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RESEARCH ARTICLE

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Genetic variants, gene expression, and soluble CD36 analysis in acute coronary syndrome: Differential protein concentration between ST-segment elevation myocardial infarction and unstable angina

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Abstract

Background: Atherosclerosis plays an important role in the pathophysiology of acute coronary syndrome (ACS). CD36 is a scavenger receptor involved in lipid metabolism. Some single-nucleotide variants in the non-coding region could indirectly alter the expression and the function of the protein.

Objective: The aim of this study was to investigate the gene and protein expression associated with *CD36* variants (rs1194182;C>G; rs1049654;C>A, rs1334512;G>T, and rs3211892;G>A) in ACS patients from the western Mexican population.

Methods: We recruited 310 ACS patients and 308 subjects in the control group (CG). Genotyping was determined by TaqMan SNP genotyping assays. CD36 expression at the mRNA level was quantified by TaqMan gene expression assays. Soluble CD36 (sCD36) was measured by enzyme-linked immunosorbent assay.

Results: We show that rs1194182G>C variant provides a protective effect with a 1.7-fold lower susceptibility to develop ACS ($p = 0.03$); however, this association was masked by diabetes and dyslipidemia. We observed a higher sCD36 concentration in patient with ST-segment elevation myocardial infarction (STEMI) compared with patients with unstable angina (UA) ($p = 0.038$). Likewise, in diabetic patients versus non-diabetic ($p < 0.001$). We observed in patients an increase in *CD36* mRNA expression (1.91 times higher) than in the CG ($p = 0.02$).

Conclusion: The rs1194182 seems to be associated with diabetes in a risky manner, in ACS patients and protective for dyslipidemia in both groups. The concentration of sCD36 seems to be associated with the clinical spectrum of the ACS patients and the presence of diabetes, since patients with STEMI present significantly elevated level compared with UA.

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KEYWORDS
acute coronary syndrome, *CD36* gene, clinical spectrum, risk factors, soluble CD36

1 | INTRODUCTION

Cardiovascular diseases are the leading cause of death worldwide, and acute coronary syndrome (ACS) causes important morbidity and mortality. ACS is an episode of myocardial necrosis or ischemia and is primarily triggered by rupture or disruption of an atheroma and includes different clinical spectrum such as unstable angina (UA), non-ST-segment elevation acute myocardial infarction (NSTEMI), and ST-segment elevation acute myocardial infarction (STEMI).[1,2]

ACS is a multifactorial disease; however, recent studies have demonstrated the relevance of the genetic component.[3,4]

CD36 is a glycoprotein that plays an important role in the early and late stages in the development of atherosclerosis and is the main scavenger receptor found on the membrane of many different cell types such as monocytes, macrophages, platelets, and adipocytes, among others. In macrophages, CD36 is responsible for the uptake of oxidized low-density lipoprotein (oxLDL), which generates signaling cascades of inflammatory responses leading to foam cell accumulation and atherosclerotic plaque growth.[5,6]

A soluble form of CD36 (sCD36) has been reported in human plasma,[7] found to be elevated in patients with pathological conditions associated with metabolic disorders, such as insulin resistance, diabetes, dyslipidemia, obesity, and atherosclerosis.[8–11] Notwithstanding, subjects with rare CD36 null mutations may development of coronary heart disease.[12] Thus, variable results of CD36 protein levels have been associated with cardiovascular diseases.[13] In this regard, mutations in *CD36* gene can affect the uptake of fatty acids.

CD36 protein is encoded by a gene located on chromosome 7q11.2.[14] Single-nucleotide variants (SNV) in *CD36* gene could be associated with metabolic disorders and could indirectly alter the expression and the function of the protein.[15,16] We selected four informative genetic variants (allele frequency higher than 5% in a previous population reported); in addition, we considered that they have been associated with cardiovascular diseases or risk factors related and by its chromosome position, they could be related to changes in gene or protein expression. For instance, the rs1194182 was associated as a predictor of both diabetes and coronary heart disease.[15] The SNV, rs1049654, was associated with increased HDL levels in African American population.[17] The SNV, rs1334512, was studied in Indian population by Sinha and was associated with protective effect against malaria.[18] The SNV, rs3211892, was associated with older age of myocardial infarction and higher white blood cell count, but a similar study has not been conducted in a Mexican population.[19]

There has been no evident proof whether variants of the *CD36* gene protect against, or increase, the risk of ACS. To our knowledge, there are no studies that evaluate the contribution of these CD36 variants in the population of western Mexico associated with ACS.

In the present study, we found the association between CD36 variants (rs1194182 C>G; rs1049654 C>A, rs1334512 G>T and rs3211892 G>A) and its susceptibility to ACS. Furthermore, the association between these variants with CD36 expression and sCD36 levels obtained from both study groups is shown.

2 | MATERIALS AND METHODS

2.1 | Study population

In this study, we recruited 310 patients 45 years and older, with ACS. The patients were all treated in the department of Cardiology of the Hospital de Especialidades del Centro Médico Nacional de Occidente del Instituto Mexicano del Seguro Social from February 2018 to June 2020. All patients were diagnosed with ACS (unstable angina, myocardial infarction with or without ST-segment elevation) and classified according to the criteria established by the American College of Cardiology.[20] Data such as weight, height, blood pressure, comorbidities history, troponin, creatine kinase (CK), and creatine kinase MB (CK-MB) levels were collected from the patients' medical records.

