Association of rs2000999 in the haptoglobin gene with total cholesterol, HDL-C, and LDL-C levels in Mexican type 2 diabetes patients

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Abstract

Recently, studies have shown significant association between the rs2000999 polymorphism in the haptoglobin-encoding gene (HP) and low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels, which are important risk factors for cardiovascular diseases. However, the association of rs2000999 with serum lipids in Latin American diabetic populations is still uncharacterized.

Here, we analyzed the association of rs2000999 with TC, high-density lipoprotein cholesterol (HDL-C), and LDL-C levels in 546 Mexican adults with type 2 diabetes (T2D) and in 654 controls without T2D. In this observational case-control study we included adults from 4 centers of the Mexican Social Security Institute in Mexico City recruited from 2012 to 2015. TC, HDL-C, LDL-C, triglycerides (TG), and glucose levels were measured by an enzymatic colorimetric method. The variant rs2000999 was genotyped using TaqMan real time polymerase chain reaction. The percentage of Native-American ancestry showed a negative association with the rs2000999 A allele. In contrast, the rs2000999 A allele had a strong positive association with European ancestry, and to a lesser extent, with African ancestry. Linear regression was used to estimate the association between the variant rs2000999 and lipid concentrations, using different genetic models. Under codominant and recessive models, rs2000999 was significantly associated with TC and LDL-C levels in the T2D group and in controls without T2D. In addition, the group with T2D showed a significant association between the variant and HDL-C levels. In summary, the rs2000999 A allele in Mexican population is positively associated with the percentage of European and negatively associated with Native American ancestry. Carriers of the A allele have increased levels of TC and LDL-C, independently of T2D diagnosis, and also increased concentrations of HDL-C in the T2D sample.

Abbreviations: GWAS = genome-wide association study, HDL-C = high-density lipoprotein cholesterol, HP = haptoglobin-encoding gene, LDL-C = low-density lipoprotein cholesterol, SE = standard error, T2D = type 2 diabetes, TC = total cholesterol, TG = triglycerides.

Keywords: haptoglobin, HDL-C, LDL-C, Mexican population, rs2000999, total cholesterol, type 2 diabetes

1. Introduction

The National Institute of Statistics and Geography (INEGI) reported in 2018 that cardiovascular diseases are the primary cause of death in Mexico.[1] The most important risk factors for the onset of these diseases are high levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-C). Genetic studies of the loci associated with lipid levels have enabled the identification of polymorphisms that may serve to develop new approaches for the prevention and treatment of cardiovascular diseases. In particular, our research group has collaborated with other research institutions with the objective of characterizing the loci associated with dyslipidemia in the Mexican and Hispanic populations. In a genome-wide association study (GWAS), we found that the genes, CELSR2, ZNF259, APOA5, KANK2/DOCK6 and NCAN/MAU2, were associated with TC levels; CELSR2, APOB, and NCAN/MAU2 with LDL-C levels[2], and DAGLB with HDL-C levels.[3] The same GWAS study failed to find an association between the haptoglobin-encoding (HP) gene (located at chromosome 16q22.2) with lipid levels. However, previous studies have showed that the A allele of the rs2000999 polymorphism in HP is associated with high levels of TC and LDL-C. It has been proposed that the rs2000999 A allele, which is associated with reduced HP expression, has decreased antioxidant protection for APOE, contributing to elevated cholesterol levels.[4,5]
Hp is an alpha-glucoprotein commonly found in plasma; it is composed of 2 light chains (α) and 2 heavy chains (β) that are covalently bound. It is mainly produced in the liver and has been reported to play a role in cholesterol esterification, particularly with respect to the binding of apolipoprotein A-I to HDL-C, which promotes cholesterol efflux from cells and stimulates the enzyme lecithin:cholesterol acyltransferase to esterify cholesterol. Hp has been proposed to target and protect the ApoA-I effector domain of lecithin:cholesterol acyltransferase from oxidative stress; Hp can also bind to ApoE. ApoA-I and ApoE contain similar sequences, which are able to stimulate LCAT to achieve cholesterol esterification in reverse cholesterol transport.[7,8,9] Recently, Boettger et al. (2016) identified a strong association between the rs2000999 (G/A) polymorphism in HP with TC and LDL-C levels. The rs2000999 A allele decreases Hp expression, consequently reducing its antioxidant capacity, which in turn leads to higher cholesterol levels.[10] The human HP gene has 2 common alleles, Hp1 and Hp2, which are the result of a small intragenic duplication of the HP gene.[11] The Hp1 allele exhibits enhanced antioxidant activity compared to Hp2. The frequency of the alleles Hp1 and Hp2 is quite variable in world populations. In general, the Hp1 allele is less frequent in East Asian populations than in other population groups, including European, African, and Native American groups.[12-17] Studies in patients with diabetes revealed that the Hp2-1 or Hp2-2 genotypes are associated with increased risk of vascular injury compared to the Hp1-1 genotype.[18,19,10] In addition, the rs2000999 A allele is almost exclusively associated with the Hp2 isomorph and decrease in expression of Hp; on the other hand, rs2000999 (G) is associated with the Hp1 isomorph.[4] A study performed in Chinese diabetic patients reported that the variant rs2000999 is not associated with diabetic macrovascular diseases, although the A allele is associated with higher levels of LDL-C.[20]

