

Single-nucleotide polymorphisms in the promoter of the gene encoding for C-reactive protein associated with acute coronary syndrome

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Abstract. Acute coronary syndrome (ACS) is a leading cause of mortality worldwide. Several studies have shown that certain single nucleotide polymorphisms (SNPs) are linked to the development of ACS. In particular, C-reactive protein (CRP) has emerged as an important predictive biomarker for cardiovascular disease. The current study aimed to investigate four polymorphisms of the CRP gene as possible biomarkers for ACS in a sample of 252 individuals (114 patients with ACS and 138 healthy controls) from Southeastern Mexico. Multivariate analysis adjusted for clinical variables showed that the polymorphism 3872CT for the genotype CC/CT [adjusted Odds Ratio (AdOR)=3.78; 95% Confidence Interval (CI): 1.11-12.92; P=0.034] and the genotype GG/GC of the polymorphisms 2667CG (AdOR=4.82; 95% CI: 1.69-13.72; P=0.02) were associated with ACS. However, the polymorphisms 3006AC genotype AA/AC and 5237GA genotype GG/GC were not found to be associated in the multivariate

analysis with ACS (P>0.05). These results suggested that 3872CC/CT and 2667CC/CG polymorphism of the CRP gene plays a significant role in the development of ACS.

Introduction

Acute coronary syndrome (ACS) is a cardiovascular disease with a multifactorial etiology, which often involves acute myocardial infarction and unstable angina (1,2). Several factors have been linked to the development of ACS, including hypertension, diabetes, dyslipidemia, obesity, smoking, age, sex and genetic background (3). This disease imposes a heavy burden, particularly on developing countries, and is a leading cause of mortality in Mexico (4). According to the Instituto Nacional de Estadística y Geografía, in 2022 heart disease was the second cause of mortality in Mexico. In Southeastern Mexico the mortality rate is 15.9%, slightly below the national rate of 17.7% (5).

The diagnostic process of ACS is often complicated by its varied clinical features. Hence, some biomarkers, including troponins, creatine kinase MB (CK-MB), B-type natriuretic peptides, copeptin, C-reactive protein (CRP), IL-6, D-dimers and fibrinogen, have evolved as crucial instruments for the diagnosis ACS (6). CRP is an acute-phase protein that plays a key role in chronic and acute inflammation. It is used as a general biomarker of systemic inflammation and participates in the pathogenesis of cardiovascular diseases, such as endothelial dysfunction and atherosclerosis (7,8). CRP has been associated with the presence of coronary artery disease (CAD) (9) and a higher risk of future cardiovascular events in

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apparently healthy individual (10). Genetic polymorphisms in the interleukin genes as well as in the CRP gene have been associated with minor elevations in CRP (11). The single nucleotide polymorphisms (SNPs) in the promoter region, exon 2 and the 3' untranslated region (UTR) of the CRP gene have been reported to influence CRP serum levels (12-14); similarly, other SNPs have been shown to induce changes in transcription factor binding and gene promoter activity (15,16). The association of SNPs in the CRP gene with plasma CRP levels and cardiovascular disease has been reported in previous studies (17-19). A genome-wide association study found no association of variants in the CRP locus and cardiovascular heart disease, but five genetic loci were strongly associated with CRP levels (20).

Another study in an American population found no association in 1059G/C polymorphism in the human CRP gene however, it suggested that genetic and environmental determinants each importantly contribute to the vascular risk associated with inflammation (21).

In the literature, the polymorphisms of the CRP gene 2667GG/GC (rs1800947), 3872CC/CT (rs1205), 5237GG/GA (rs2808630) and 3006AA/AC (rs3093066) are known to be associated with changes of the CRP production *in vivo* and cardiovascular diseases (22,23). However, it remains to be elucidated whether polymorphisms in the CRP gene are associated with susceptibility to ACS in patients of Mexico. For this reason, the current study analyzed the relationship between four SNPs of the CRP gene, namely 2667 GG/GC, 3872CC/CT, 5237GG/GA and 3006AA/AC, and ACS in a group of individuals from Southeastern Mexico.

