



OPEN *AGTR1* rs5186 polymorphism and inflammatory biomarkers are associated with cardiovascular disease risk in an admixed Mexican population

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The angiotensin II type 1 receptor (*AGTR1*) rs5186 (*A1166C*) polymorphism has been associated with cardiovascular disease (CVD) risk. However, its relevance in genetically admixed populations, such as Mexicans, remains unclear. The aim of this study was to evaluate the association between the *AGTR1* rs5186 polymorphism and CVD risk in a Mexican population, while integrating key inflammatory and metabolic biomarkers. A case-control study was conducted with 356 participants from Nayarit, Mexico, classified into three groups: CVD ($n = 118$), hypertension free of CVD (HTN, $n = 119$), and healthy controls ($n = 119$). Genotyping of rs5186 was performed via real-time PCR. Logistic regression models were adjusted for sociodemographic, clinical, and lifestyle variables, including serum amyloid A (SAA) and homocysteine levels. The *A1166C* genotype was independently associated with CVD risk (OR = 3.42, 95% CI: 1.16, 10.08). Elevated SAA (OR = 1.01, 95% CI: 1.01, 1.02) and homocysteine (OR = 3.20, 95% CI: 1.93, 5.33) were also significant predictors. These associations persisted after adjusting for potential confounders. This study highlights the relevance of *AGTR1* rs5186 and inflammatory biomarkers in CVD susceptibility in a Mexican admixed population. Findings support the inclusion of genetic screening and inflammatory profiling in population-specific preventive strategies.

Keywords *AGTR1* rs5186 polymorphism, Cardiovascular disease, Genetic susceptibility, Inflammatory biomarkers, Mexican population, Admixture

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide, with a growing burden in low- and middle-income countries, including Mexico^{1–4}.

Growing evidence highlights the pivotal role of chronic low-grade inflammation in the pathogenesis and progression of CVD, contributing to atherosclerosis, myocardial infarction, heart failure, and related conditions⁵. Residual inflammatory risk, commonly assessed by high-sensitivity C-reactive protein (hsCRP), has been shown to predict recurrent cardiovascular events even among patients receiving optimal lipid-lowering therapy. Furthermore, elevated hsCRP levels in apparently healthy individuals identify a subgroup at increased cardiovascular risk, in whom statin therapy may reduce the incidence of first major cardiovascular events, even in the absence of elevated low-density lipoprotein cholesterol (LDL-C)⁵.

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In this context, the interplay between genetic susceptibility and modifiable risk factors is increasingly recognized as a key determinant of CVD development and progression⁶. Among genetic contributors, polymorphisms within the renin–angiotensin system (RAS), particularly in the angiotensin II type 1 receptor (*AGTR1*) gene, have attracted considerable attention due to their potential impact on vascular homeostasis^{7,8}. The *AGTR1* gene encodes the angiotensin II type 1 receptor, a central mediator of vasoconstriction, sodium retention, and vascular remodeling^{9,10}.

The rs5186 polymorphism (*A1166C*), located in the 3' untranslated region (UTR) of *AGTR1*, has been associated with altered receptor expression and/or signaling efficiency, which may enhance angiotensin II-mediated effects and contribute to hypertension and other cardiovascular risk phenotypes^{11–14}.

Specifically, the C allele at position 1166 disrupts a binding site for microRNA-155 (miR-155), a post-transcriptional regulator that normally suppresses *AGTR1* expression. As a result, carriers of the C allele exhibit reduced miR-155-mediated downregulation, leading to increased receptor density and enhanced angiotensin II signaling, with downstream effects on vasoconstriction, vascular inflammation, and endothelial dysfunction¹⁵.

The frequency of the C allele varies considerably across ethnic groups, ranging from 0.19 to 0.31 in populations of European descent of 0.03 to 0.11 in Asian populations, with lower frequencies reported in populations of African ancestry¹⁶. This variability in allele distribution, combined with differences in linkage disequilibrium patterns and genetic background, suggests that the cardiovascular consequences of the rs5186 polymorphism may not be uniform across populations, underscoring the need to evaluate its effects within specific contexts. Previous studies in Asian and European populations have reported associations between the C allele of rs5186 and myocardial infarction, arterial stiffness, and poor outcomes in heart failure^{12,14,17,18}.