To date, the association of rs2000999 of HP with serum lipids in the Latin American diabetic population is uncharacterized. Therefore, the aim of this study was to analyze the association of rs2000999 with TC, HDL-C, and LDL-C and in the Mexican population with type 2 diabetes (T2D).

2. Material and methods

2.1. Study population

We investigated 1,200 Mexican adults of both sexes (546 adults with T2D, the T2D group; and 654 controls without T2D, the No T2D group) from 4 centers of social security in Mexico City from 2012 to 2015. The T2D group was selected based on fasting glucose levels, in accordance with American Diabetes Association (ADA) guidelines.[21] The present work was designed as an observational case-control study with a convenience sample size.

The study protocol complies with the ethical guidelines of the 1975 Declaration of Helsinki. The study was authorized by the Instituto Mexicano del Seguro Social ethics committee and informed consent was obtained from all participants.

2.2. Procedure

All participants were weighed using a digital scale (Seca, Hamburg, Germany). Height was measured with a portable stadiometer (Seca 225, Hamburg, Germany). Body mass index (BMI), calculated as weight (kg)/height (m)². Waist circumference was measured taken after expiration at the midpoint between the low rib margin and iliac crest. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercurial sphygmomanometer (ALPK2, Tokyo, Japan). Data regarding age and smoking status (current smokers/non-smokers) was determined by self-report.

Two blood samples were collected from each participant to estimate the biochemical parameters and for DNA extraction. The IL-650 equipment (Instrument Laboratory, Bedford, MA, USA) was used to quantify TC, HDL-C, LDL-C, triglycerides (TG), and glucose levels measured by enzymatic colorimetric method (kit numbers 0018250540, 0018255740, 0018256040, 0018480500 and 0018250740 of Werfen Czech s.r.o, respectively). According with Grundy et al.[22] metabolic syndrome was defined with the presence of 3 or more risk factors (waist circumference ≥102 cm in men or ≥88 cm in women, triglycerides ≥150 mg/dl, HDL-C < 40 mg/dl in men or < 50 in women, systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg and fasting glucose ≥100 mg/dl).

DNA isolation was performed in the AutoGen Flex Star (AutoGen, MA, USA) following the manufacturer’s recommendations. DNA quantity (260nm) and purity (260/280nm ratio) were evaluated in the Epoch Microplate Spectrophotometer using Gen5 Microplate Data Analysis (BioCell, VT, USA). DNA integrity was also assessed by electrophoresis in agarose gels (.8%).

Genotyping of rs2000999 was performed by real time polymerase chain reaction using the TaqMan allelic discrimination assay C_11439054_10 on the 7900HT Fast Real-Time PCR system (Applied Biosystems, CA, USA), following standard protocols. Duplicates were performed in 10% of the samples. Genotype discrimination was evaluated using the SDS software (Applied Biosystems, CA, USA).

Estimation of individual ancestral proportions: Given that the individuals in this sample are of mixed ancestry, the Axiom LAT microarray (Affymetrix, CA, USA) was used to determine the Native-American (NAM), European and African ancestry proportions of the participants using the program ADMIXTURE.[23] We added individual ancestral proportions as a covariate in the association analyses.

