Characterization of LDLR rs5925 and PCSK9 rs505151 genetic variants frequencies in healthy subjects from northern Chile: Influence on plasma lipid levels

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Abstract
Background: Identification and characterization of genetic variants and their effects on human health may allow to establish relationships between genetic background and susceptibility to developing cardiovascular diseases. LDLR and PCSK9 polymorphisms have been associated with higher lipid levels and risk of cardiovascular diseases. Thus, the main aim of this study was to evaluate genotype distribution and relative allelic frequency of LDLR rs5925 (1959C > T) and PCSK9 rs505151 (23968 A > G) genetic variants and their effects on lipid levels of healthy subjects from northern Chile.

Methods: A total of 178 healthy individuals were recruited for this study. The genotyping of rs5925 (LDLR) and rs505151 (PCSK9) polymorphisms was performed by PCR-RFLP and qPCR, respectively. In addition, glucose and lipid levels were determined and associated with the genetic data.

Results: Genotype distribution for LDLR rs5925 polymorphism was as follows: CC = 19%; CT = 53%; and TT = 28% (HWE: $\chi^2 = 0.80; P = .37$), and for PCSK9 rs505151 genetic variant was as follows: AA = 93%; AG = 7%; and GG = 0% (HWE: $\chi^2 = 0.22; P = .64$). The frequency of T (rs5925) and G (rs505151) mutated alleles was 0.55 and 0.03, respectively. Data showed that individuals carrying LDLR mutated allele (T) presented lower values of total cholesterol, triglycerides, and LDL-cholesterol when compared to CC homozygous genotype ($P < .05$). Subgroup analysis revealed that women carrying the PCSK9 mutated allele (G) exhibited higher values of total cholesterol, triglycerides, HDL-C, and LDL-C when compared to male group carrying the same genotype ($P < .05$).

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; DBP, diastolic blood pressure; DNA, deoxyribonucleic acid; dNTPs, deoxyribonucleotides; HBP, high blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MgCl₂, magnesium chloride; PCR, polymerase chain reaction; PCSK9, proprotein convertase subtilisin/kexin type 9; SNP, single nucleotide polymorphism; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

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1 | INTRODUCTION

A growing number of human diseases have been associated with genetic variants; therefore, a key goal of biomedical research consists of correlating the genotype with the phenotype for ailments affecting the population. While the precise magnitude of inheritance is specific for each disease kind, other factors also can influence disease outcome, such as the pathology subtype, age onset, and environmental factors. These considerations can be particularly important for cardiovascular diseases (CVD), such as high blood pressure (HBP), cardiac ischemia, or stroke, which are the third cause of death in Chile. Most of these CVD are associated with increased plasma levels of low-density lipoprotein cholesterol (LDL-C). On this issue, LDL receptor (LDLR) plays an important role in the lipoprotein metabolism, since LDL-C is removed from blood vessels through LDLR endocytosis. This receptor is a 160-kDa transmembrane glycoprotein, ubiquitously distributed and encoded by LDLR gene located in chromosome 19, which spans 45 Kb and contains 18 exons encoding for six functional domains in the mature protein. LDLR gene mutations represent the main genetic cause of familial hypercholesterolemia suggesting a mechanism involving genetic factors. In this study, we evaluated LDLR rs5925 (1959C>T or Avll) genetic variant, previously reported to be associated with increased total cholesterol (TC) and LDL-C in Chinese, Brazilian, Hispanic, and non-Hispanic white individuals. Besides, this genetic variant was strongly associated with differences on plasma lipid levels in subjects with high risk of CVD and lower response to fluvastatin treatment. On the other hand, this LDLR variant was associated with low LDL-C in Italian individuals and was not associated with change in lipid profile in European subjects from Germany, the Netherlands, and Denmark. Moreover, no association was found between LDLR rs5925 genetic variant and atorvastatin response in Chilean Amerindian subjects, suggesting that LDLR polymorphism can influence plasma lipid levels and account for CVD risk, but that effect could also be partially influenced by ethnic characteristics in the studied individuals.