Materials and methods

Subjects. The present retrospective and descriptive study included patients who attended the cardiology department at the Hospital Regional de Alta Especialidad-Ciudad Salud (HRAE-CS; Chiapas, Mexico) between March 2010 and December 2012. It included a cohort of 252 patients, 159 male (63.1%) and 93 female (36.9%), with an age range of 22-81 years and a median age of 53.5. In total, 114 patients were diagnosed with primary ACS according to the American College of Cardiology (ACA) criteria (24) and were included in the ACS group under the following criteria: i) Persistent chest pain for >20 min; ii) typical electrocardiographic changes including new pathologic Q waves; iii) ST-segment elevation of >1 mm; and iv) increased plasma levels of CK-MB >2-fold higher than the upper reference limit and/or troponin-T >0.1 µg/ml.

In parallel, 138 healthy individuals were included in the control group according to the ACA criteria (24): i) Individuals without a history of cardiovascular disease; and ii) individuals without evidence of other systemic diseases.

Based on their medical history, no subjects in the control group suffered from ACS or any related pathology. The age and sex of the control and the ACS groups were matched. The selection of patients is shown in the STROBE flow (Fig. 1). The current study was conducted in accordance with The Declaration of Helsinki. All participants provided written informed consent before taking their blood samples. The study was approved by the HRAE-CS Ethics Committee (approval no. 02/2010).

DNA extraction and genotyping. For each patient, 5 ml of blood was drawn into a purple top EDTA tube and stored at 4°C for a maximum of 1 week to prevent degradation of nucleic acids. DNA was extracted from EDTA-treated venous blood samples using the QIAamp DNA Blood Mini kit (Qiagen GmbH) and stored at -20°C until use. DNA was quantified in an Eppendorf 6131 Biophotometer (Eppendorf SE) and sample concentrations were adjusted to 50 ng/µl. Genotyping was performed in a StepOne Real-Time PCR System with the TaqMan Genotyping Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). Specific assays for the SNPs 3872C>T [rs1205; genotyping primer: ACTTCCAGTTTGGCTTCTGTCCTCA(C/T)AGTCTCTCTCCATGTGGCAAACAAG], 5237G>A [rs2808630; genotyping primer: AGGCCAGAGGCTGTCTACCAGACTA(G/A)GTATAGTAAGATGCAAGCAACTGAA], 2667C>G [rs1800947; genotyping primer: AGATGGTGTTAATCTCATCTGGTGA(C/G)AGCACAAGTCCCACATGTTTCACAT] and 3006A>C [rs3093066; genotyping primer: ATGTCAGTGGCCTCATGCTTTGCA C(A/C)TTACAAAGTGAGTAATGTGTGCTGA] previously associated with cardiovascular disease (CVD) in the CRP gene (25) were purchased from Thermo Fisher Scientific, Inc. with ID C_7479334_10, C_12035003_10, C_11663840_10 and C_27452296_10, respectively. PCR protocol included initial denaturation 1 cycle at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 1 min. The genotyping was performed following the manufacturer's protocol. Genotype calls were made upon visualization of the allelic discrimination charts in which the clusters were identified.