Exclusion criteria in ACS: individuals with overlapping other cardiac diseases (e.g., myocarditis, pericarditis, hypertrophic cardiomyopathy, valvular heart disease, Tako-Tsubo cardiomyopathy, cardiac trauma, and congestive heart failure) or noncardiac diseases (e.g., pulmonary embolism, pulmonary infarction, pneumothorax, pleurisy, pneumonia, anemia, aortic dissection, aortic aneurysm, esophageal spasm, and cerebrovascular disease), individuals with familial hypercholesterolemia and genetically related individuals.

The 308 participants in the control group (CG) were randomly selected from Hospital de Especialidades del Centro Médico Nacional de Occidente del Instituto Mexicano del Seguro Social during the same period as ACS patients. The inclusion criterion for the controls was the absence of symptoms or medical history of ischemic cardiopathy (ascertained by questionnaire), with similar age to ACS patient. All the individuals included in the study were randomly selected, and they were from western Mexico.

A fasting blood sample (20 ml) was collected for all measurements in this study.

For ACS patients, the blood sample was collected approximately 48 h after the ischemic event; it should be noted that all patients were under pharmacological treatment. The medication intake included antiplatelet (acetylsalicylic acid and clopidogrel), statins, anticoagulants (heparin and enoxaparin), and antihypertensive agents (captopril, enalapril, spironolactone, and furosemide).

The fasting blood sample was taken for measurements of serum glucose, lipid profile (triacylglycerols, total cholesterol, and high- and low-density lipoprotein cholesterol), apolipoprotein A-I (ApoA1), apolipoprotein B (ApoB), and high-sensitivity C-reactive protein (hsCRP), which were measured using Biosystems reagents (Biosystems S.A. Barcelona,) in a Mindray BS-120 analyzer. Quality controls were acquired as recommended.

Type of study: it was a case–control study.

2.2 | Ethical considerations

The study was conducted in accordance with the 2013 Helsinki Declaration. All individuals agreed to participate in the study and signed informed written consents. The ethical approval was obtained by the Centro Universitario de Ciencias de La Salud (CUCS), UdeG (CI/065/2014).

2.3 | DNA isolation

Genomic DNA was extracted of whole blood samples as previously described (Miller, 1988) using a modified Miller's technique.[21] gDNA concentration was determined spectrophotometrically at a wavelength of 260 nm (absorbance of nucleic acids) and 280 nm (absorbance of proteins). Once gDNA concentration was obtained, the samples were stored at −20°C until ready to use.

2.4 | Genotyping and quality control

We choose four SNVs of *CD36* (rs1194182, rs1049654, rs1334512, and rs3211892).

Genetic variants were genotyped using TaqMan Assays for CD36 gene (rs1194182, C_8314408_1; rs1049654, C_8315074_10; rs1334512, C_8314964_10, and rs3211892, C_25644352_10), using TaqMan Genotyping Master Mix (Applied Biosystems,), following the manufacturer's protocol.

The context sequence [VIC/]FAM] of each SNVs: rs1194182, CATGCGTCCGAAGCTCTGGAAGGCT[C/G]AGGATGTCAA TGGGCTTTCAGATGTC; rs1049654, GATTCTTTCTGTGACTCATC AGTTC[C/A]TTTCCTGTAAAATTCATGTCTTGCT; rs1334512, AG AGTGCTTTCTCTTCTCTTTTTTT[G/T]GGGGGGGGAGGGGGT GTGGTTGCAT; rs3211892, AATGTTTTGAATTTTGTTTACTGCT [A/G]TTTCTTTAGAGTTCGTTTTCTAGCC.

Thermal cycling conditions were set in a Roche Light Cycler 96® device as follows: we used a 10 min waiting time for enzyme activation at 95°C and, finally, 40 cycles for denaturation and hybridization/extension (95°C, 15 s; 60°C, 60 s, respectively).

As quality control, a double-blind genotyping of 1/4 of the samples was performed for all variants, with no variation in the genotype assignment.

2.5 | Real-time qPCR analysis

Total RNA was extracted from peripheral blood leukocytes using TRIzol reagent (Invitrogen,) according to the manufacturer's instruction to obtain total RNA according to the method of Chomczynski and Sacchi.[22] One microgram of total RNA was reverse transcribed using reverse transcription reagents M-MLV Reverse Transcriptase, dNTP Mix, oligo (dt) 15 Primer, RNasin® Ribonuclease Inhibitor and Ribonuclease H (Promega Corporation,) to obtain cDNA following the manufacturer's protocol. Real-time PCR was performed using TaqMan probes: glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (Hs02786624_g1) and *CD36* (Hs00354519_m1) according to the conditions indicated in TaqMan Gene Expression Assay Protocol: 20× TaqMan® Gene Expression Assay, 2× TaqMan® Gene Expression Master Mix, cDNA template (1–100 ng) and RNase-free water (Applied Biosystems,) using a Roche LightCycler 96® System.

All samples were run in triplicate under the following conditions: initial denaturation at 95°C for 10 min, followed by 45 cycles including heating at 95°C for 15 s and alignment + extension at 60°C for 1 min.

Relative expression was normalized to the expression level using a housekeeping gene (*GAPDH)* as an internal control and was evaluated using the $2^{-\Delta\Delta Cq}$ and $2^{-\Delta Cq}$ methods.[23] Using the following equation:

ΔCq = (average Cq of the gene of interest–average Cq of reference gene).

We calculated $\Delta\Delta Cq = (\Delta Cq - \Delta Cq\ \text{calibrator})$, where ΔCq calibrator refers to the average ΔCq of the control group.

Results are expressed as a relative fold increase compared with control and unit relative of expression (URE), respectively.