Beside the aforementioned, plasma lipid levels are regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), a secretory serine endoprotease mainly synthesized and mainly secreted from liver with lower expression in intestine, kidney, and brain. PCSK9 plays a role as chaperone binding to LDL receptor (LDLR) to promote its lysosomal degradation in hepatic cells, mediating approximately 70% of LDL-C clearance. The PCSK9 gene comprises 12 exons and 11 introns located in chromosome 1, and is highly polymorphic showing a total of 163 mutations and polymorphisms that are distributed in every PCSK9 domains. PCSK9 missense mutations determine a gain of function that accentuated PCSK9 activity and cause a rare form of autosomal hypercholesterolemia; on the other hand, loss-of-function mutations, identified at relatively high frequencies (2%-4%) in certain ethnic groups, were associated with lowered plasma LDL-C levels and significant protection from CHD. PCSK9 rs505151 polymorphism (23968 A>G) is a gain-of-function mutation located in exon 12 that spans cysteine-rich C-terminal domain and appears to be involved in autoprocessing. A substitution from glutamate to glycine at position 670 results in accumulation of processed PCSK9 and consequent increased plasma LDL levels in some population. This PCSK9 genetic variant was recently associated with higher triglycerides (TG) and LDL-C levels as well as cardiovascular risk; associated with severity of coronary atherosclerosis in Caucasian, African-Americans, and American Indian; and also associated with hypercholesterolemia in men but not in women in European population. Previously reported, PCSK9 rs505151 genetic variant was associated with higher LDL-C levels and affected lipid-lowering response to atorvastatin in individuals from the south of Chile. On the other hand, no association was found between PCSK9 rs505151 genetic variant and LDL-C in old individuals with vascular disease from Scotland, Ireland, and the Netherlands, and young and middle age African-American and non-Hispanic black population.

All these discrepancies are mostly related to the prevalence of the mutant allele in different ethnicities varying their impact on the incidence of cardiovascular risk; however, life style habits and environmental factors could not be ruled out. Thus, we consider those important reasons to evaluate the actual prevalence of these genetic variants in healthy Chilean individuals along with their relationships to plasma lipid levels as important predictors of cardiovascular risk. Then, this study was focused to determine the prevalence of LDLR rs5925 and PCSK9 rs505151 genetic variants in healthy subjects from the north of Chile and the effect of those variants on plasma lipid levels.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 178 unrelated individuals (129 females and 49 males) were selected randomly for this study. All subjects included were healthy university students born in the region of Antofagasta (northern Chile), with no diagnosis of CVD, and were informed about the study.
design and goals. All the subjects signed a written informed consent prior to enrolling the study and blood extraction. All participants filled out a standardized questionnaire concerning basic cardiovascular risk factors, the collected data included age, gender and considered smoking, drinking, recreational drug consumption, exercise habits, and history of CVD based on self-reports. The type of recreational drugs used was not specified. Individuals enrolled in the study required to have normal fasting glucose (levels lower than 100 mg/dL), systolic blood pressure lower than 120 mm Hg, diastolic blood pressure lower than 80 mm Hg, and body mass index lower than 25. Individuals taking anti-hypercholesterolemic, anti-hypertensive, or hypoglycemic medicines or those who declared to have any personal or family history of CVD were excluded from the study. All procedures were approved by the Ethics Committee of Universidad de Antofagasta (Chile) and performed according to institutional guidelines. Anthropometric and clinical parameters as well as blood sample analyses were processed following standard procedures. Briefly, the body mass index (BMI) was defined as the body weight in kilograms divided by the square of the body height in meters and expressed in units of kg/m². Blood samples were obtained and separated in two fractions, a plasma fraction for biochemical analyses was centrifugal separated and stored at −20°C, and another fraction for total genomic DNA analyses was maintained at 4°C until processing.

### 2.2 Biochemical measurements

Venous blood was drawn from antecubital vein in all subjects after overnight fasting. The plasma levels of glucose, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined using enzymatic-colorimetric commercial available kits from Human Diagnostics Worldwide, Germany. Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald’s formula if TG did not exceed 400 mg/dL.

