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

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Received October 24, 2023; Accepted July 26, 2024

DOI: 10.3892/br.2024.1838

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.

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Key words: acute coronary syndrome, C-reactive protein, risk, polymorphisms, quantitative PCR

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 Geografia, 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

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 hearth 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: ACTTCCAGTTTGGCTTCT GTCCTCA(C/T)AGTCTCTCTCCATGTGGCAAACAAG], 5237G>A [rs2808630; genotyping primer: AGGCCAGAG GCTGTCTACCAGACTA(G/A)GTATAGTAAGATGCA AGCAACTGAA], 2667C>G [rs1800947; genotyping primer: AGATGGTGTTAATCTCATCTGGTGA(C/G)AGCACA AAGTCCCACATGTTCACAT] and 3006A>C [rs3093066; genotyping primer: ATGTCACTGGCCTCATGCTTTGCA 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 seg 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

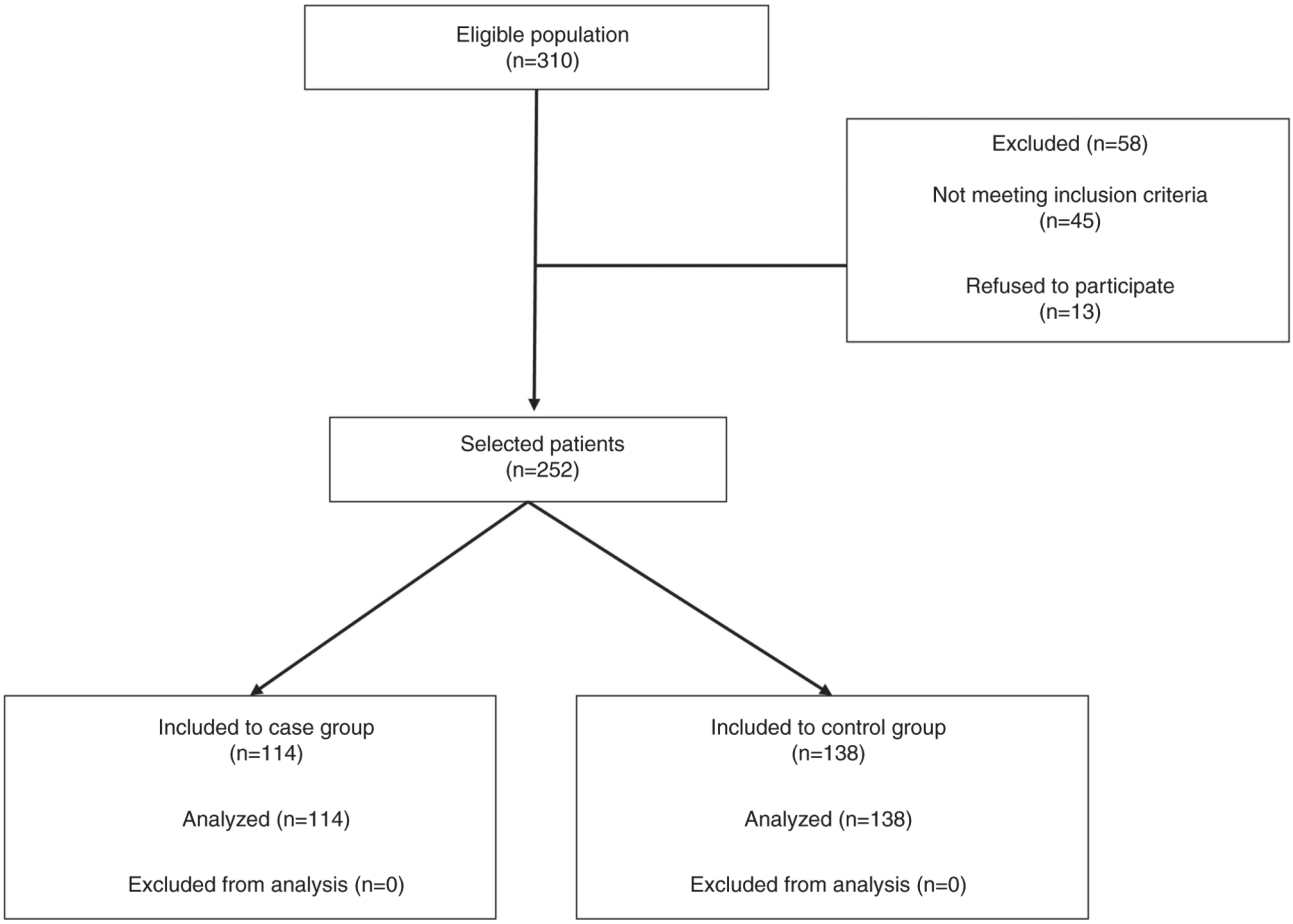

Figure 1. Strobe flow diagram. Flowchart of eligible participants for the current study.