2.3. Statistical analysis

The normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test. For the traits that significantly deviate from normality, rank based inverse normal transformations were applied. Differences between the cases and controls for continuous and categorical traits were evaluated using the Student t test and Chi-Squared test, respectively. We tested the association of rs2000999 with individual ancestral proportions, using an additive model, with adjustments for sex, age and T2D diagnosis. The association of rs2000999 with lipid concentrations was assessed using linear regression under different genetic models (codominant, dominant and recessive). All statistical analyses were performed using SPSS software (version 22.0, IBM, Armonk, NY, USA). Two-sided P values < .05 were considered significant.

3. Results

3.1. Characteristics of the study population

The characteristics of the 1200 participants included in the sample are presented in Table 1. Compared with non-diabetic
individuals, the T2D group exhibited significantly higher frequency of women, age, waist circumference, BMI, TG, ratios of TC/HDL-C, NonHDL-C/HDL and TG/HDL-C, blood pressure, glucose, and frequency of current smokers and individuals with metabolic syndrome. However, the non-diabetic individuals showed significantly higher levels of TC, HDL-C and LDL-C than the T2D group. LDL-C/HDL-C ratio and NonHDL-C did not display significant difference between the non-diabetic and T2D groups. Similarly, the non-diabetic and T2D groups had similar frequencies of the rs2000999 A. The polymorphism was in Hardy–Weinberg equilibrium in both groups (P_{No-T2D}=.530 and P_{T2D}=.991).

### 3.2. Association of rs2000999 A allele with individual ancestry proportions

The rs2000999 A allele had a strong positive association with the percentage of European ancestry (β±SE [standard error] = 0.028 ± 0.011, P = .013) and to a lesser extent African ancestry (β±SE = 0.003 ± 0.002, P = .041). On the other hand, the percentage of Native-American ancestry showed a strong negative association with the A allele of rs2000999 of HP (β±SE = −0.031 ± 0.012, P = .012).

### 3.3. Association of rs2000999 with serum lipids

We tested the association of the variant rs2000999 with serum lipids under different genetic models, using the A allele as the effect allele. Under the codominant model (AA vs AG vs GG) the rs2000999 A allele was associated with higher TC and LDL-C levels in the No T2D group (TC: β±SE = 9.102 ± 3.677, P = .014; LDL-C: β±SE = 6.423 ± 3.144, P = .042) and the T2D group (TC: β±SE = 8.918 ± 3.955, P = .025; LDL-C: β±SE = 8.222 ± 3.240, P = .012) (Table 2). Under the recessive model (AA vs AG + GG), AA homozygotes are significantly associated with higher TC and LDL-C levels in the No T2D group (TC: β±SE = 10.102 ± 4.038, P = .013; LDL-C: β±SE = 7.287 ± 3.453, P = .035) and T2D group (TC: β±SE = 10.196 ± 5.535, P = .026; LDL-C: β±SE = 8.662 ± 3.736, P = .021) (Table 2). For HDL-C, there are also significant associations under the codominant and recessive models in the T2D group (codominant: β±SE = 2.402 ± 1.126, P = .034; recessive: β±SE = 3.283 ± 1.295, P = .012), but not in the non-diabetic group (Table 2). Under the dominant model, rs2000999 was not significantly associated with lipid levels, although we saw similar trends indicating higher lipid levels in carriers of the A allele, particularly for TC and LDL-C (Table 2).

### 4. Discussion

This is the first study to describe an association of the rs2000999 variant of HP with serum lipids in a Latin American diabetic population. The allelic frequency of the variant was quite similar in the No T2D and T2D groups. In this work, we show that the percentage of Native-American ancestry is negatively associated

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**Table 1**

|                     | Without T2D | T2D |
|---------------------|-------------|-----|
|                     | N = 654     | N = 546 |
| Woman n (%)         | 307 (46.9)  | 322 (58.9) |
| Age (years)         | 49.615 ± 9.281 | 57.077 ± 10.006 |
| Current smokers n (%) | 65 (9.9)  | 142 (26.0) |
| Waist circumference (cm) | 90.8 ± 13.2 | 97.3 ± 12.4 |
| BMI (kg/m²)         | 27.944 ± 4.712 | 29.427 ± 5.140 |
| TC (mg/dL)          | 195.751 ± 39.605 | 168.659 ± 43.873 |
| HDL-C (mg/dL)       | 49.418 ± 13.595 | 43.977 ± 13.039 |
| LDL-C (mg/dL)       | 135.582 ± 33.539 | 127.967 ± 45.479 |
| TG/HDL-C            | 4.2 ± 1.3   | 4.5 ± 1.4   |
| NonHDL-C            | 2.0 ± 0.9   | 1.3 ± 0.9   |
| HDL (mg/dL)         | 146.3 ± 36.2 | 145.1 ± 40.1 |
| HDL-C               | 3.2 ± 1.3   | 3.5 ± 1.4   |
| TG (mg/dl)          | 151.5 ± 74.9 | 184.36 ± 93.4 |
| Glucose (mg/dl)     | 92.2 ± 26.8 | 148.6 ± 16.0 |
| With metabolic syndrome n (%) | 118.0 ± 13.7 | 123.7 ± 16.0 |
| Variant rs2000999   |             |     |
| A (A) (%)           | 18.8        | 19.9   |
| African (%)         | 3.5 ± 2.3   | 3.4 ± 2.4   |
| European (%)        | 32.8 ± 17.9 | 32.2 ± 17.0 |
| Amerindian (%)      | 63.7 ± 19.3 | 64.3 ± 18.2 |