We determined the relative CD36 mRNA expression in peripheral blood leukocytes from ACS patients ($n = 40$) and control group ($n = 27$). ACS patients were included for each of the three clinical entities of ACS ($n = 13$ UA, $n = 12$ NSTEMI, and $n = 15$ STEMI). The sample size was calculated in the Epi Info™7.1.4 program (difference between means), taking as reference the data reported in controls by Zhang et al. 2014.[24] The selection of the individuals in the control group was randomized. The minimum sample size was 8 individuals per group.

2.6 | sCD36

sCD36 levels were determined in duplicate plasma samples using enzyme immunosorbent assays (ELISA) (MyBioSource Human Soluble CD36 kit) according to the manufacturer's instructions. The assay sensitivity was 0.1 ng/ml. As a quality control, a coefficient of variation of less than 20% between replicates was considered.

2.7 | Statistical analysis

Statistical analysis was fulfilled by SPSS V.22.0 (IBM Corp.,). Data were presented as a median and interquartile range (IQR) unless

otherwise indicated. The Mann–Whitney *U* test and Kruskal–Wallis test were used to compare differences between groups. Genotype and allele frequencies of CD36 genetic variants were compared between the ACS patients and control group. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated using Chi-squared and Fisher's exact tests when appropriate. Bonferroni's correction was applied to reduce the statistical type 1 error (pc) in multiple comparisons. Hardy–Weinberg equilibrium was calculated from genotype distribution, and the linkage disequilibrium (LD) between the polymorphisms was calculated using Lewontin's D′ and r2 between genetic markers. The haplotypes and their frequencies were estimated using the SHEsis software platform (http://analysis.bio-x.cn/myAnalysis.php). A multilinear regression model was used to estimate the association of common risk factors (comorbidities, gender, medication intake, etc.) with sCD36 and mRNA expression levels.

3 | RESULTS

3.1 | Clinical features

The clinical description is shown in Table 1. The mean age of ACS and CG was 62 (±10 standard deviation [SD]) and 56 (±10 SD) years, respectively. In the ACS group, males were 3.4-fold more affected than females.

We found higher levels of cholesterol total, triglycerides, LDL-C, HDL-C, ApoAI, and ApoB in the CG compared with ACS group. On the contrary, ACS patients showed higher levels of both glucose and CRP.

The most relevant risk factor was hypertension in both study groups.

STEMI was the most frequent type of ACS (70%), followed by NSTEMI (18%) and UA (12%). The medication intake included acetylsalicylic acid, clopidogrel, statins, heparin enoxaparin, captopril, enalapril, spironolactone, and furosemide.

3.2 | Genotype and allele analysis

The genotype and allelic frequencies of *CD36* variants (rs1194182 C>G; rs1334512 G>T, rs1049654 C>A, and rs3211892 G>A) are shown in Table 2. Genotype distributions were consistent with the Hardy–Weinberg equilibrium (HWE) for all SNVs.

The genotypic and allelic frequencies of the rs1194182 variants showed a significant difference between groups, in which a protective contribution was attributed by the C allele (OR = 0.760, 95% CI 0.605–0.955, $p = 0.01$), the C/C genotype (OR = 0.597, 95% CI 0.371.962 $p = 0.03$), and the dominant model (OR = 0.682, 95% CI 0.488–0.954 $p = 0.02$).

For the rest of the variants, neither the genotypes nor the alleles distribution was statistically different between study groups.

In order to rule out a confounder effect in genetic associations, we performed a binary logistic regression. According to the dominant model, this variant seems to be associated with diabetes in a risky manner, in ACS patients (OR = 1.98, 95% CI 1.1–3.52 $p = 0.02$). On the contrary, also it is associated with dyslipidemia in both groups in a protective manner. GC (OR = 0.237, 95% CI 0.09–0.62 $p < 0.01$). ACS (OR = 0.533, 95% CI 0.32–0.88 $p = 0.14$).

In an attempt to evaluate a genetic interaction, we performed a univariate analysis of variance; however, this did not show significant differences (Table S1).

3.3 | Haplotype analysis of CD36

Analysis showed that rs1194182 C>G, rs1049654 C>A, and rs3211892 G>A of *CD36* were in linkage disequilibrium (D′ = 0.85, r2 = 0.73, $p < 0.05$). We analyzed the association between haplotype distribution. However, no significant differences in haplotype distribution were found. Table 3 shows the haplotypes with a frequency >3%.

3.4 | CD36 mRNA expression

The quantitative real-time PCR analysis of the *CD36* gene expression was performed for ACS patients and individuals of control group. The expression of CD36 was at least 1.91-fold higher in all analyzed cases of ACS in comparison with control group (Figure 1A). The mean amount of CD36 gene transcripts in peripheral blood leukocytes was 2.96 URE in the ACS patients, while in the control group it was 1.94 URE (Figure 1B). This result was statistically significant ($p = 0.021$).

Subsequently, ACS patients were stratified according to clinical spectrum. We did not observe differences in expression according to diagnosis (UA: 2; NSTEMI: 2.29; STEMI: 2.53) (Figure 1C); Figure 1D shows the comparison by the $2^{-\Delta Cq}$ method, and although it is observed that expression increases with the severity of the clinical spectrum (UA: 2.74 URE; NSTEMI: 2.73 URE; STEMI: 3.34 URE; $p = 0.413$), these differences were not statistically significant.

We then analyzed the data from ACS patients and individuals of control group stratifying the results by dominant model; however, we found no differences in the level of CD36 expression among genetic variants in any group (Table S2).

