseases), drinkers (alcohol intake > 20 g/day for women and > 40 g/day for men), and subjects taking any prescribed medication that could affect lipid or glucose levels. We also excluded smokers and pregnant or breastfeeding women.

FABP2 *and* MTTP *genotyping*

DNA was extracted from leucocytes using the salting-out method.[15] The Ala54Thr polymorphism in the *FABP2* gene (rs1799883) and the -493 G/T polymorphism in the *MTTP* gene (rs1800591) were detected by a real-time RT-PCR system using allelic discrimination (assay numbers C_761961_10 and C_8934089_10; TaqMan®; Applied Biosystems, Foster City, CA, USA) in a 96-well format and were read by a Roche LightCycler 96 system (Roche, Mannheim, Germany). DNA at a final concentration of 50 ng was used. PCR conditions were 95°C for 60 seconds, 40 cycles of amplification at 95°C for 15 seconds, and annealing/extension at 60°C for 60 seconds. Genotyping was verified using positive controls of the DNA samples (representing three possible genotypes) and negative controls (water) in each 96-well plate. Additionally, 10% of the samples were rerun and found to be 100% concordant.

Anthropometric measurements

Anthropometric parameters were measured after 12 hours of fasting and a final bowel movement. Height measurements were taken using a stadiometer (Rochester Clinical Research, Rochester, NY, USA). Tetrapolar electrical bioimpedance was used to assess body composition (InBody 3.0 body composition analyser; Biospace, Seoul, Korea).

BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured at the midpoint between the lowermost rib and the upper part of the iliac crest or at the point between those two bones, which produced the narrowest diameter. Subjects were classified has having abdominal obesity if their waist circumference was ≥ 80 cm in the case of women and ≥ 90 cm in the case of men, in accordance with International Diabetes Federation criteria.[16]

Biochemical analyses

Venous blood samples were taken after overnight fasting, and serum was immediately separated by centrifugation, analysed, and frozen at -70°C. Measurements of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), glucose, alanine aminotransferase, and aspartate aminotransferase were performed using a Vitros-250 dry chemistry analyser (Ortho-Clinical Diagnostics, Johnson & Johnson Services, Inc., Rochester, NY, USA). LDL-C levels were calculated using the Friedewald formula. For quality control purposes, we used a commercial control serum and a human pooled serum to ensure the accuracy of biochemical measurements. High molecular weight adiponectin

isoform and insulin levels were measured by ELISA assay (ALPCO Diagnostics, Salem, NH, USA, and Monobind Inc., Lake Forest, CA, USA, respectively). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as described by Matthews.[17]

Definitions and cutoffs

The following abnormalities were considered to exist in the presence of the stated factors and conditions: insulin resistance when HOMA-IR was ≥ 2.5;[18] hypoadiponectinaemia when adiponectin levels were ≤ 3 μg/mL;[18] hypertriglyceridaemia when triglyceride levels were ≥ 150 mg/dL; hypercholesterolaemia when total cholesterol levels were ≥ 200 mg/dL; high LDL-C when levels were ≥ 130 mg/dL; and hypoalphalipoproteinaemia when HDL-C levels were ≤ 35mg/dL.[19] Additionally, physical activity was considered to be present when subjects reported at least 150 minutes of moderate intensity aerobic exercise per week, or at least 75 minutes of vigorous intensity aerobic exercise per week (according to the Global Recommendations on Physical Activity for Health for adults).[20] Otherwise, subjects were considered as sedentary. A questionnaire used to assess physical activity was based on the Rapid Assessment of Physical Activity Mexican Spanish version.[21]

Statistical analyses

For calculating the sample size, we considered quantitative variables (allele frequencies), with 90% statistical power ($\beta = 0.10$ and $\alpha = 0.05$). Quantitative variables are expressed as mean ± standard deviation (SD), whereas qualitative variables are expressed as number and percentage. Statistical differences between groups were analysed using the Student's t-test, while the chi-square test was used for quantitative and qualitative variables. Hardy–Weinberg equilibrium and genetic inference analyses for the genetically at-risk group were performed using Arlequin mathematical algorithm software.[22] We categorized the two groups into the wild-type group and the genetically at-risk group according to results obtained from Arlequin software. Subjects were included in the wild-type group when they had three or more wild-type alleles (according to Arlequin software results). Subjects were included in the genetically at-risk group when they had two or more polymorphic alleles.