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 ASC (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%	

[a]Student's t test; [b]Fisher's exact test; [c]Stratum 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=7x10^{-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: No (%)	Acute coronary syndrome: Yes (%)	P-value[a]
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	

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

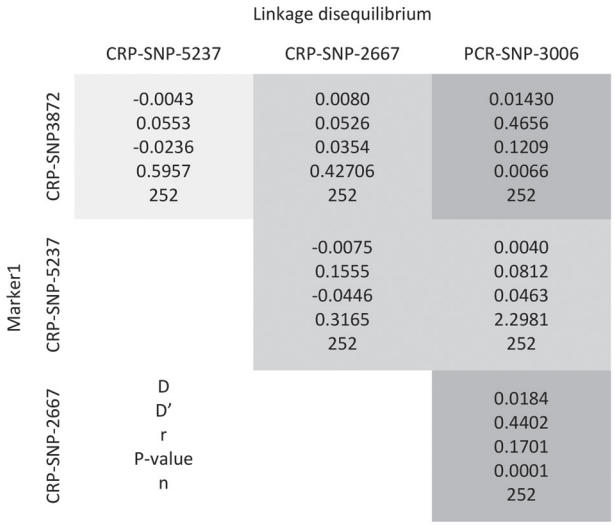

Figure 2. Linkage disequilibrium analysis of polymorphisms.

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).

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/AA 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. [a]Stratum generally considered a risk factor. [b]Analysis 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)	$7x10^{-4}$
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.

Acknowledgements

Not applicable.

Funding

No funding was received.

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.

References

1. Birnbaum Y, Wilson JM, Fiol M, de Luna AB A, Eskola M and Nikus K: ECG diagnosis and classification of acute coronary syndromes. Ann Noninvasive Electrocardiol 19: 4-14, 2014.
2. Crea F and Liuzzo G: Pathogenesis of acute coronary syndromes. J Am Coll Cardiol 61: 1-11, 2013.
3. Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, Caso P, Dudek D, Gielen S, Huber K, *et al*: Israrci ST-segment yükselmesi belirtileri göstermeyen hastalarda akut koronersendromlarin (AKS) tedavi kilavuzlari. Turk Kardiyol Dern Ars 39: 73-128, 2011.
4. Sanchis-Gomar F, Perez-Quilis C, Leischik R and Lucia A: Epidemiology of coronary heart disease and acute coronary syndrome. Ann Transl Med 4: 256, 2016.
5. INEGI: Techninal note on statistics of registered deaths. 2021. Mexico, 2022. Available at: https://www.inegi.org.mx/contenidos/programas/mortalidad/doc/defunciones_registradas_202 1_nota_tecnica.pdf.
6. Katsioupa M, Kourampi I, Oikonomou E. Tsigkou V, Theofilis P, Charalambous G, Marinos G, Gialamas I, Zisimos K, Anastasiou A, *et al*: Novel biomarkers and their role in the diagnosis and prognosis of acute coronary syndrome. Life (Basel) 13: 1992, 2023.
7. Momiyama Y, Ohmori R, Fayad ZA, Kihara T, Tanaka N, Kato R, Taniguchi H, Nagata M, Nakamura H and Ohsuzu F: Associations between plasma C-reactive protein levels and the severities of coronary and aortic atherosclerosis. J Atheroscler Thromb 17: 460-467, 2010.
8. Szmitko PE, Wang CH, Weisel RD, de Almeida JR, Anderson TJ and Verma S: New markers of inflammation and endothelial cell activation: Part I. Circulation 108: 1917-1923, 2003.
9. Gupta S, Gupta VK, Gupta R, Arora S and Gupta V: Relationship of high-sensitive C-reactive protein with cardiovascular risk factors, clinical presentation and angiographic profile in patients with acute coronary syndrome: An Indian perspective. Indian Heart J 65: 359-365, 2013.