3.5 | sCD36

Plasma concentration of sCD36 was assessed in both study groups, and it was observed that sCD36 was slightly lower in ACS patients compared with the CG (Figure 2A); however, this difference was not significant ($p = 0.416$). In addition, we observed that sCD36 levels were higher according to the severity of the clinical spectrum of ACS; thus, patients with STEMI presented higher levels than patients with NSTEMI and UA (9.38, 8.04 and 7.43 ng/ml, respectively). Nevertheless, we just found significant differences between STEMI and UA. ($p = 0.038$) (Figure 2B).

TABLE 1 Clinical and demographic data

Variable	ACS (*N* = 308)	CG (*N* = 150)	Reference values	*p*
Age (years), mean ± SD	**62 ± 10**	**56 ± 10**	**–**	**<0.001**
Male/female ratio	3.4	1.06	–	<0.001
Total cholesterol (mg/dl), median (IQR)	113(91–136)	154(135–180)	150–199	<0.001
Glucose (mg/dl), median (IQR)	118.5(96.5–165)	92(81–109)	75–105	<0.001
Triglycerides (mg/dl), median (IQR)	85(72–103)	99(78–130)	≤250	<0.001
LDL-C (mg/dl), median (IQR)	42(34–53)	69(51–95)	<130	<0.001
HDL-C (mg/dl), median (IQR)	19(14–24)	39(24–56)	>40	<0.001
CRP (mg/dl), median (IQR)	23.5(6–36)	2(1–3.9)	1–10	<0.001
ApoA I (mg/dl), median (IQR)	165.4(151–179)	198(181–213)	94–178	<0.001
ApoB (mg/dl), median (IQR)	132.7(110–157)	167(147–185)	63–133	<0.001
ApoAI/ApoB ratio, median (IQR)	0.81(0.68–0.96)	0.85(0.72–0.99)	†	0.08
sCD36 (ng/ml), median (IQR)	9.09(6.9–11.9)	9.3(7.1–11.2)		0.416
CK (IU/ml), median (IQR)	365.5(141–1017.5)	N.A.	22–195	N.A.
CK-MB (IU/ml), median (IQR)	51(23–125)	N.A.	<130	N.A.
Troponin I (ng/ml), median (IQR)	3.9(0.9–9.9)	N.A.	0.1–0.4	N.A.
Risk factor	ACS *n* (%)	CG *n* (%)		*p*
Obesity	88(29)	45(15)		<0.001
T2DM	161(53)	70(23)		<0.001
Dyslipidemia	144(47)	59(20)		<0.001
Hypertension	204(67)	97(32)		<0.001
Smoking	142(46)	60(20)		<0.001
Sedentary lifestyle	176(58)	76(25)		<0.001
Metabolic syndrome	111(36)	71(23)		<0.001
ACS diagnosis	*n* (%)	ACS treatment		*n* (%)
UA	37 (12)	Acetyl salicylic acid		292(94)
NSTEMI	56 (18)	Statins		277(89)
STEMI	217 (70)	Clopidogrel		279 (90)
		Heparin		34(10)
		Enoxaparin		185(60)
		Captopril		29(9)
		Spironolactone		46(14)

Abbreviations: ACS, acute coronary syndrome; ApoA I, apolipoprotein A I; ApoB, apolipoprotein B; CG, control group; CK-MB, creatinine kinase muscle and brain; CRP, C-reactive protein; HDL-c, high-density lipoprotein; K, creatinine kinase; LDL-c, low-density lipoprotein; N.A., not applicable; p*, Mann–Whitney *U* tests; SD, standard deviation; T2DM, type 2 diabetes mellitus.

†values established by the AMORIS and INTERHEART studies: 0.4–0.6 low risk, 0.7–0.8 moderate risk, and 0.9–1.1 high risk.

After stratifying by risk factors, we observed differences in plasma levels of sCD36 between ACS patients with diabetes and without diabetes (9.50 vs 6.47 ng/ml; $p < 0.001$) (Figure 2C). Likewise, we classified ACS patients with their clinical spectrum and observed increased sCD36 values in diabetic ACS patients compared with non-diabetic ACS patients. We also observed significant differences between patients with diabetic UA vs non-diabetic UA (9.09 vs. 5.49 ng/ml; $p < 0.001$), as well as with diabetic STEMI vs non-diabetic STEMI patients (10.36 vs. 7.23 ng/ml; $p = 0.013$) (Figure 2D).

We also stratified according to treatment. It is worth mentioning that we found slightly higher levels of sCD36 in patients without statin treatment compared with patients with treatment (8.56 vs. 8.04 ng/ml; $p = 0.443$); likewise, for patients without acetylsalicylic acid (ASA) treatment compared with patients with treatment (8.28 vs. 8.03 ng/ml; $p = 0.248$); however, we did not observe significant differences. Additionally, we analyzed the association between genetic variants on *CD36* and plasma levels of sCD36 in both groups of study without any evidence of association ($p > 0.05$).