Emerging evidence indicates that inflammatory and metabolic mediators can directly interact with the angiotensin II receptor type 1 (AT1R) receptor and potentiate its downstream effects. Specifically, homocysteine (Hcys) has been shown to directly bind and activate the AT1 receptor, displacing angiotensin II and inducing distinct conformational changes that aggravate vascular injury¹⁹. Similarly, serum amyloid A (SAA), an acute-phase apolipoprotein produced in response to systemic inflammation, has been shown to modulate angiotensin II-induced vascular damage, contributing to endothelial dysfunction, foam cell formation, and atherogenesis²⁰. These interactions suggest that elevated levels of these mediators may amplify AT1R-mediated cardiovascular risk, particularly in individuals carrying genetic variants that enhance receptor expression or signaling.

The Mexican population is characterized by a complex genetic admixture of Indigenous American, European, and African ancestries²¹. This heterogeneity influences the distribution and expression of genetic variants and may modulate their biological effects^{22–24}. To date, studies on *AGTR1* rs5186 in Mexican cohorts are scarce and inconclusive. Conflicting findings have been reported regarding its association with hypertension and myocardial infarction^{22–24}, and no prior study has comprehensively evaluated its interaction with biomarkers such as SAA and Hcys. Beyond their role as indicators of systemic inflammation and endothelial dysfunction^{25,26}, both mediators have been shown to directly modulate AT1R activity and angiotensin II-mediated vascular pathways^{19,20}, making its assessment combined with rs5186 polymorphism biologically relevant and clinically significant.

Therefore, this study aimed to assess the association between the *AGTR1* rs5186 polymorphism and CVD risk in an admixed Mexican population. Unlike prior reports, we integrated genotypic data with biochemical profiling and clinical variables in a multivariable framework. This approach offers novel insights into the genetic architecture of CVD risk in a historically understudied and ethnically diverse population, contributing to precision prevention strategies tailored to Latin American contexts.

Results

General characteristics of the study population are summarized in the Table 1. All groups had a considerable prevalence of overweight according to the criteria of the world health organization. Also, harmful habits such as smoking, and alcohol consumption occurred in the population. Likewise, physical activity in the hypertension (HTN) group was lower ($p < 0.01$) than in the control group. On the other hand, the systolic blood pressure (SBP) of the HTN group was significantly higher ($p < 0.01$) as compared to the control group, while the CVD group had the lowest diastolic pressure (DBP). In addition, medication use was observed in the HTN and CVD groups as part of treatment for hypertension, dyslipidemia, and diabetes.

At the clinical level, the CVD group exhibited significantly higher levels of glucose, creatinine, serum calcium, SAA, and Hcys (all $p < 0.01$). In contrast, this group had lower concentrations of total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and apolipoprotein A1 and B (ApoA and ApoB) compared to the other groups. Meanwhile, the HTN group had the highest uric acid levels (Table 2).

Allelic and genotypic frequencies of *A1166C* (rs5186) polymorphism for *AGTR1* is summarized in Table 3. No differences were observed for the different genotypes among the study groups in the descriptive analysis. In addition, the performed analysis using the Hardy-Weinberg principle showed that there is no disequilibrium ($p = 0.34$) in *AGTR1 A1166C* polymorphism evaluated in the population. The genotype frequencies of *AGTR1* (rs5186) were in Hardy-Weinberg equilibrium in all three study groups ($p > 0.05$).