Odds ratios (ORs) and linear logistic regression tests were used to analyse associations between the genetically at-risk group and metabolic disorders. Potential interactions between *FABP2* and *MTTP* polymorphisms affecting total cholesterol and LDL-C levels were screened using logistic regression analysis in which the corresponding interaction terms were introduced into the models. Values were corrected for multiple comparisons using the Bonferroni test. An estimation of individual ancestry was calculated using Structure software (version 2.3.4) based on the allelic frequencies of the *FABP2* and *MTTP* gene polymorphisms reported in African, European (http://www.ensembl.org), and Amerindian (unpublished data) ancestral populations. Accordingly, the proportions of ancestral components were compared using hypercholesterolaemia categories. A p value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 20 for Windows (SPSS Inc., Chicago, IL, USA) and Epi-info™ 7 statistical software (CDC, Atlanta, GA, USA).

Ethical guidelines

All subjects signed an informed consent before participating in the study, which was conducted according to Declaration

of Helsinki guidelines. This study was approved by the Ethics Committee for Human Research of the University Center of Health Sciences, University of Guadalajara (Registration number: CI/019/2010).

Results

Population

The study's 523 subjects included 152 (29%) men and 371 (71%) women. The mean age was 36.4 ± 12.2 years. Of the total population, 63% presented with overweight and obesity according to their BMI. Additionally, 58% of subjects had a sedentary lifestyle (Table 1). The prevalence rates of metabolic disorders were as follows: hypoadiponectinaemia (51%), hypertriglyceridaemia (37%), insulin resistance (37%), hypercholesterolaemia (35%), high LDL-C levels (29%), and hypoalphalipoproteinaemia (23%).

Genetic characteristics

The genotype frequencies of the *FABP2* polymorphism were as follows: Ala54Ala (52.2%), Ala54Thr (41.1%), and Thr54Thr (6.7%). The allele frequencies among the study population were 73% for the Ala54 allele and 27% for the Thr54 allele. For the *MTTP* polymorphism, the genotype frequencies were as follows: G/G (58.2%), G/T (38.1%), and T/T (3.7%). The allele frequencies were 77% for the G allele and 23% for the T allele. The distribution of genotypes for the *FABP2* and *MTTP* polymorphisms were concordant with the Hardy–Weinberg equilibrium ($p = 0.91$ and $p = 0.07$, respectively).

Genotype frequencies were 58% for wild-type Ala54Ala/GG, 36% for Ala54Thr/GT, and 6% for Thr54Thr/TT. Therefore, the frequency of the genetically at-risk group was 42%.

There were no significant differences in demographic characteristics, such as sex and age of the studied subjects, between the genetically at-risk and wild-type groups. However, weight, BMI, and waist circumference were significantly higher in the wild-type group compared with the genetically at-risk group (all $p < 0.05$, Table 2).

Table 1. Demographic characteristics of the study population

Variable	General population
Age (years)	36.4 ± 12.2
Sex (F/M) (%)	71/29
Waist circumference (cm)	90.4 ± 15.8
Weight (kg)	73.7 ± 17.1
BMI (kg/m^2)	27.5 ± 5.8
Physical inactivity (%)	58.0

Data are shown as mean ± standard deviation or percentage.
F: female, M: male, BMI: body mass index.

Associations between the genetically at-risk group and metabolic disorders

Subjects who were carriers in the genetically at-risk group had higher levels of total cholesterol and LDL-C than did non-carriers (both $p < 0.01$, Table 3). Furthermore, these carriers also had a higher frequency of hypercholesterolaemia and high LDL-C levels than did non-carrier subjects (both $p < 0.05$, Table 4). Additionally, the genetically at-risk group had a higher probability of developing hypercholesterolaemia (OR = 1.57, 95% confidence interval [CI] 1.04–2.35, $p < 0.05$) and high LDL-C levels (OR = 1.68, 95% CI 1.08–2.62, $p < 0.05$) than did the wild-type group.