Statistical analysis. Data are presented as mean ± standard deviation (age) or percentages. For inferential statistics, the normal distribution of the data was first determined using the Kolmogorov-Smirnov test and the equality of variances was confirmed using the Levene test. The age of the groups was compared using the Student's unpaired t-test. Fisher's exact test was used to compare categorical values. The association between various cardiovascular risk variables (age, male, diabetes, smoking, alcoholism, arterial hypertension, dyslipidemia, sedentary lifestyle and obesity), or CRP genotypes, with the presence of ACS was determined using odds ratios (OR) and 95% confidence intervals (95% CI) using univariate binary logistic regression analyses. Subsequently, significant predictors were added to the multivariate model and with forward stepwise logistic regression the most parsimonious model was identified. The probability used for the stepwise regression was set at 0.15 for variable input and 0.25 for removal. Analysis involving a two-way interaction (A x B, where A and B are the genotypes 2667CC/CG and 3872CC/CT) was made in a separate multivariate model that did not include genotypes of other polymorphisms. The multivariate analysis was considered pertinent because various metabolic and vascular disorders have been previously reported to be associated with both the exposure variable of interest (CRP genotypes) and the outcome variable (ACS) (26-29). Multivariate logistic regression allowed for the necessary statistical adjustments for probable confounding variables, resulting in an adjusted OR (AdOR). Data were analyzed using SPSS V.21 (IBM Corp.) apart from linkage disequilibrium (LD) and haplotype analyses, which