On the other hand, the model performed by multivariable logistic analysis and adjusted by sex, age, smoking, current alcohol consumption, and clinical parameters, showed a positive association of *A1166C* (odds ratio [OR] = 3.42, 95% confidence interval [95% CI]: 1.16, 10.08; $p = 0.04$) genotype, SAA (OR = 1.01, 95% CI: 1.01, 1.02; $p < 0.01$), Hcys (OR = 3.20, 95% CI: 1.93, 5.33; $p < 0.01$), and serum calcium (OR = 5.60, 95% CI: 1.70, 18.46; $p = 0.01$) with the presence of CVD, meanwhile the body mass index (BMI) had a positive association (OR = 1.19, 95% CI: 1.10, 1.30; $p < 0.01$) with the presence of hypertension (Table 4).

Characteristic	Control (n=119) ^a	HTN (n=119) ^b	CVD (n=118) ^c	<i>p</i>
Sex n (%)	87 (83.11)	86 (72.27)	88 (74.58)	0.92*
Male	32 (26.89)	33 (27.73)	30 (25.42)	
Female				
Age, years	65 (56, 74)	67 (58, 74)	67 (58, 75)	0.63**
BMI, kg/m ²	26.61 (24.22, 29.17)	28.73 (26.31, 31.14) ^a	27.56 (25.03, 30.92) ^b	<0.01**
Current Smoking n (%)	17 (14.29)	7 (5.88) ^a	5 (4.24) ^a	0.01*
Current alcohol drinking n (%)	68 (57.14)	44 (36.97) ^a	31 (26.27) ^a	<0.01*
Physical activity index	7.63 (6.63, 8.50)	7.00 (5.88, 7.88) ^a	7.25 (6.25, 8.75)	<0.01**
Hypertension n (%)	--	119 (100) ^a	93 (78.81) ^{a,b}	<0.01*
Systolic pressure (mmHg)	120 (115, 120)	120 (120, 130) ^a	120 (110, 136)	<0.01**
Diastolic pressure (mmHg)	80 (75, 80)	80 (70, 80)	70 (70, 80) ^{a,b}	<0.01**
Diabetes n (%)	--	--	51 (43.22)	-
Statins n (%)	--	28 (24.14) ^a	96 (81.36) ^{a,b}	<0.01*
Fibrates n (%)	3 (2.52)	9 (7.89)	9 (7.63)	0.15*
CAI n (%)	--	--	4 (3.39)	-
β-Blockers n (%)	--	25 (22.12) ^a	73 (61.86) ^{a,b}	<0.01*
IACE n (%)	--	28 (25.00) ^a	39 (33.05) ^a	<0.01*
Angiotensin-II Antagonist n (%)	--	65 (58.04) ^a	50 (42.37) ^{a,b}	<0.01*
Diuretics n (%)	--	20 (17.86) ^a	17 (14.53) ^a	<0.01*
Clopidogrel n (%)	--	2 (1.75) ^a	42 (35.90) ^{a,b}	<0.01*
Aspirin n (%)	--	26 (22.61) ^a	55 (47.01) ^{a,b}	<0.01*
Calcium antagonist n (%)	--	25 (22.12) ^a	21 (17.80) ^a	<0.01*

Table 1. General characteristics of the study groups. Results are expressed as medians (interquartile range) or percentages. *p* values were determined through *Chi-squared test, and **Kruskal-Wallis with post hoc Dunn's test. *p* < 0.05 was considered statistically significant. BMI, body mass index. CAI, cholesterol absorption inhibitors. IACE, inhibitors of angiotensin converting enzyme. HTN, hypertension free of CVD group, CVD, Cardiovascular Disease Group. ^aSignificant difference compared to control group (*p* < 0.05). ^bSignificant difference compared to HTN group (*p* < 0.05).