10. Casas JP, Shah T, Hingorani AD, Danesh J and Pepys MB: C-reactive protein and coronary heart disease: A critical review. J Intern Med 264: 295-314, 2008.
11. Eklund CM: Proinflammatory cytokines in CRP baseline regulation. Adv Clin Chem 48: 111-136, 2009.
12. Pai JK, Mukamal KJ, Rexrode KM and Rimm EB: C-reactive protein (CRP) gene polymorphisms, CRP levels, and risk of incident coronary heart disease in two nested case-control studies. PLoS One 3: e1395, 2008.
13. Rios DL, Cerqueira CC, Bonfim-Silva R, Araújo LJ, Pereira JF, Gadelha SR and Barbosa AA: Interleukin-1 beta and interleukin-6 gene polymorphism associations with angiographically assessed coronary artery disease in Brazilians. Cytokine 50: 292-296, 2010.
14. Tonet AC, Karnikowski M, Moraes CF, Gomez L, Karnikowski MGO, Córdova C and Nóbrega OT: Association between the-174 G/C promoter polymorphism of the interleukin-6 gene and cardiovascular disease risk factors in Brazilian older women. Braz J Med Biol Res 41: 47-53, 2008.
15. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, Sharma P, Lowe GD, World MJ, Humphries SE and Hingorani AD: Human CRP gene polymorphism influences CRP levels: Implications for the prediction and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol 23: 2063-2069, 2003.
16. Kovacs A, Green F, Hansson LO, Lundman P, Samnegård A, Boquist S, Ericsson CG, Watkins H, Hamsten A and Tornvall P: A novel common single nucleotide polymorphism in the promoter region of the C-reactive protein gene associated with the plasma concentration of C-reactive protein. Atherosclerosis 178: 193-198, 2005.
17. Volanakis JE: Human C-reactive protein: Expression, structure, and function. Mol Immunol 38: 189-197, 2001.
18. NACB LMPG Committee Members; Myers GL, Christenson RH, Cushman M, Ballantyne CM, Cooper GR, Pfeiffer CM, Grundy SM, Labarthe DR, Levy D, *et al*: National academy of clinical biochemistry laboratory medicine practice guidelines: Emerging biomarkers for primary prevention of cardiovascular disease. Clin Chem 55: 378-384, 2009.
19. Wang XH, Liu SQ, Wang YL and Jin Y: Correlation of serum high-sensitivity C-reactive protein and interleukin-6 in patients with acute coronary syndrome. Genet Mol Res 13: 4260-4266, 2014.
20. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert, JC, Bennett D, *et al*: Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA 302: 37-48, 2009.
21. Zee RYL and Ridker PM: Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. Atherosclerosis 162: 217-219, 2002.
22. Liu C, Jin P, Luo Y, Xu J, Kong C, Chen J, Xie H and Zhou G: Association of single-nucleotide polymorphisms of C-reactive protein gene with susceptibility to infantile sepsis in Southern China. Med Sci Monit 24: 590-595, 2018.
23. Lange LA, Carlson CS, Hindorff LA, Lange EM, Walston J, Durda JP, Cushman M, Bis JC, Zeng D, Lin D, *et al*: Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. JAMA 296: 2703-2711, 2006.
24. Cannon CP, Brindis RG, Chaitman BR, Cohen DJ, Cross JT Jr, Drozda JP Jr, Fesmire FM, Fintel DJ, Fonarow GC, Fox KA, *et al*: 2013 ACCF/AHA key data elements and definitions for measuring the clinical management and outcomes of patients with acute coronary syndromes and coronary artery disease: A report of the American college of cardiology foundation/American heart association task force on clinical data standards (writing committee to develop acute coronary syndromes and coronary artery disease clinical data standards). Circulation 127: 1052-1089, 2013.
25. Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, Liu K, Williams OD, Iribarren C, Lewis EC, *et al*: Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. Am J Hum Genet 77: 64-77, 2005.
26. Zhao Y, Wang H, Liu S, Zhao X, Chen Y, Yang Y, Wang W, Wu Y, Chen A, Tang J, *et al*: Association study of CRP gene polymorphism and hypertension in Han Chinese population. Gene 512: 41-46, 2013.
27. Jebur HB, Masroor M, Ahmad H, Khan NA, Akther J, Bharali D, Singh VK, Verma A, Khan S, Khan V, *et al*: CRP gene polymorphism and their risk association with type 2 diabetes mellitus. Open Access Maced J Med Sci 7: 33-37, 2018.