### 2.3 DNA genotyping

Genomic DNA was extracted from peripheral blood leukocytes by using salting out procedure optimized by Salazar et al.²⁶ LDLR rs5925 involves a substitution of C for T in the third base of codon 632 located in exon 13, which introduces a synonymous mutation that creates a recognition site for Avell endonuclease.²⁶ Genotyping for LDLR polymorphism was carried out by PCR amplification of a 228 bp amplicon, using the forward primer of 5'-GTCATCTTCTTGCTGTGAC-3' and a reverse primer of 5'-GGTTCCCACAAGGAGGGTTCAAGGT-3', described by Ahn et al.²⁶ PCR amplification reaction was performed with 50 ng of genomic DNA, 0.2 mmol/L of dNTPs mix, 2 mmol/L of MgCl₂, 200 mmol/L of each primer, 1 U Taq polymerase (New England Biolabs Inc), and PCR buffer (20 mmol/L Tris-HCl, 10 mmol/L (NH₄)₂SO₄, 10 mmol/L KCl, 2 mmol/L MgSO₄, 0.1% Triton®-X-100, pH 8.8). The thermocycling conditions were as follows: 35 cycles consisting on denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute. PCR products were evaluated in 2% agarose gel electrophoresis, and subsequently, 7 µL of PCR products was digested with 1 U of Avell (New England Biolabs Inc). Reactions were incubated at 37°C for 12 hours and detected in 2% agarose gel electrophoresis.

PCSK9 gene mutation involves a substitution of A for G in the second base of codon 670 located in exon 12, which introduces an amino acid substitution from glutamate to glycine.²⁷ Genotyping analysis for PCSK9 rs23968 A > G polymorphism was determined by real-time PCR using 4351379 TaqMan® SNP Genotyping Assays, Human, SM (ID 998744, Applied Biosystems). The PCR reaction was performed following standard conditions from the manufacturer. Briefly, assays contained 1 µL DNA (20 ng). 6.25 µL 2x TaqMan® PCR master mix, 0.625 µL 20 TaqMan® genotyping assay, and 5.125 µL nuclease-free water for 13 µL total volume. Thermal cycling conditions for real-time system were initial denaturation at 95°C for 10 minutes, followed by 50 cycles of denaturation at 95°C for 15 seconds, annealing and extension at 60°C for 1 minute, and a final extension at 60°C for 30 seconds.

### 2.4 Statistical analyses

Genotype and allele frequencies were obtained by direct gene counting. The genotype distribution for the Hardy-Weinberg equilibrium (HWE) was assessed by chi-squared analysis. The Shapiro-Wilk test was performed to test for normal distribution of continuous variables. Continuous variables were represented as means ± standard deviation (SD). Comparison of continuous variables was performed using Student’s t test or ANOVA one-way test. The multiple comparisons were performed by Bonferroni method. The statistical analyses were performed using the Sigma Stat statistical software (Systat Software Inc). A P < .05 was regarded as statistically significant.

### 3 RESULTS

Anthropometrics and clinical characteristics of 178 individuals (28% male and 72% female) recruited for this study are summarized in Table 1. The average age was 22.8 ± 0.6 years (25.3 ± 1.6 years for male and 21.8 ± 0.5 years for female). All participants in this study had normal value for anthropometric body mass index (BMI) 24.1 ± 3.7 kg/m² (24.6 ± 3.5 kg/m² for male and 23.6 ± 3.7 kg/m² for female). Clinical characteristics were normal as well, with systolic blood pressure (SBP) of 116.8 ± 14.8 mm Hg (118.3 ± 13.6 mm Hg for male and 115.6 ± 15.7 mm Hg for female) and diastolic blood pressure (DBP) of 69.9 ± 12.6 mm Hg (70.3 ± 15.0 mm Hg for male and 69.5 ± 10.4 mm Hg for female). Plasma glucose and lipid profile were all in the reference values, glucose 85.5 ± 1.9 mg/dL; cholesterol 163.1 ± 10.0 mg/dL; triglycerides 94.4 ± 7.7 mg/dL; HDL-cholesterol 46.7 ± 0.6 mg/dL; and LDL-cholesterol 84.3 ± 2.7 mg/dL. Analysis by gender is shown in Table 1. No significant differences were found among groups.
The genotype distribution of LDLR rs5925 and PCSK9 rs505151 genetic variants was consistent with the Hardy-Weinberg equilibrium as shown in Table 2. The relative frequency of LDLR rs5925 polymorphism in the studied group was 0.55 for mutated allele (T). The relative frequency of rs505151 PCSK9 polymorphism was 0.03 for mutated allele (G).