Parameter	Control ^a	HTN ^b	CVD ^c	<i>p</i>
Glucose (mg/dL)	96.00 (91.00, 104.00)	100.00 (92.00, 107.00)	107.00 (94.00, 132.00) ^{a,b}	<0.01**
Total cholesterol (mg/dL)	192.10 (170.40, 217.20)	187.60 (157.40, 206.40)	149.50 (123.30, 176.70) ^{a,b}	<0.01**
LDL cholesterol (mg/dL)	127.66 (105.66, 143.83)	117.52 (95.73, 139.83)	84.79 (65.95, 112.95) ^{a,b}	<0.01**
HDL cholesterol (mg/dL)	40.70 (35.40, 48.60)	39.30 (33.30, 46.90)	37.40 (31.20, 44.30)	0.07**
Triglycerides (mg/dL)	140.60 (96.70, 182.30)	140.95 (98.00, 183.20)	125.35 (95.20, 175.80)	0.67**
ApoA (mg/dL)	131.80 (120.40, 146.60)	131.75 (117.90, 148.30)	122.90 (112.40, 148.20) ^{a,b}	0.01**
ApoB (mg/dL)	111.20 (96.70, 124.00)	104.15 (87.30, 118.60)	77.60 (65.20, 92.60) ^{a,b}	<0.01**
Creatinine (mg/dL)	0.80 (0.70, 0.90)	0.90 (0.70, 1.00)	0.95 (0.80, 1.10) ^{a,b}	<0.01**
Uric acid (mg/dL)	5.40 (4.50, 6.30)	5.90 (4.60, 6.90) ^a	5.75 (4.70, 6.50)	0.04**
Serum calcium (mg/dL)	9.40 (9.20, 9.60)	9.30 (9.20, 9.70)	9.50 (9.30, 9.80) ^{a,b}	<0.01**
SAA (ng/mL)	336.84 (295.80, 392.10)	369.52 (320.32, 422.74)	522.35 (434.71, 576.47) ^{a,b}	<0.01**
Hcys (ng/mL)	31.50 (25.16, 34.07)	32.49 (26.27, 34.09)	38.10 (37.85, 39.04) ^{a,b}	<0.01**

Table 2. Clinical parameters of the study groups. Results are expressed as medians (interquartile range). *p* values were determined through Kruskal-Wallis with post hoc Dunn's test. *p* < 0.05 was considered as statistically significant. LDL, low density lipoprotein. HDL, high density lipoprotein. ApoA, apolipoprotein A. ApoB, apolipoprotein B. SAA, serum amyloid A. Hcys, homocysteine. HTN, hypertension free of CVD, CVD, cardiovascular disease group. ^aSignificant difference compared to control group (*p* < 0.05). ^bSignificant difference compared to HTN group (*p* < 0.05).

Discussion

This study provides meaningful and novel contributions to our understanding of CVD risk by examining the *AGTR1* rs5186 polymorphism within an admixed Mexican population—a group often underrepresented in genomic research. Although no statistically significant differences were observed in genotype frequencies across clinical groups, the multivariable analysis revealed that carriers of the C allele (1166AC/CC genotypes) exhibited a significantly increased likelihood of CVD, independent of conventional risk factors.

Genotype	Control (n=107)	HTN (n=107)	CVD (n=112)	p
A1166C (rs5186) n (%)				
AA	58 (54.21)	56 (52.34)	60 (53.57)	0.96
AC	39 (36.45)	43 (40.19)	44 (39.29)	
CC	10 (9.35)	8 (7.48)	8 (7.14)	
Allele frequency				
A	0.72	0.72	0.73	
C	0.28	0.28	0.27	

Table 3. *AGTR1* genotype in the study groups. Values are expressed as percentage. p values were calculated through Chi-squared test. HTN, hypertension free of CVD, CVD, Cardiovascular Disease Group. $p < 0.05$ was considered as statistically significant.

Parameter	HTN OR (95% CI)	p	CVD OR (95% CI)	p
A1166C (rs5186) AC/CC	1.48 (0.63, 3.47)	0.36	2.51 (1.07, 5.91)	0.03
Hcys	0.83 (0.65, 1.06)	0.14	2.69 (1.70, 4.23)	<0.01
SAA	0.99 (0.99, 1.00)	0.47	1.01 (1.01, 1.01)	<0.01
Cholesterol	1.01 (0.99, 0.02)	0.46	0.97 (0.96, 0.99)	<0.01
Serum calcium	--		2.81 (1.09, 7.20)	0.03
BMI	1.24 (1.05, 1.45)	0.01	1.03 (0.92, 1.16)	0.59