A multivariable linear regression was performed after adjusting for age, gender, TC, glucose HDL, LDL, TG, CRP, APOAI, APOB, body mass index, dyslipidemia, hypertension, diabetes mellitus 2,

TABLE 2 Genotype, allele, and haplotype distribution

Variable	ACS n(%)	CG *n*(%)	OR (CI 95%)	*p*
rs1194182 C>G				
Genotype				
G/G	126(41)	98 (32)	–	–
G/C	141(45)	154 (50)	0.712 [0.502–1.010]	0.06
C/C	43(14)	56 (18)	**0.597[0.371.962]**	**0.03**
Allele				
G	393 (63)	350 (57)	**0.760 [0.605–0.955]**	**0.01**
C	227 (37)	266 (43)		
Dominant Model			HWE	0.81
G/G	126 (41)	98 (32)	**0.682 [0.488–0.954]**	**0.02**
G/C+C/C	184 (59)	210 (68)		
Recessive Model				
G/G+G/C	267 (86)	252 (82)	0.725[0.470–1.118]	0.14
C/C	43 (14)	56 (18)		
rs1334512 G>T				
Genotype				
T/T	308(99.4)	307 (99.7)	–	–
T/G	2(0.6)	2 (0.3)	0.914 [0.410–2.036]	0.82
G/G	0(0)	0 (0)	0.990 [0.020–50.055]	1.00
Allele				
T	618 (99.7)	615(99.8)	0.915 [0.414–2.023]	0.82
G	2 (0.3)	1 (0.2)		
Dominant Model			HWE	0.59
T/T	308 (99.4)	307 (99.7)	0.914 [0.410–2.036]	0.82
T/G+G/G	2 (0.6)	1 (0.3)		
Recessive Model				
T/T+T/G	310 (100)	308 (100)	1.006 [0.020–50.884]	1.00
G/G	0 (0)	0 (0)		
rs1049654 C>A				
Genotype				
C/C	86 (28)	82 (27)	–	–
C/A	161 (52)	151 (49)	1.017 [0.699–1.480]	0.93
A/A	63 (20)	75 (24)	0.801[0.510–1.258]	0.33
Allele				
C	333 (54)	315 (51)	0.902 [0.721–1.128]	0.36
A	287 (46)	301 (49)		
Dominant Model			HWE	0.73
C/C	86(28)	82 (27)	0.953[0.667–1.362]	0.79
C/A+A/A	224 (72)	226 (73)		
Recessive Model				
C/C+C/A	247 (80)	233 (76)	0.753 [0.513–1.105]	0.15
A/A	63 (20)	75 (24)		
rs3211892 G>A				
Genotype				
G/G	298(96)	295 (97.6)	–	–

TABLE 2 (Continued)

Variable	ACS n(%)	CG *n*(%)	OR (CI 95%)	*p*
G/A	12(4)	13 (2.4)	0.914 [0.410–2.036]	0.82
A/A	0(0)	0 (0)	0.990 [0.020–50.055]	1.0
Allele				
G	608 (98)	603 (98)	0.915 [0.414–2.023]	0.82
A	12 (2)	13 (2)		
Dominant Model			HWE	0.97
G/G	298 (96)	295 (96)	1.994 [0.180–22.099]	0.56
G/A + A/A	12 (4)	13 (4)		
Recessive Model				
G/G + G/A	310 (100)	308 (100)	1.006 [0.020–50.722]	1.0
A/A	0 (0)	0 (0)		

Abbreviations: ACS,acute coronary syndrome; CG, control group; CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium; results with significant correlation ($p < 0.05$) are emphasized by the bold values.

TABLE 3 *CD36* haplotype distribution in the ACS patients and control group

Haplotype	ACS n(%)	CG n(%)	OR (CI 95%)	*p*
GCG	319(51)	294(47)	–	*1*
CAG	202(32)	234(38)	0.9335 (0.7299–1.1939)	0.5833
GAG	74(12)	56(9)	1.4289 (0.9757–2.0927)	0.0667

Note: Haplotype is represented by rs1194182, rs1049654 y rs3111892, respectively.

Abbreviations: ACS, acute coronary syndrome; CG, control group; CI, confidence interval; OR, odds ratio.

smoking, sedentary lifestyle, metabolic syndrome, and treatment. A significant association between diabetes and sCD36 was seen in ACS patients (β:3.094, $p = 0.010$). On the contrary, we observed a positive association in the CG with obesity and cholesterol (β:1,03, $p = 0.02$; β:1,23, $p = 0.02$, respectively).

Finally, we did not find a correlation between sCD36 levels and relative CD36 mRNA expression ($r = 0.032$, $p > 0.05$).

4 | DISCUSSION

Cardiovascular diseases are still leading first death cause all over the world. In Mexico, ACS represents the main cause of death in general population. In this study, we found that the CG presented a higher lipid concentration compared with ACS group. However, these are within the reference values. These results can be attributed to the effect of pharmacological therapy of patients with ACS, which includes nitrates, antithrombotic, antiplatelets, beta-blockers, statins, and antihypertensive drugs. In addition, after a myocardial infarction, the plasmatic concentration of total cholesterol, LDL-c, and HDL-c decrease below the baseline value.[25–27] Therefore, we assume that these two variables probably contributed to our results.

In our results, we identified that the C allele of the variant rs1194182 provides a protective effect with a 1.7-fold lower susceptibility to develop ACS. Furthermore, the dominant model was significant to produce a change in susceptibility to protection; in other words, both heterozygotes (GC) and carriers (CC) have the effect of 1.47-fold lower risk of developing ACS compared with wild allele carriers in this population. This variant rs1194182 is in the 5' UTR, which is an essential site of transcriptional regulation and could indirectly modify the concentration of its protein.[16] Due to CD36 participating in the process of internalizing oxLDL, forming foam cells that promote inflammatory responses and subsequently narrowing of the arterial lumen, its inhibition may reduce atheroma.[28] However, in our results, we did not find a significance between rs1191182 and sCD36 levels and this may be explained by the fact that the concentration of sCD36 could have been modified by the presence of comorbidities such as diabetes, obesity, and metabolic syndrome, which have been reported to increase the concentration of CD36.[7,29,30] Furthermore, in silico analysis associated this variant with the transcription factor SOX4,[31] which is a transcriptional activator that binds with high affinity to the T-cell enhancer motif. Studies in mice demonstrated that T-cell deficiency decreases atherosclerotic lesions, and it has been observed that all Cd4 + Cd8+ cells exclusively expressed Sox4 suggesting that aortic CD4 + CD8+ lymphocytes may be immature.[32] Therefore, according to our outcomes, the homozygous C/C mutation may lead to the dysfunction of CD36 and could be beneficial for the carrier. However, this association should be taken with caution due to binary logistic regression showed an association with dyslipidemia and diabetes. Contrary to our results, Gong described that there was no association in the lipids analyzed with this variant.[33] A possible explanation for our results on the relationship with dyslipidemia is that a reduction in CD36 expression levels has been associated with an increased influx of fatty acids to the liver.[30] On the contrary, we observed an