Based on previous reports, we studied the potential association of both rs5925 LDLR and rs505151 PCSK9 genetic variants with plasma lipid levels. The results showed significant lower values of TC, TG, and LDL-C (P < .05) in individuals carrying LDLR rs5925 mutated allele (T) in either homozygous TT or heterozygous CT when compared to CC homozygous genotype. Moreover, similar results were observed for TG, and LDL-C in the female group (P < .05).

Analysis of PCSK9 rs505151 genetic variant showed no changes in lipid profile for the total group of individuals studied. However, the presence of mutated allele (G) in the male group was associated with lower values in TC (P < .05) and TG (P < .05) when compared to men carrying AA genotype. On the other hand, in the female group we found higher values of TC (P < .05) associated with the presence of mutated allele (G) when compared to women carrying the AA genotype. In addition, the female carrying AG heterozygous genotype showed significant higher values of TC and TG (P < .05) when compared to men carrying AA genotype. On the other hand, in the female group we found higher values of TC (P < .05) associated with the presence of mutated allele (G) when compared to women carrying the AA genotype. In addition, the female carrying AG heterozygous genotype showed significant higher values of TC, TG, HDL-C, and LDL-C when compared to male group carrying the same genotype. The results are shown in Table 3.

4 | DISCUSSION

This is the first study highlighting the frequency of LDLR and PCSK9 genetic variants and their relationship with plasma lipid levels in healthy individuals from Antofagasta city (northern Chile). In the studied group, the relative frequency for mutated allele T of rs5925 (LDLR) was 0.55, which is not very different for that found in individuals from southeastern Brazil, non-Hispanic white population in North America, and normocholesterolemic individuals from Italy, Germany, and the Netherlands. However, this rate was different from the allele frequency found in Chilean Amerindian hypercholesterolemic subjects (0.44) from southern Chile, healthy Caucasian Brazilian individuals, and Chinese population. The analysis of PCSK9 mutated allele (G) frequency was 0.03, similar to the previously described for healthy and coronary disease patients from southern Chile, American Indians, European

| Parameter | Total (178) | Male (49) | Female (129) |
|-----------|------------|-----------|--------------|
| Age (years) | 22.8 ± 0.6 | 25.3 ± 1.6 | 21.8 ± 0.5 |
| BMI (kg/m²) | 24.1 ± 3.7 | 24.6 ± 3.5 | 23.3 ± 3.7 |
| Cigarette smoking (%) | 21.3 | 15.5 | 23.5 |
| Alcohol consumption (%) | 71 | 77.6 | 68.5 |
| Drug consumption (%) | 14 | 12.1 | 14.7 |
| Physical activity (%) | 64 | 85 | 57 |
| SBP (mm Hg) | 116.8 ± 14.8 | 118.3 ± 13.6 | 115.6 ± 15.7 |
| DBP (mm Hg) | 69.9 ± 12.6 | 70.3 ± 15.0 | 69.5 ± 10.4 |
| Glucose (mg/dL) | 85.5 ± 1.9 | 84.8 ± 3.0 | 90.7 ± 5.2 |
| Cholesterol (mg/dL) | 163.1 ± 10.0 | 176.9 ± 25.1 | 157.8 ± 10.0 |
| Triglycerides (mg/dL) | 94.4 ± 7.7 | 96.7 ± 10.0 | 93.5 ± 10.1 |
| HDL-Cholesterol (mg/dL) | 46.7 ± 0.6 | 45.8 ± 1.2 | 46.9 ± 0.7 |
| LDL-Cholesterol (mg/dL) | 84.3 ± 2.7 | 88.4 ± 4.9 | 82.7 ± 3.2 |

Note: Number of subjects in parentheses. Values are expressed as mean ± SD.

Abbreviations: BMI, body mass index; DBP, diastolic Blood Pressure; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic Blood Pressure.