Table 4. Firth’s penalized multivariable logistic regression analysis of CVD and hypertension risk. Results are expressed as Odds Ratio (OR) and 95% Confidence Interval (95% CI). Firth’s penalized logistic regression was used for both models. The HTN model was adjusted for sex, age, current alcohol consumption, and cardiovascular medication use. The CVD model was adjusted for sex, current alcohol consumption, physical activity index, and cardiovascular medication use. SAA, serum amyloid A. Hcys, homocysteine. BMI, body mass index. HTN, hypertension free of CVD. CVD, cardiovascular disease group. $p < 0.05$ was considered statistically significant.

The findings of this study provide significant insights into the prevalence and impact of the rs5186 polymorphism in the *AGTR1* gene within a Mexican cohort. The general characteristics of the study population reveal notable trends and risk factors associated with CVD and HTN, highlighting the intricate interplay between genetic predispositions and lifestyle factors.

The Hardy-Weinberg equilibrium analysis showed no deviation from equilibrium for *AGTR1* (rs5186) A1166C polymorphism ($p = 0.34$), supporting an adequate genotype distribution in the study population. Previous studies have indicated that the effect of the *AGTR1* (rs5186) polymorphism on cardiovascular outcomes may be influenced by gene–gene and gene–environment interactions, which vary across populations¹².

With respect to the allele frequency of rs5186, the C allele frequency observed in the present study (0.27, 0.28) is consistent with frequencies reported in other Latin American admixed populations. Data from the 1000 Genomes Project indicate C allele frequencies of 0.266 in Mexican Americans from Los Angeles, 0.265 in Peruvians from Lima, 0.212 in Puerto Ricans, and 0.207 in Colombians from Medellín²⁷. Additionally, a recent study in a Mexican population from Mexico City reported a C allele frequency of 0.312²². These frequencies are substantially higher than those reported in East Asian (0.019, 0.091) and African (0.005, 0.090) populations, and comparable to those observed in European populations (0.207, 0.313), reflecting the significant European genetic component of the Mexican mestizo population. These findings underscore the biological relevance of evaluating this variant in Latin American admixed populations, where the C allele reaches intermediate-to-high frequencies that may confer cardiovascular susceptibility.

In addition, the C allele in the *AGTR1* (rs5186) polymorphism has been associated with enhanced AT1R activity through disruption of the miR-155 binding site, leading to increased receptor density and enhanced angiotensin II signaling, which can contribute to elevated blood pressure and increased CVD risk^{12,13}. Accordingly, the 1166AC/CC genotype showed a significant association with CVD (OR = 2.51, 95% CI: 1.07, 5.91; $p = 0.03$) in the multivariable logistic regression analysis, adjusted for sex, BMI, current alcohol consumption, physical activity index, clinical parameters, and cardiovascular medication use. In this regard, Semianiv et al.¹³ found a linkage between the C allele of *AGTR1* (rs5186) and arterial hypertension severity, highlighting the importance of this genetic variant as a potential marker for cardiovascular risk.

Although the case-control design does not allow mechanistic conclusions, the observed associations are consistent with a plausible physiological framework supported by previous literature. Within this context, the Hcys has been shown to directly bind and activate the AT1R, displacing angiotensin II and inducing conformational changes that aggravate vascular injury¹⁹. Similarly, SAA modulates angiotensin II-induced vascular damage, contributing to endothelial dysfunction, foam cell formation, and atherogenesis²⁰. These findings suggest that carriers of the AC/CC genotype with elevated SAA and Hcys levels may face a compounded cardiovascular risk, in which genetic susceptibility and systemic inflammation converge through the AT1R signaling axis.

No significant association was found between the *AGTR1* (rs5186) polymorphism and hypertension in the present study. However, a significant association with CVD was observed after adjusting for cardiovascular risk factors, suggesting that this polymorphism may be more closely related to overall cardiovascular susceptibility than to hypertension alone in this population. Differences in blood pressure observed across groups likely reflect the effects of antihypertensive treatment, particularly among participants in the CVD group. The management of blood pressure is crucial in reducing the risk of cardiovascular events, and the observed differences in blood pressure parameters highlight the need for effective hypertension control strategies in these populations²⁸.