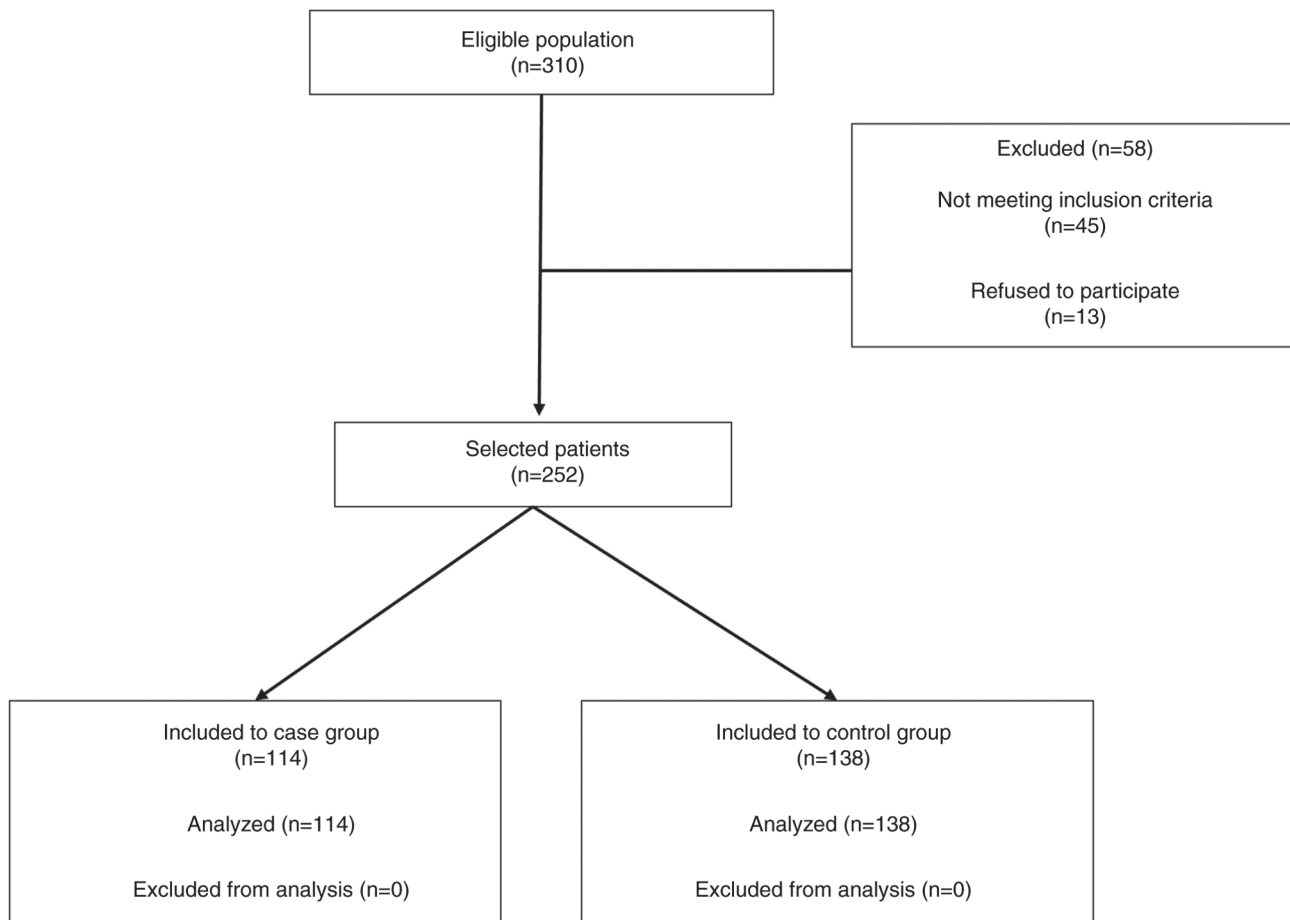


Figure 1. Strobe flow diagram. Flowchart of eligible participants for the current study.

were performed using SNPStats software (30). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Main clinical characteristics in ACS. Demographic and clinical parameters of the patients with ACS and control group are presented in Table I. In total, 252 participants were included in the present study. Male individuals were the majority in both groups, with 55.8% in the control and 71.9% in the ACS group. Significant differences were found between the group control and ACS in age, sex, T2DM, smoking, alcohol, HBP, dyslipidemia and sedentary lifestyle ($P < 0.05$). On the other hand, the obesity incidence was not found significantly different ($P = 0.876$). Except for T2DM, the aforementioned variables are considered risk factors for ACS.

Genotype of the C-reactive protein in ACS. Allele and genotype frequencies of the four polymorphisms in the patients with ACS and controls were in Hardy-Weinberg equilibrium ($P > 0.05$; data not shown). The frequency of the genotypes 2667CC/CG, 3872CC/CT, and 3006AA/AC were significantly higher in the group of patients with ACS, compared with the control group (26.1 vs. 57.0%, $P < 0.001$; 66.7 vs. 80.7%, $P = 0.015$; and 8.0 vs. 16.7%, $P = 0.049$; respectively). The genotype 5237GG/GA did not show a statistical significance between groups ($P = 0.467$; Table II). Furthermore, the analysis involved a two-way

interaction between the 2667*3872 polymorphisms, showing that the combination of genotypes 2667CC/CG*3872CC/CT was significantly higher in the group with ACS (43.0%) compared with the control group (18.1%) ($P < 0.001$; Table II).

Association of C-reactive protein polymorphisms with acute coronary syndrome. The univariate logistic regression analysis showed that age, maleness, T2DM, smoking, alcohol, HBP, dyslipidemia and sedentary lifestyle were factors associated with ACS (Table III). The multivariate analysis reveals that only age, T2DM, alcohol, HBP and sedentary lifestyle had an association with ACS (Table III). The univariate model reveals that genotypes 2667CC/CG, 3872CC/CT and 3006AA/AC were ACS-associated (OR=3.75, 95% CI: 2.21-6.39; OR=2.09, 95% CI: 1.16-3.75; and OR=2.3, 95% CI: 1.04-5.08, respectively; Table III). The multivariate model showed that genotypes 2667CC/CG (AdOR=4.82, 95% CI: 1.69-13.72) and 3872CCGG/CTGA (AdOR=3.78, 95% CI: 1.1-12.92) remain associated to ACS independently of other clinical variables or genotypes analyzed; while the 3006AA/AC genotype lost relevance and was not included in the multivariate model. The interaction of the 2667CC/CG and 3872CC/CT genotypes increased the risk for ACS in the univariate and multivariate model (OR=3.40, 95% CI: 1.92-6.02; and AdOR=4.14, 95% CI: 1.49-11.52, respectively). However, the probability of developing ACS generated by this combination of genotypes was not higher than that generated by the 2667CC/CG genotype alone (Table III).

Table I. Main clinical characteristics of the participating subjects according to the presence or absence of acute coronary syndrome.