Furthermore, the genetically at-risk group was associated with an increase in cholesterol levels of 10.6 mg/dL ($\beta = 10.6$, 95% CI 3.46–17.85, $p = 0.004$) and an increase in LDL-C levels of 8.94 mg/dL ($\beta = 8.94$, 95% CI 2.27–15.60, $p = 0.009$). Significant interactions between *FABP2* and *MTTP* polymorphisms for total

Table 2. Comparison of demographic and anthropometrical variables by genetic group

Variable	Wild-type group (n = 389)	Genetically at-risk group (n = 134)	p value
Age (years)	36.8 ± 12.1	35.4 ± 12.2	0.246
Sex (F/M) (%)	70.4/29.6	73.1/26.9	0.582
Waist circumference (cm)	91.3 ± 15.7	87.5 ± 15.7	**0.023**
Weight (kg)	74.7 ± 17.2	70.7 ± 16.4	**0.019**
BMI (kg/m^2)	27.8 ± 5.9	26.6 ± 5.6	**0.037**
Physical activity (%)	43.2	42.3	0.918

Data are shown as mean ± standard deviation or percentage.
F: female, M: male, BMI: body mass index.

Table 3. Comparison of biochemical variables by genetic group

Variable	Wild-type group (n = 389)	Genetically at-risk group (n = 134)	p value
Glucose (mg/dL)	88.9 ± 10.5	88.6 ± 9.7	0.735
Uric acid (mg/dL)	5.1 ± 2.1	5.0 ± 1.3	0.791
AdipoQ (μg/mL)	3.3 ± 2.1	3.5 ± 2.4	0.330
Insulin	11.3 ± 8.6	11.9 ± 8.0	0.608
HOMA-IR	2.5 ± 2.1	2.1 ± 1.8	0.500
Cholesterol(mg/dL)	182.8 ± 36.8	193.5 ± 35.6	**0.004**
HDL-C (mg/dL)	44.2 ± 19.2	45.8 ± 12.6	0.395
LDL-C (mg/dL)	110.7 ± 31.8	119.6 ± 31.4	**0.008**
VLDL-C (mg/dL)	28.4± 16.8	29.3 ± 16.8	0.594
Triglycerides (mg/dL)	143.5 ± 84.1	145.8 ± 83.8	0.785
ALT	29.8 ± 17.2	32.5 ± 26.0	0.275
AST	28.0 ± 11.1	29.6 ± 13.5	0.202

Data are shown as mean ± standard deviation.
AdipoQ: adiponectin, HOMA-IR: homeostatic model assessment of insulin resistance, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, VLDL-C: very low-density lipoprotein cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Table 4. Prevalence of metabolic disorders associated with the genetically at-risk group

Variable	Wild-type group (n = 389)	Genetically at-risk group (n = 134)	p value
Hypoadiponectinaemia	192 (58.7)	60 (50.0)	0.100
HOMA-IR	119 (36.0)	48 (41.0)	0.329
Hypercholesterolaemia	124 (32.0)	57 (42.5)	**0.028**
Hypoalphalipoproteinaemia	88 (24.7)	21 (17.5)	0.104
High LDL-C	92 (26.4)	44 (37.6)	**0.021**
Hypertriglyceridaemia	142 (36.6)	48 (36.1)	0.916
Abdominal obesity	224 (65.5)	67 (55.4)	**0.049**

Variables are expressed as number (%). HOMA-IR: homeostatic model assessment of insulin resistance, LDL-C: low-density lipoprotein cholesterol.

cholesterol (interaction, $p = 0.041$) and LDL-C (interaction, $p = 0.036$) serum levels were found.

Ancestry analyses

Association analysis did not show any differences in ancestral components (European, African, and Amerindian). Therefore, we concluded that population stratification was not a confounding factor in the association with hypercholesterolaemia.