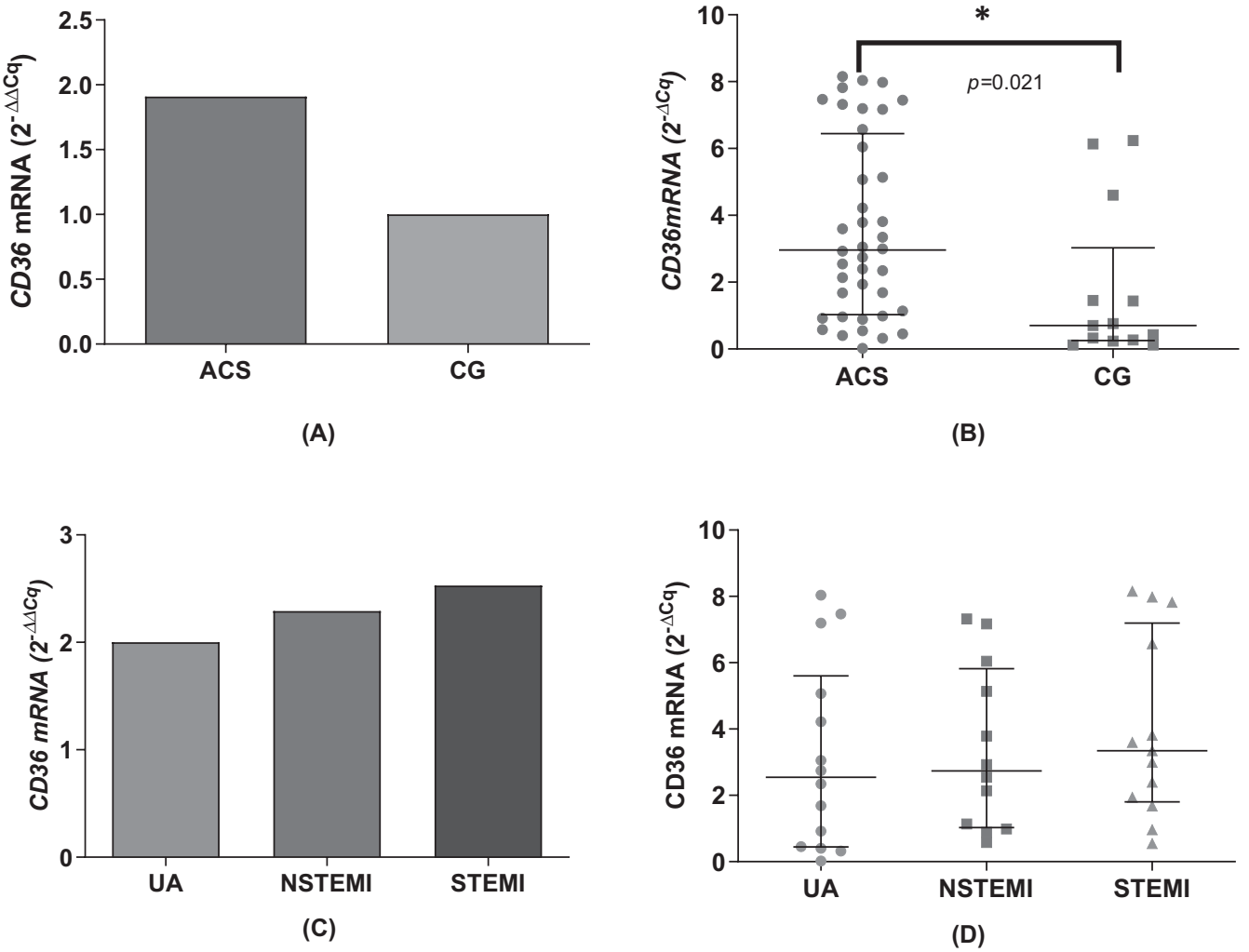

FIGURE 1 Comparison of CD36 mRNA expression. (A) Comparison of ACS patients and CG by the $2^{-\Delta\Delta Cq}$ method. (B) Comparison of ACS patients and CG by the $2^{-\Delta Cq}$ method. (C) Comparison by clinical entities using the $2^{-\Delta\Delta Cq}$ method. (D) Comparison by clinical entities using the $2^{-\Delta Cq}$ method. CD36 mRNA expression in ACS patients ($n = 40$), CG ($n = 27$), UA ($n = 13$), NSTEMI ($n = 12$) and STEMI ($n = 15$) were included. Data were analyzed by the $2^{-\Delta\Delta Cq}$ and $2^{-\Delta Cq}$ methods normalized to *GADPH*. ACS: acute coronary syndrome; CG: control group. $^{*}p < 0.05$ by the Mann–Whitney *U* test

association of this variant with diabetes risk. In agreement with our study, Farook et al. associated this variant with metabolic syndrome, which is a predictor of type 2 diabetes mellitus (T2DM).[15]