Variable	Total	Acute coronary syndrome		P-value
		No	Yes	
Age (years)	51.8±13.5	45.8±11.9	59.2±11.6	<0.001 ^a
Sex				0.009 ^b
Male ^c	63.1%	55.8%	71.9%	
Female	36.9%	44.2%	28.1%	
T2DM				<0.001 ^b
Yes	30.6%	13.0%	51.8%	
No	69.4%	87.0%	48.2%	
Smoking				0.008 ^b
Yes ^c	35.7%	28.3%	44.7%	
No	64.3%	71.7%	55.3%	
Alcohol				<0.001 ^b
Yes ^c	52.0%	38.4%	74.7%	
No	48.0%	61.6%	25.3%	
HBP				<0.001 ^b
Yes ^c	42.1%	17.4%	71.9%	
No	57.9%	82.6%	28.1%	
Dyslip				<0.001 ^b
Yes ^c	27.4%	10.1%	48.2%	
No	72.6%	89.9%	51.8%	
Sedentarism				<0.001 ^b
Yes ^c	38.7%	23.9%	63.1%	
No	61.3%	76.1%	36.9%	
Obesity				0.876 ^b
Yes ^c	26.4%	26.8%	25.6%	
No	73.6%	73.2%	74.4%	

^aStudent's t test; ^bFisher's exact test; ^cStratum generally considered a risk factor. HBP, high blood pressure; T2DM, type 2 diabetes mellitus; Alcohol, alcoholism; Dyslip, dyslipidemia.

Haplotype analysis and LD. The most frequent haplotype in the population was H1 T/A/G/C, with 29.15% of occurrence, followed by H2 C/A/G/C with 28.46%, H3 C/A/C/C with 11.92% and H4 T/A/C/C with 10.68%; together, they represent 80.21% of the total haplotypes. Due to the frequency of the remaining haplotypes in the population, only four were analyzed. Haplotypes numbers H2, H3, and H4 showed an association with risk of ACS (OR=14.86, 95% CI: 2.62-84.15, P=0.0026; OR=29.98, 95% CI: 4.38-205.34, P=7×10⁻⁴ and OR=100.56, 95% CI: 4.64-2177.88, P=0.0037 respectively) after adjusting for age, sex and other clinical variables (T2DM, smoking, alcohol, hypertension, dyslipidemia, sedentary lifestyle and obesity), as shown in Table IV. The polymorphism 3006 exhibited a strong LD with 3872 and 2667 (with D=0.0143, D'=0.4556, P=0.006; and D=0.0184, D'=0.4402, P<0.001, respectively), as shown in Fig. 2. The genotype 3006AA/AC rs3093066 was associated with ACS in the univariate model. This polymorphism was previously associated with CRP levels in Caucasian American and African American young adults (25). However, this polymorphism does not seem to

be an independent risk factor for the genesis of ACS in the population in the present study, since other clinical or genetic factors studied here make it lose relevance in a multivariate analysis. A probable explanation for this fact is that polymorphism 3006 exhibited a strong LD with 3872 and 2667 (with D=0.0143, D'=0.4556, P<0.05; and D=0.0184, D'=0.4402, P<0.05, respectively). This means that the alleles of the 3006 polymorphisms are nonrandomly associated with the alleles at nearby polymorphic sites 3872 and 2667. Therefore, it is likely that the 3006AA/AC genotype is not an independent risk factor, but rather a 'linked or predicted' factor by the genotypes 2667CC/CG and 3872CC/CT, an aspect that was detected in the multivariate model.

Discussion

Clinical parameters. ACS is a spectrum of myocardial ischemic disorders, including myocardial infarction with ST-segment elevation, myocardial infarction with non-ST segment elevation and unstable angina, with a multifactorial origin. In

Table II. Distribution of the analyzed genotypes of CRP according to the presence or absence of acute coronary syndrome.