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Based on previous reports, we studied the potential association of both rs5925 LDLR and rs505151 PCSK9 genetic variants with plasma lipid levels. The results showed significant lower values of TC, TG, and LDL-C (P < .05) in individuals carrying LDLR rs5925 mutated allele (T) in either homozygous TT or heterozygous CT when compared to CC homozygous genotype. Moreover, similar results were observed for TG, and LDL-C in the female group (P < .05).

However, in the male group only TC showed lower values for heterozygous CT subjects (P < .05). The results are shown in Table 3.

Analysis of PCSK9 rs505151 genetic variant showed no changes in lipid profile for the total group of individuals studied. However, the presence of mutated allele (G) in the male group was associated with lower values in TC (P < .05) and TG (P < .05) when compared to men carrying AA genotype. On the other hand, in the female group we found higher values of TC (P < .05) associated with the presence of mutated allele (G) when compared to women carrying the AA genotype. In addition, the female carrying AG heterozygous genotype showed significant higher values of TC and TG (P < .05) when compared to men carrying AA genotype. The results are shown in Table 3.

## TABLE 2 Genotype distribution and relative allele frequency for the evaluated genetic variants

| Genotype (%) | CC | CT | TT | P-value* | Allele frequency |
|--------------|----|----|----|---------|-----------------|
| LDLR (rs5925) n = 172 |    |    |    |         |                 |
| Total        | 32 (19) | 91 (53) | 49 (28) | .37 | 0.45 | 0.55 |
| Male         | 9 (20) | 23 (50) | 14 (30) | .93 |       |     |
| Female       | 23 (18) | 68 (54) | 35 (28) | .32 |       |     |
| PCSK9 (rs505151) n = 178 |    |    |    |         |                 |
| Total        | 166 (93) | 12 (7) | 0 (0) | .64 | 0.97 | 0.03 |
| Male         | 46 (94) | 3 (6) | 0 (0) | .83 |       |     |
| Female       | 120 (93) | 9 (7) | 0 (0) | .75 |       |     |

*Chi-square analysis was used to test Hardy-Weinberg Equilibrium.
TABLE 3 Lipid profile (mg/dL) according to LDLR and PCSK9 polymorphisms in healthy individuals from the north of Chile

|                  | TC          | TG          | HDL-C       | LDL-C       |
|------------------|-------------|-------------|-------------|-------------|
|                  | C/C         | C/T         | T/T         | C/C         | C/T         | C/C         | C/T         |
| LDLR (rs5925)    |             |             |             |             |             |             |             |
| Total            | 171.5 ± 50.9 | 143.3 ± 33.4 | 149.0 ± 29.1 | 96.1 ± 58.6 | 85.4 ± 48.2 | 68.9 ± 28.2 | 44.6 ± 8.8  | 46.8 ± 7.9 | 47.4 ± 9.3 | 103.8 ± 55.0 | 79.3 ± 32.5 | 78.2 ± 25.9 |
| Male             | 169.4 ± 53.6 | 140.4 ± 28.8 | 159.4 ± 29.8 | 100.8 ± 30.1 | 84.6 ± 30.9 | 86.0 ± 32.8 | 46.4 ± 7.7  | 46.3 ± 8.7 | 45.3 ± 9.6 | 103.7 ± 51.5 | 75.1 ± 29.9 | 97.2 ± 34.2 |
| Female           | 167.0 ± 53.3 | 144.4 ± 35.1 | 144.3 ± 28.3 | 94.2 ± 66.3  | 86.4 ± 52.3 | 66.6 ± 28.1 | 44.2 ± 9.3  | 46.9 ± 7.8 | 49.5 ± 8.6 | 102.9 ± 56.6 | 88.2 ± 71.7 | 79.7 ± 30.8 |
| PCSK9 (rs505151) |             |             |             |             |             |             |             |             |             |             |             |             |
| Total            | 137.9 ± 36.2 | 156.7 ± 48.6 | 894 ± 79.5  | 78.5 ± 57.6 | 55.2 ± 19.1 | 57.9 ± 11.8 | 69.3 ± 30.3 | 83.1 ± 43.2 | 72.0 ± 30.1 | 51.6 ± 19.5 |             |             |
| Male             | 137.6 ± 30.8 | 108.6 ± 14.0 | 80.1 ± 33.8 | 43.0 ± 21.2  | 49.1 ± 12.9 | 48.5 ± 5.9  | 70.2 ± 30.1 | 51.6 ± 19.5 |             |             |             |             |
| Female           | 136.2 ± 39.1 | 174.7 ± 44.2 | 83.8 ± 63.3 | 86.1 ± 60.6  | 56.9 ± 21.0 | 59.3 ± 12.6 | 66.0 ± 37.9 | 94.9 ± 44.4 |             |             |             |             |