28. Wei W, Yang S, Qiu Y, Wang H, Zhao X, Zhao Y, Li Y, Wu M, Chen Y, Wang W, *et al*: CRP gene polymorphism contributes genetic susceptibility to dyslipidemia in Han Chinese population. Mol Biol Rep 41: 2335-2343, 2014.
29. Todendi PF, Klinger EI, Ferreira MB, Reuter CP, Burgos MS, Possuelo LG and Valim AR: Association of IL-6 and CRP gene polymorphisms with obesity and metabolic disorders in children and adolescents. An Acad Bras Cienc 87: 915-924, 2015.
30. Solé X, Guinó E, Valls J, Iniesta R and Moreno V: SNPStats: A web tool for the analysis of association studies. Bioinformatics 22: 1928-1929, 2006.
31. Libby P: Mechanisms of acute coronary syndromes and their implications for therapy. N Engl J Med 368: 2004-2013, 2013.
32. Hackam DG and Anand SS: Emerging risk factors for atherosclerotic vascular disease: A critical review of the evidence. JAMA 290: 932-940, 2003.
33. Miller DT, Zee RY, Suk Danik J, Kozlowski P, Chasman DI, Lazarus R, Cook NR, Ridker PM and Kwiatkowski DJ: Association of common CRP gene variants with CRP levels and cardiovascular events. Ann Hum Genet 69: 623-638, 2005.
34. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GDO, Pepys MB and Gudnason V: C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 350: 1387-1397, 2004.
35. Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, Garside DB and Wilson PW: Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 290: 891-897, 2003.
36. Schulz S, Rehm S, Schlitt A, Lierath M, Lüdike H, Hofmann B, Bitter K and Reichert S: C-reactive protein level and the genetic variant rs1130864 in the CRP gene as prognostic factors for 10-year cardiovascular outcome. Cells 12: 1775, 2023.
37. Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC); European Association for the Study of Diabetes (EASD); Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, *et al*: ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD-summary. Diabetes Vasc Dis Res 11: 133-173, 2014.
38. Babes EE, Bustea C, Behl T, Abdel-Daim MM, Nechifor AC, Stoicescu M, Brisc CM, Moisi M, Gitea D, Iovanovici DC, *et al*: Acute coronary syndromes in diabetic patients, outcome, revascularization, and antithrombotic therapy. Biomed Pharmacother 148: 112772, 2022.
39. Reynoso-Villalpando GL, Padilla-Gutiérrez JR, Valdez-Haro A, Casillas-Muñoz F, Muñoz-Valle JF, Castellanos-Nuñez E, Chávez-Herrera JC and Valle Y: Relationship between C-reactive protein serum concentration and the 1846 C>T (rs1205) polymorphism in patients with acute coronary syndrome from Western Mexico. Genet Test Mol Biomarkers 21: 334-340, 2017.
40. Suk Danik J, Chasman DI, Cannon CP, Miller DT, Zee RY, Kozlowski P, Kwiatkowski DJ and Ridker PM: Influence of genetic variation in the C-reactive protein gene on the inflammatory response during and after acute coronary ischemia. Ann Hum Genet 70: 705-716, 2006.
41. Kardys I, de Maat MP, Uitterlinden AG, Hofman A and Witteman JC: C-reactive protein gene haplotypes and risk of coronary heart disease: The rotterdam study. Eur Heart J 27: 1331-1337, 2006.
42. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA and Braunwald E: Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) Investigators. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 352: 20-28, 2005.
43. Russell AI, Cunninghame Graham DS, Shepherd C, Roberton CA, Whittaker J, Meeks J, Powell RJ, Isenberg DA, Walport MJ and Vyse TJ: Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. Hum Mol Genet 13: 137-147, 2004.
44. Hernández-Díaz Y, Tovilla-Zárate CA, Juárez-Rojop I, Baños-González MA, Torres-Hernández ME, López-Narváez ML, Yañez-Rivera TG and González-Castro TB: The role of gene variants of the inflammatory markers CRP and TNF-α in cardiovascular heart disease: Systematic review and meta-analysis. Int J Clin Exp Med 15: 11958-11984, 2015.
45. Grammer TB, März W, Renner W, Böhm BO and Hoffmann MM: C-reactive protein genotypes associated with circulating C-reactive protein but not with angiographic coronary artery disease: the LURIC study. Eur Heart J 30: 170-182, 2009.