CRP genotypes	Total (%)	Acute coronary syndrome		P-value ^a
		No (%)	Yes (%)	
2667				
2667CC/CG	40.1	26.1	57.0	<0.001
2667GG	59.9	73.9	43.0	
3872				
3872CC/CT	73.0	66.7	80.7	0.015
3872TT	27.0	33.3	19.3	
5237				
5237GG/GA	29.4	29.0	29.8	0.467
5237AA	70.6	71.0	70.2	
3006				
3006AA/AC	11.9	8.0	16.7	0.049
3006CC	88.1	92.0	83.3	
2667*3872 ^b				
CC/CG*CC/CT*	29.4	18.1	43.0	<0.001
CC/CG*TT	10.7	8.0	14.0	
GG*CC/CT	43.7	48.6	37.7	
GG*TT	16.3	25.4	5.3	

^aFisher's exact test; ^banalysis involved a two-way interaction (2,667x3,872 genotypes). P<0.05 was considered to indicate a statistically significant difference. CRP, C-reactive protein.

addition, previous works have linked genetic polymorphisms with the development of ACS (13,14,31). The present study analyzed the association between four polymorphisms in the promoter region of the CRP gene and ACS in a group of individuals from Southern Mexico. In the present study variables such as age, maleness, T2DM, alcohol consumption, smoking, HBP, dyslipidemia and sedentary lifestyle showed significant differences between the groups (Table I), a result which is consistent with previous reports (16,32-34). Hypertension, dyslipidemia and smoking are the three modifiable risk factors most strongly and independently associated with coronary heart disease (CHD) (35). Recently, a study showed that factors such as age 70 years old, femaleness sex, HBP, T2DM and hypertension are more frequent in patients with combined endpoint (cardiovascular mortality, mortality from stroke, myocardial infarction and stroke/transient ischemic attack in 10-year follow-up) in comparison with the control group in German patients (P<0.05) (36). DM is linked with comorbidities such as CAD (37) and represents 25-30% of the patients with ACS. Patients with ACS and DM, present poorer outcomes regarding CV morbidity and mortality, compared with individuals with ACS but not with DM. In addition, T2DM prolongs hospitalization time in patients with ACS and disruption of the normal glucose homeostasis has been found in ~70-75% of patients with CAD (38).

		Linkage disequilibrium		
		CRP-SNP-5237	CRP-SNP-2667	PCR-SNP-3006
Marker1	CRP-SNP3872	-0.0043	0.0080	0.01430
		0.0553	0.0526	0.4656
		-0.0236	0.0354	0.1209
		0.5957	0.42706	0.0066
		252	252	252
	CRP-SNP-5237		-0.0075	0.0040
			0.1555	0.0812
			-0.0446	0.0463
			0.3165	2.2981
			252	252
	CRP-SNP-2667			D
				D'
				r
				P-value
				n
				0.0184
				0.4402
				0.1701
				0.0001
				252

Figure 2. Linkage disequilibrium analysis of polymorphisms.

CRP polymorphisms and ACS. Few studies have been conducted in the Hispanic population to establish an association between gene polymorphisms and ACS. In the present study, the genotype 3872CC/CT (rs1205) was found to be a risk factor for ACS (AdOR=3.78; 95% CI: 1.11-12.92, P=0.034). The only study conducted in Mexico with this SNP did not show any association with ACS in individuals from Western Mexico; however, the allele T is associated with lower CRP concentration (38). 3872CT has been associated with low levels of CRP (33,39-42) myocardial infarction (33) and CHD (12), but other studies have not shown an association with CHD (40) or aortic stenosis (30). The current results reported that the 5237GG/GA genotype did not show an association with ACS (OR=1.04; 95% CI: 1.60-1.79, P=0.884); which agrees with a previous report (12).

The 2667CC/CG genotypes (rs1800947) were found to be a risk factor for ACS in the present study population (AdOR=4.82, 95%CI: 1.69-13.72, P=0.003). These polymorphisms have been associated with CHD (43), coronary artery disease (44), myocardial infarction (45), cerebrovascular ischemia-related disease (46), cardiovascular disease mortality (23) and other diseases such as diabetes (47), prostatic (48) and colorectal cancer (49) and microangiopathic stroke (50). In addition, other investigations have not found an association (33,15,51,52). Allele G from rs1800947 has been associated with low CRP levels in coronary artery disease (44), myocardial infarction (53) and adverse cardiovascular (54).