The reason for the differences reported in these findings may be the interactions of rs1194182 variant with other variants in *CD36* gene. A recent study showed that rs1194182 variant is in high linkage disequilibrium with the rs1761667 variant (r2 = 0.97), which has been associated with diabetes.[15] Regarding dyslipidemia, some variants have been associated with CD36 deficiency.[34] Studies have shown that HDL concentrations are elevated in mice with CD36 deficiency.[35] Recent studies have shown that CD36 can bind to HDL.[36] A possible mechanism explaining the protection is the increase in HDL cholesterol in CD36-deficient patients because without its binding site it cannot be removed.[37]

Moreover, the variants analyzed were found to be in linkage disequilibrium, suggesting that the associations may not be independent and should be analyzed together. Nevertheless, no significant differences in haplotype distribution were found. The lack of association of the haplotypes could suggest that the individual effect may be masked by the presence of other variants close to the linkage disequilibrium block of the gene or by the presence of risk factors in ACS as corroborated by binary regression analysis.

The plasma concentration of sCD36 was not different between the study groups; contrary to our results, previous studies observed higher concentrations of sCD36 in patients with atherosclerotic carotid stenosis with clinical symptoms[9] and it has been proposed as a marker of plaque instability.[30] A possible explanation for our findings is that patients with ACS were already under treatment. It has been described that the use of statins[38] and ASA[39] could decrease sCD36 levels. Our patients with ACS were treated with both drugs in a high percentage, so this could have influenced our results. Although we observed reduced sCD36 levels in treated patients, these were not significant compared with untreated patients (statins; 8.4 vs. 8.6 ng/ml; $p = 0.443$; ASA; 8.0 vs. 8.3 $p = 0.443$). However, one factor