Note: Lipid values are expressed as mean ± SD. Data were analyzed by one-way ANOVA or Student's t test.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

rs5925 [P < .05 or **P < .01 when compared to CC homozygous genotype (Bonferroni)]; rs505151 [P < .05 when compared to AA homozygous genotype; †P < .05 or ††P < .01 when compared to the same genotypes by gender].

Regarding to lipid profiles, the results showed lower levels of TC and LDL-C compared to healthy age-matched individuals from central and south regions of Chile. Although controversial seasonal variation might contribute to change in plasma levels, our study showed significant association between LDL rs5925 mutated allele and plasma levels of TC and LDL-C in men and female group prior to atorvastatin treatment. On the other hand, the present study showed significant association between PCSK9 rs505151 mutated allele and plasma levels of TC, HDL-C and LDL-C in men and female group compared to reference allele (C). Similar results were observed in studied subjects from Italy. However, no differences in TC or LDL-C levels found in the study could account for lower TC and LDL-C values found in the north of Chile than central and southern regions, with no distinct seasons and having temperature range 3°C to 5°C higher than central and southern regions. The north of Chile is a hot and dry desertic region, with temperatures ranging 3°C to 5°C higher than central and southern regions. The north is different to those found in individuals from Italy.

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genotype contributing to the manifestation of a disease. Knowledge of gene-environment interactions allows for the identification of new genetic variants, enables predictive studies of diseases in high-risk populations, and helps to stimulate public health policies or strategies for the prevention of diseases.\(^{38}\) Finally, several studies have introduced evidence that genetic factors contribute to manifestation of cardiovascular diseases in an equivalent way to environmental factors.\(^{39}\)

In summary, the results suggest that LDLR and PCSK9 polymorphisms play a significant role in regulating plasma lipid levels, particularly TC and LDL-C, in healthy individuals from the north of Chile. The data together support the evidence that presence of mutated allele (T) for LDLR rs5925 genetic variant could be an important predictor of high level of lipids and therefore risk of CVD especially in females. However, in order to confirm the influence of LDLR and PSCK9 genotypes on plasma lipid levels, enrolling a greater number of individuals as well as case-control study are required. Overall, we suggest this genomic approach could contribute to understand susceptibility to develop CVD and to help as cardiovascular risk markers in population from the northern Chile. Knowing new factors that predispose to cardiovascular diseases, such as polymorphisms of candidate genes, can help to elaborate risk score tables similar to the Framingham Model, study to take preventive or therapeutic measures, and predict the risk beyond the conventional.\(^{30}\)

5 | CONCLUSIONS

The present study shows that genotype distribution for LDLR rs5925 and PCSK9 rs505151 genetic variants was consistent with the Hardy-Weinberg equilibrium. No differences by gender of genotype distribution or relative allele frequencies were observed. The effect of LDLR rs5925 and PCSK9 rs505151 gene polymorphisms on plasma lipid levels is associated with gender among healthy subjects from northern Chile. The clinical utility of these genetic variants in the prediction of cardiovascular risk needs to be investigated in a future case-control study including a major number of individuals.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHOR’S CONTRIBUTIONS

All authors have read the manuscript and agreed with the content. JEV conceived and designed the study; CR and HR performed the experiments; CR, HR, and ASG analyzed the data; LAS participated in the design of the study and contributed reagents/materials and analysis tools; LAS and AMK reviewed and edited the manuscript; and ASG and JEV wrote the paper.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of Universidad de Antofagasta (Chile), and all participants signed an informed consent.

DATA AVAILABILITY STATEMENT

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

CONSENT FOR PUBLICATION

Not applicable.

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