Discussion

This study showed an association between the genetically at-risk group (Thr54/-493T) and the presence of lipid disorders, particularly those disorders associated with total cholesterol and LDL-C levels. Lipid disorders, as well as obesity, are considered to be significant factors contributing to the development of cardiovascular disease.[23]

Our study did not show any significant differences between the wild-type and genetically at-risk groups regarding variables that may contribute to lipid-level disorders, such as sex, age, and physical activity level. This finding provides strong evidence for the presence of a genetic component in the genetically at-risk group, mainly regarding total cholesterol and LDL-C levels. Waist circumference and BMI are well-known factors of significance in development of metabolic disorders. Despite the fact that abdominal obesity (as measured by waist circumference) was more prevalent in the wild-type group, biochemical disorders (high cholesterol and LDL-C levels) occurred more frequently in the genetically at-risk group. However, in a previous study, we found that identical dietary patterns were present in normal weight and overweight subjects.[24] These results highlight the importance of considering genetic background when prescribing an integral treatment for metabolic disorders. To the best of our knowledge, this is the first study to report the synergic effect of both genetic variants (Ala54Thr of the *FABP2* gene and -493 G/T of the *MTTP* gene) involved in lipid metabolism.

Both single nucleotide polymorphisms that were investigated in our study play a role in lipid metabolism. The *FABP2* polymorphism is involved in the transport of dietary fatty acids to enterocytes[7] and the *MTTP* polymorphism is involved in transport and assembly of chylomicrons and VLDL.[13] Therefore, an association could be established between the genetically at-risk group and plasma lipid level disorders in our study.

With regard to the -493 G/T polymorphism of the *MTTP* gene, MTTP has a higher level of activity and a greater role in the assembly and secretion of VLDL compared with the wild-type variant. VLDL can be catabolized into LDL particles. Therefore, MTTP could contribute to an increase in total cholesterol and LDL-C levels.[25] Dietary triglycerides enter the bloodstream, where the lipoprotein lipase enzyme hydrolyses them into VLDL. VLDL subsequently becomes LDL, which is the most responsible lipoprotein for delivering cholesterol to tissues, thus contributing to cardiovascular disease.[26]

I-FABP is expressed in enterocytes in the small intestine. The main function of I-FABP is related to internalization of long-chain fatty acids into enterocytes. Carrier subjects of the Thr54 variant could thus have a two-fold greater affinity for long-chain fatty acids and an associated rise in intestinal fat absorption, which is related to diseases that involve lipid metabolism[27], such as dyslipidaemia.[28] Although some researchers have found an association between the Ala54Thr polymorphism and insulin resistance,[29] such results are inconsistent with other studies that showed no association between insulin resistance and this genetic variant.[28] Nonetheless, because

the mechanism by which the *FABP2* Thr54 variant is capable of raising fasting LDL-C levels is unknown, more research is needed to identify this mechanism. Baier et al.,[30] using genetically modified Caco-2 cells and intestinal biopsies, demonstrated the effectiveness of the I-FABP Thr54 variant in increasing intestinal fat absorption. We hypothesize that the presence of the Thr54 variant contributes to the rise in postprandial fatty acid levels that must be transported by chylomicrons and chylomicron remnants. This could result in an increment of hepatic synthesis of VLDLs that are catabolized into LDL lipoproteins.[30,31]

In this study, we did not find any significant associations between the genetically at-risk group and the metabolic disorders of hypoadiponectinaemia, hypertriglyceridaemia, insulin resistance, and hypoalphalipoproteinaemia. We only found that the genetically at-risk group had a higher probability of developing hypercholesterolaemia and high LDL-C levels, which is consistent with a previous study by Stand et al.[31]

Mexico has a high prevalence of lipid disorders. Among the most common of these disorders is hypercholesterolaemia, which could be the result of interactions between multiple genetic and environmental factors. Obesity is also highly prevalent in Mexico, and obesity is increasing, despite implementation of specific health strategies and/or treatments. Such strategies may not take into consideration the interaction between genetic and environmental factors. We suggest that genetic factors should be given greater consideration because their effect on the health of individuals is quite considerable.

Conclusions

In our study, the genetically at-risk group was associated with hypercholesterolaemia and high LDL-C levels. These findings highlight the importance of designing new personalized nutritional strategies for prevention and treatment of chronic disease.

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Authors' contributions

AP and EM designed the study. KG and OR collected and processed the data. KG, MG, and EM analysed the data. KG, OR, MG, EB, AP, MG, and EM participated in interpretation of data and preparation of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

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