The genotype 3006AA/AC rs3093066 was associated with ACS in the univariate model. This polymorphism was previously associated with CRP levels in European American and African American young adults (25). However, this polymorphism does not seem to be an independent risk factor for the genesis of ACS in the present population, since other clinical or genetic factors studied here make it lose relevance in a multivariate analysis. A probable explanation for this is that polymorphism 3006 exhibited a strong LD with 3872 and 2667 (with D=0.0143, D'=0.4556, P<0.05; and D=0.0184, D'=0.4402, P<0.05, respectively). This meant that the alleles of the 3006 polymorphism were non-randomly associated

Table III. Univariate and multivariate logistic regression analyses of factors associated with ACS.

	Univariate model				Multivariable model			
	cOR	95% CI		P-value	aOR	95% CI		P-value
Age	1.09	1.06	1.12	<0.001	1.15	1.08	1.21	<0.001
Male	2.03	1.19	3.44	0.009				
T2DM	7.15	3.86	13.25	<0.001	4.76	1.56	14.49	0.006
Smoking	2.05	1.21	3.46	0.007				
Alcohol ^a	4.74	2.59	8.64	<0.001	9.15	3.14	26.63	<0.001
HBP	12.17	6.67	22.1	<0.001	5.40	1.89	15.30	0.002
Dyslip	8.25	4.25	16.0	<0.001	2.87	0.96	8.99	0.060
Sedentarism	5.44	3.01	9.82	<0.001	16.36	4.74	56.42	<0.001
Obesity	0.94	0.50	1.75	0.845				
CRP genotypes								
2667CC/CG	3.75	2.21	6.39	<0.001	4.82	1.69	13.72	0.003
3872CC/CT	2.09	1.16	3.75	0.013	3.78	1.11	12.92	0.034
5237GG/GA	1.04	1.60	1.79	0.884				
3006AA/AC	2.30	1.04	5.08	0.038				
2667*3872 ^b	3.40	1.92	6.02	<0.001	4.14	1.49	11.52	0.006

Important variables in the univariate model were subsequently added to the multivariate model and a forward stepwise logistic regression identified the most parsimonious model. The probability used for the stepwise regression was set at 0.15 for entry of variables and 0.25 for removal.

^aStratum generally considered a risk factor. ^bAnalysis involved a two-way interaction (2667CC/CG*3872CC/CT), which was performed in a separate multivariate model that did not include genotypes of other polymorphisms. $P < 0.05$ was considered to indicate a statistically significant difference. ACS, acute coronary syndrome; cOR, crude odds ratio; CI, lower and upper bounds of the 95% confidence interval; aOR, adjusted odds ratio; T2DM, type 2 diabetes mellitus; Alcohol, alcoholism; Dyslip, dyslipidemia; HBP, high blood pressure; CRP, C-reactive protein.

Table IV. Haplotype association with ACS (n=252, adjusted by sex, age, T2DM, smoking, alcohol, hypertension, dyslipidemia, sedentarism and obesity).

Haplotype	CRP3872	CRP5237	CRP2667	CRP3006	Freq	OR (95% CI)	P-value
H1	T	A	G	C	0.2915	1.00	-
H2	C	A	G	C	0.2846	14.86 (2.62-84.15)	0.0026
H3	C	A	C	C	0.1192	29.98 (4.38-205.34)	7×10^{-4}
H4	T	A	C	C	0.1068	100.56 (4.64-2177.88)	0.0037

Global haplotype association. $P < 0.05$ was considered to indicate a statistically significant difference. ACS, acute coronary syndrome; T2DM, type 2 diabetes mellitus; OR, adjusted odds ratio; CI, Lower and upper bounds of the 95% confidence interval; freq, genotype frequency.