46. Schulz S, Lüdike H, Lierath M, Schlitt A, Werdan K, Hofmann B, Gläser C, Schaller HG and Reichert S: C-reactive protein levels and genetic variants of CRP as prognostic markers for combined cardiovascular endpoint (cardiovascular death, death from stroke, myocardial infarction, and stroke/TIA). Cytokine 88: 71-76, 2016.
47. Chen Z, Yu D, Xu ZW, Li SS, Li XF, Li J and Yang X: C-reactive protein gene polymorphisms and gene-environment interactions in ischaemic stroke. Neurol Res 37: 979-984, 2015.
48. Lee CC, You NC, Song Y, Hsu YH, Manson J, Nathan L, Tinker L and Liu S: Relation of genetic variation in the gene coding for C-reactive protein with its plasma protein concentrations: Findings from the women's health initiative observational cohort. Clin Chem 55: 351-360, 2009.
49. Markt SC, Rider JR, Penney KL, Schumacher FR, Epstein MM, Fall K, Sesso HD, Stampfer MJ and Mucci LA: Genetic variation across C-reactive protein and risk of prostate cancer. Prostate 74: 1034-1042, 014.
50. Chen XL, Liao YQ and Liu JR: Genotype CC of rs1800947 in the C-reactive protein gene may increase susceptibility to colorectal cancer: a meta-analysis. Asian Pac J Cancer Prev 15: 2663-2667, 2014.
51. Kuhlenbaeumer G, Huge A, Berger K, Kessler C, Voelzke H, Funke H, Stoegbauer F, Stoll M and Ringelstein EB: Genetic variants in the C-reactive protein gene are associated with micro-angiopathic ischemic stroke. Cerebrovasc Dis 30: 476-482, 2010.
52. Dai DF, Chiang FT, Lin JL, Huang LY, Chen CL, Chang CJ, Lai LP, Hsu KL, Tseng CD, Tseng YZ and Hwang JJ: Human C-reactive protein (CRP) gene 1059G>C polymorphism is associated with plasma CRP concentration in patients receiving coronary angiography. J Formos Med Assoc 106: 347-354, 2007.
53. Kolz M, Koenig W, Müller M, Andreani M, Greven S, Illig T, Khuseyinova N, Panagiotakos D, Pershagen G, Salomaa V, *et al*: DNA variants, plasma levels and variability of C-reactive protein in myocardial infarction survivors: Results from the AIRGENE study. Eur Heart J 29: 1250-1258, 2008.
54. Rizzello V, Liuzzo G, Giannuario GD, Trabetti E, Brugaletta S, Santamaria M, Piro M, Pignatti PF, Maseri A, Biasucci LM and Crea F: 1059G/C polymorphism within the exon 2 of the C-reactive protein gene: Relationship to C-reactive protein levels and prognosis in unstable angina. Coron Artery Dis 18: 533-538, 2007.
55. Slatkin M: Linkage disequilibrium-understanding the evolutionary past and mapping the medical future. Nat Rev Genet 9: 477-485, 2008.
56. Martínez-Cortés G, Salazar-Flores J, Fernández-Rodríguez LG, Rubi-Castellanos R, Rodríguez-Loya C, Velarde-Félix JS, Muñoz-Valle JF, Parra-Rojas I and Rangel-Villalobos H: Admixture and population structure in Mexican-Mestizos based on paternal lineages. J Hum Genet 57: 568-574, 2012.
57. Obisesan TO, Leeuwenburgh C, Phillips T, Ferrell RE, Phares DA, Prior SJ and Hagberg JM: C-reactive protein genotypes affect baseline, but not exercise training-induced changes, in C-reactive protein levels. Arterioscler Thromb Vasc Biol 24: 1874-1879, 2004.
58. Wang Q, Hunt SC, Xu Q, Chen YE, Province MA, Eckfeldt JH, Pankow JS and Song Q: Association study of CRP gene polymorphisms with serum CRP level and cardiovascular risk in the NHLBI family heart study. Am J Physiol Heart Circ Physiol 291: H2752-H2757, 2006.
59. Szalai AJ, Wu J, Lange EM, McCrory MA, Langefeld CD, Williams A, Zakharkin SO, George V, Allison DB, Cooper GS, *et al*: Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. J Mol Med (Berl) 83: 440-447, 2005.
60. Shipe ME, Deppen SA, Farjah F and Grogan EL: Developing prediction models for clinical use using logistic regression: An overview. J Thorac Dis 11 (Suppl 4): S574-S584, 2019.
61. Van Smeden M, de Groot JAH, Moons KG, Collins GS, Altman DG, Eijkemans MJ and Reitsma JB: No rationale for 1 variable per 10 events criterion for binary logistic regression analysis. BMC Med Res Methodol 16: 163, 2016.
62. Van Smeden M, Moons KG, de Groot JA, Collins GS, Altman DG, Eijkemans MJ and Reitsma JB: Sample size for binary logistic prediction models: Beyond events per variable criteria. Stat Methods Med Res 28: 2455-2474, 2019.