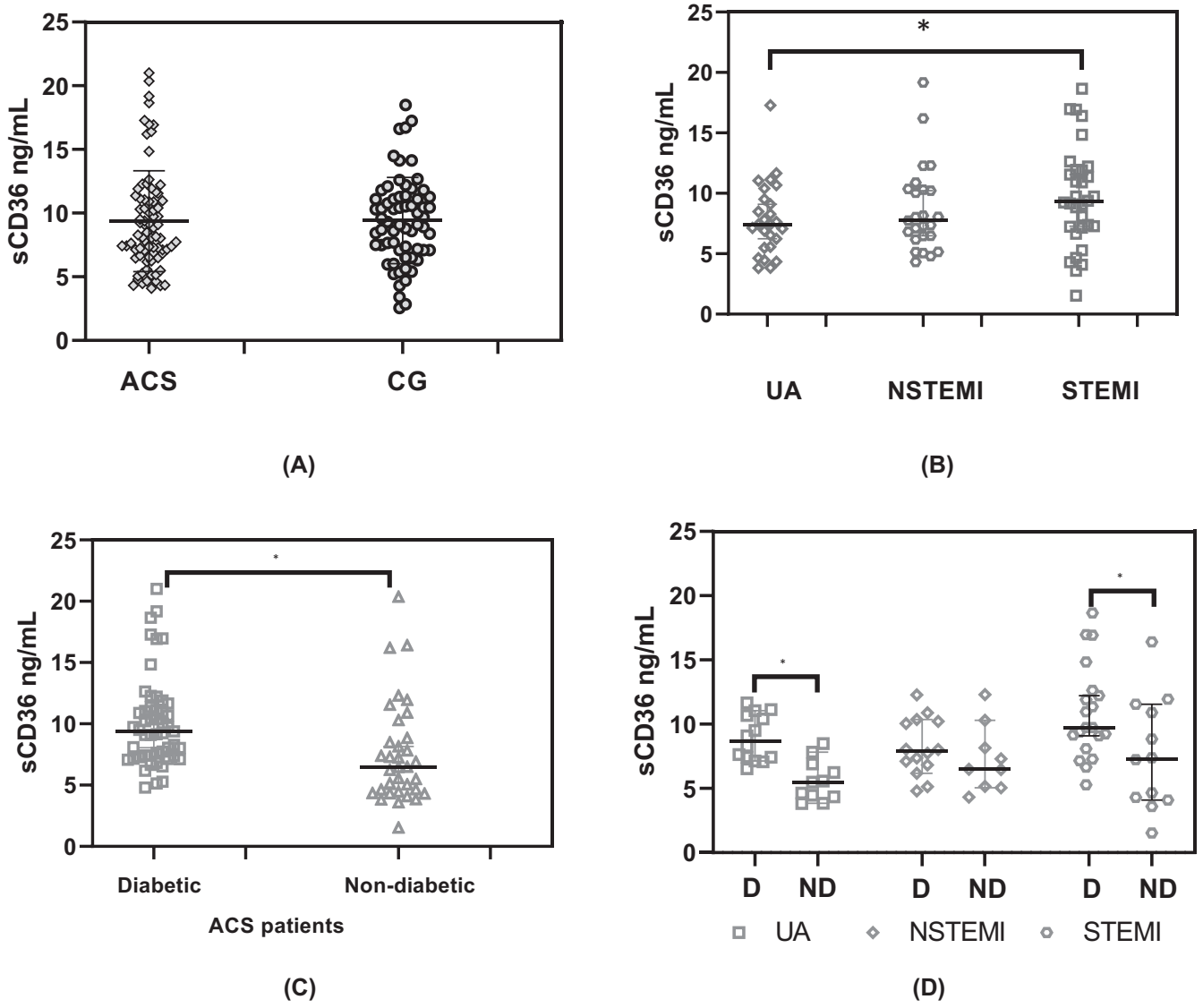

FIGURE 2 Comparison of plasma sCD36 concentrations. (A) ACS patients ($n = 90$) versus CG ($n = 80$). (B) ACS patients stratified according to clinical spectrum, STEMI vs UA $p < 0.038$ (UA $n = 28$, NSTEMI = 28 STEMI n = 34). (C) ACS patients with diabetes $n = 55$ vs patients without diabetes $n = 35$ $p < 0.0001$. (D) UA diabetic vs non-diabetic patients $p < 0.0001$; NSTEMI diabetic vs non-diabetic patients $p = 0.587$; STEMI diabetic vs non-diabetic patients $p = 0.013$. ACS: acute coronary syndrome; UA: unstable angina; NSTEMI: non-ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction CG: control group; D: diabetic; ND: non-diabetic $^*p < 0.05$ by the Mann–Whitney U test

that could have influenced this finding is that CD36 is a substrate of matrix metalloproteinase 9; therefore, during the time course post-myocardial infarction, the protein level decreases[40] as a mechanism to control apoptosis and neutrophil clearance for myocardial repair,[41] which may have further decreased the concentration of sCD36 and could explain the results in our ACS patients.

Additionally, we observed increased levels of sCD36 in ACS patients according to the clinical spectrum. STEMI patients had higher levels of sCD36 than NSTEMI and UA. It has been hypothesized that sCD36 is released into the circulation as part of the low-grade inflammatory state in insulin resistance, or during cellular apoptosis of foam cells, in microparticles derived from monocytes, platelets or endothelial cells.[8] Patients with coronary artery disease are reported to have circulating activated platelets, platelet-derived microparticles, monocyte and platelet aggregates, and increased platelet reactivity. Activated platelets and platelets and leukocyte aggregates were also found to be statistically elevated in subjects with UA compared with patients with stable angina.[42] As aforementioned, sCD36 is a protein associated with circulating microparticles, and in healthy individuals, 90% has been associated with platelets; however, it has been observed that in pathological conditions such as metabolic syndrome, T2DM and cardiovascular diseases it could change the cellular source.[43,44] Thus, due to the nature of each clinical spectrum, it could be that sCD36 concentration increases in accordance with the inflammation that occurs in each of clinical spectrum. To the best of our

knowledge, there are no studies that compare each clinical spectrum of ACS (UA, NSTEMI and STEMI) with the concentration of sCD36.

Multivariable regression analysis revealed an association with diabetes in ACS patients; diabetic patients presented higher levels of sCD36 than non-diabetic patients. Increased CD36-mediating fatty acids influx impairs insulin sensitivity in liver and skeletal muscle, and it could lead to the development of T2DM.[45] Moreover, in response to elevated glucose levels, increases in peroxisome proliferator-activated receptor γ (PPAR-γ) could lead to an increase in CD36 expression (specifically in macrophages) and this could contribute to accelerated atherosclerosis in diabetic patients.[29] Also, CD36 has been described to mediate pancreatic β-cell dysfunction, as well as contribute to decreased β-cell mass,[46] leading to reduced insulin secretion and progression of diabetes.[47]

In agreement with our results, other authors observed an increase in sCD36 in diabetic individuals compared with non-diabetics.[7,8] However, Castelblanco et al. found no differences in sCD36 concentration between subjects with T2DM and healthy subjects.[48] The variability in the different results could be explained by the diversity in the characteristics of the studies such as the different populations analyzed, the age of the individuals included in each study, the different complications derived from diabetes, as well as the length of time the disease has been present and the medications used to treat diabetes.

On the contrary, we found a positive correlation between sCD36, obesity, and cholesterol in the CG. Similarly, to our study, Handberg et al. demonstrated that plasma sCD36 levels were three times higher in obese controls compared with controls without obesity.[7] This increase could be explained since these individuals present inflammation and, therefore, high levels of leukocytes[49] and platelet.[50]

The data obtained in our investigation show that CD36 mRNA expression is significantly increased in ACS patients. Our result agrees with those described by other authors, who describe that in ACS patients CD36 expression is upregulated.[51,52] On the contrary, it has been hypothesized that CD36 expression increases significantly during the evolution of monocytes to macrophages.[53] In patients with ACS, CD36 receptor expression on circulating monocytes may be strongly associated with the late stage of atherosclerosis, because the onset of acute symptoms is characterized by increased monocyte and macrophage activity, as well as a very high level of inflammation in the body.[51] In addition, a positive correlation between CD36 expression in the aorta and CD36 expression on peripheral blood mononuclear cells has also been described.[54]

The results should be interpreted with considerations since patients were under treatment. In addition, only four *CD36* variants were evaluated, cross-sectional nature of the analysis, and possibility of false positives due to multiple statistical testing. Furthermore, we have to consider that the size of samples analyzed for the quantification of sCD36 and mRNA expression is small. However, a more specialized methodological approach is required, since our patients received pharmacological treatment as part of their medical follow-up and it would be unethical to request that patients leave it for the purposes of this study.

5 | CONCLUSIONS

The rs1194182 seems to be associated with diabetes in a risky manner, in ACS patients and protective for dyslipidemia in both groups. The concentration of sCD36 seems to be associated with the clinical spectrum of the ACS patients and the presence of diabetes, since patients with STEMI present significantly elevated level compared with UA. Clinical ACS spectrum is indistinguishable; thus, sCD36 could be analyzed in a longitudinal study as a perspective in order to test whether it could be a clinically useful marker of complementary diagnosis.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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