with the alleles at nearby polymorphic sites 3872 and 2667. Therefore, it is likely that the 3006AA/AC genotype is not an independent risk factor, but rather a 'linked or predicted' factor by the genotypes 2667CC/CG and 3872CC/CT, an aspect that was detected in the multivariate model. The LD between polymorphism 3006 with polymorphisms 3872 and 2667 may reflect the natural selection history, gene conversion and mutation history of the analyzed population (from Chiapas, southeast of Mexico) (55), which although it can be classified as mestizo (individuals of mixed ancestry with a European and an indigenous background), has a strong Mayan ethnological and linguistic affiliations (56). Future analyses will be necessary in this respect. On the other hand, the present results showed that haplotype numbers H2 (C/A/G/C),

H3 (C/A/C/C) and H4 (T/A/C/C) showed an association with risk ACS. Similar results have been shown in the US population (12).

Polymorphisms in the CRP promoter region would increase the risk of ACS by favoring an elevation of this protein, which is a clear sign of systemic inflammation (57,58). The expression of the CRP gene is modulated by several SNPs in the promoter region and these SNPs affects changes in gene promoter activity, RNA structure or turnover, transcription factor binding and transcriptional activity (25,59). The mechanisms by which SNP 2667 affects the expression of CRP are unclear, so it remains possible that 2667 is in strong LD with a polymorphism in a distal regulatory element not within the region scanned for polymorphisms (25). However, a study showed

that the minor alleles of the 2667 and 3872 polymorphisms linked with CRP haplotypes are associated with decreased promoter activity (23). As aforementioned, several SNPs have been associated with CHD and with CRP levels. CRP is an inflammatory marker associated with cardiovascular disease, T2DM and other pathology. It is probable that CRP genotypes affect CRP synthesis and influence the onset of clinical CVD or ACS (23).

Due to the aforementioned factors, it is hypothesized that the number of subjects included in the present study may be a limitation. However, the results shown can be considered reliable, considering that it has previously been postulated that it is necessary to have ≤ 10 individuals with the event of interest for each predictor variable included in logistic regression models (60) to improve the performance of the model and reduce the risk of false positive results. In the multivariate logistic regression model performed in the present study (Table III), eight predictor variables were included in the multivariate model, analyzing 114 individuals with ACS and 138 subjects in the control group. Therefore, the present study complied with the rule of having ≤ 10 events per variable. However, some reports have suggested that this rule is controversial (61,62). Another limitation was that the serum levels of CRP were not determined in the participants, so it would be relevant for future research to quantify this protein to evaluate whether the risk conferred by certain genotypes is directly related to the elevation of CRP.

In conclusion, four polymorphisms in the promoter region of the CRP gene in association with ACS were analyzed in the present study. Variables such as age, T2DM, alcohol, HBP and sedentary lifestyle were associated with ACS. To the best of the authors' knowledge, this work provided the first evidence of the association of CRP genotypes 2667CC/CG and 3872CC/CT with ACS in Mexico. These genotypes were evidenced as risk factors independent of other clinical risk factors in the multivariate analysis. It is possible that a set of polymorphisms, along with environmental factors, contribute to the development of chronic degenerative diseases, modulating the increased risk for heart disease. However, further studies are required to ascertain whether these polymorphisms participate in the development of ACS.

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Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Authors' contributions

ALR and LMCA were responsible for conceptualization. IDE, ESG, ALR, BHO and ICQC were responsible for methodology. JAF, ALR, IDE and VOG were responsible for

software. ALR, LMCA, IDE and ESG were responsible for formal analysis. ALR, RSGB, SGM and NLL were responsible for investigation. JAF, IDE and VOG were responsible for data curation. ALR, RSGB and NLL were responsible for writing and preparation of the original draft. ALR, LMCA, IDE, ICQC, SGM and NLL were responsible for writing, reviewing and editing. LMCA, IDE and BHO were responsible for supervision. LMCA was responsible for project administration. ALR and LMCA confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the HRAE-CS Ethics Committee, with a permit number 02/2010. Informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

Not applicable.

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