Analysis by Student t and Chi-Squared.

Data are represented as n (%) and mean ± standard deviation. BMI = body mass index, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, NonHDL-C = ratio of nonHDL-C and HDL-C, T2D = type 2 diabetes, TC/HDL-C = ratio of TC and HDL-C, TC = total cholesterol, TG/HDL-C = ratio of TG and HDL-C, TG = triglycerides.

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**Table 2**

|                      | TC (mg/dL) | P      | HDL (mg/dL) | P      | LDL (mg/dL) | P      |
|----------------------|------------|--------|-------------|--------|-------------|--------|
|                      | AA/AG/GG   |        | AA/AG+GG    |        | AA+AG/GG    |        |
| TC (mg/dL) NO        | 9.102 ± 3.677 | .014  | 10.102 ± 4.038 | .013  | 10.680 ± 14.162 | .451  |
|                      | 8.918 ± 3.955 | .025  | 10.196 ± 5.555 | .026  | 12.339 ± 12.580 | .327  |
| HDL (mg/dL) NO       | 1.279 ± 1.160 | .271  | 1.156 ± 1.275 | .325  | 3.494 ± 4.444 | .432  |
|                      | 2.402 ± 1.126 | .034  | 3.283 ± 1.295 | .012  | 0.793 ± 3.585 | .837  |
| LDL (mg/dL) NO       | 6.425 ± 3.144 | .042  | 7.287 ± 3.453 | .035  | 5.650 ± 12.093 | .641  |
|                      | 8.222 ± 3.240 | .012  | 8.662 ± 3.736 | .021  | 16.961 ± 10.301 | .100  |

Analysis by linear regression adjusted for age, sex, obesity diagnosis, smoke status and individual ancestry proportions.

Data as β±SE. HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, SE = standard error, T2D = Type 2 diabetes, TC = total cholesterol.
with the rs2000999 A allele. In contrast, the rs2000999 A allele has a strong positive association with European ancestry, and to a lesser extent, with African ancestry (it is important to note that the average African ancestry in the Mexican sample is quite low, around 3.5%). Our results are quite consistent with data from different world populations. In the allele frequency database ALFRED (https://alfred.med.yale.edu/alfred/index.aspx), rs2000999 A allele frequencies in Southern European populations are higher than 20%, and in Native American groups from Mexico (Pima, Maya) do not surpass 10%. The frequencies of the A allele in African populations range from 0% to 12.8%. Given that the rs2000999 A allele is present almost exclusively on Hp2 haplotypes,[4] our results indicate that that frequency of the Hp2 allele is lower in the Native American ancestral population than in the European or African ancestral populations. This is consistent with studies that have reported that the frequencies of the Hp1 allele are quite high in some Native American groups, including indigenous groups from Mexico.[16,17] Further studies are needed to describe in detail the linkage disequilibrium patterns between rs2000999 and the Hp1 and Hp2 alleles in different population groups, including indigenous groups from the Americas.

We observed that under codominant and recessive models, rs2000999 is associated with TC and LDL-C levels in both the No T2D and T2D groups. Interestingly, under codominant and recessive models, rs2000999 was also significantly associated with HDL-C levels in the T2D group, but not in the non-diabetic group. Our results indicate that carriers of the A allele have higher lipid concentrations than non-carriers, although dominant group. Our results indicate that carriers of the A allele have increased levels of TC and LDL-C, independently of T2D diagnosis, and also increased concentrations of HDL-C in the T2D sample, but not in the non-diabetic group.

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