Elevated levels of SAA (OR = 1.01, 95% CI: 1.01, 1.02; $p < 0.01$) and Hcys (OR = 3.20, 95% CI: 1.93, 5.33; $p < 0.01$) were significantly associated with CVD, which is consistent with their roles as inflammatory and metabolic markers, respectively^{29,30}. Additionally, serum calcium levels (OR = 5.60, 95% CI: 1.70, 18.46; $p = 0.01$) were positively associated with CVD, suggesting that mineral imbalances may contribute to cardiovascular risk. In addition, a meta-analysis demonstrated an association of circulating calcium levels with vascular disease and mortality. This association was attenuated when was adjusted for some cardiovascular risk factors³¹. Nevertheless, the risk may be consistently increased depending on the population and geographical region of residence.

The biochemical profile of the CVD group indicated significantly higher levels of glucose, creatinine, serum calcium, SAA, and Hcys compared to the other groups ($p < 0.01$). These elevated markers are well-known indicators of increased cardiovascular risk and are associated with the pathophysiology of atherosclerosis and other CVD conditions^{29,30}. Additionally, the CVD group exhibited lower levels of total cholesterol, LDL-C, HDL-C, ApoA, and ApoB, which might reflect the impact of lipid-lowering therapies commonly prescribed to manage hyperlipidemia in these patients³².

Furthermore, BMI was found to have a positive association with hypertension

(OR = 1.19, 95% CI: 1.10, 1.30; $p < 0.01$), highlighting the impact of obesity on blood pressure and cardiovascular health³³. Likewise, the high prevalence of overweight and harmful behaviors, such as smoking and alcohol consumption, in the study population is consistent with the multifactorial nature of CVD^{4,34}. These findings further support the role of weight management as a key component of hypertension prevention and control strategies. The frequent use of medications in the HTN and CVD groups also reflects the clinical complexity of these conditions.

Medication use was prevalent in both the HTN and CVD groups, particularly for the treatment of hypertension, dyslipidemia, and diabetes. The use of medications in these groups likely reflects efforts to mitigate the impact of these chronic conditions and prevent further cardiovascular complications³⁵. Inhibitors of angiotensin converting enzyme (ACE) and angiotensin II receptor blockers used by 33.05% and 42.37% of CVD group, respectively; directly target the RAS pathway, with angiotensin II receptor blockers specifically antagonizing the AT1R encoded by *AGTR1*. The *AGTR1* A1166C polymorphism has been recognized as a relevant determinant of response to RAS-targeted therapies. Carriers of the C allele have been shown to exhibit differential responses to candesartan, including greater reductions in inflammatory and neurohormonal markers compared to AA genotype carriers³⁶. This pharmacogenomic interaction implies that angiotensin II receptor blockers treatment in C allele carriers may attenuate the phenotypic cardiovascular consequences of increased AT1R density, potentially modifying the observable relationship between the rs5186 polymorphism and CVD outcomes in treated individuals. Adjustment for cardiovascular medication use as a covariate in the multivariable model allowed estimation of the genetic effect independent of pharmacological modulation.

The findings of this study are significant as they provide insights into the prevalence and impact of the *AGTR1* (rs5186) polymorphism in the *AGTR1* gene within a Mexican population, contributing to the understanding of genetic and environmental factors influencing cardiovascular risk in this population. In this context, there have been some studies in Latin America exploring the relationship between polymorphisms in the RAS genes and CVD, though the extent and focus of these studies vary across countries. For example, research in Brazil has identified associations between certain RAS gene polymorphisms and hypertension, indicating the genetic complexity of cardiovascular risk in Brazilian populations³⁷. In Argentina, studies have also examined the influence of RAS polymorphisms on cardiovascular conditions, including hypertension and heart failure³⁸. Additionally, in Chile and Colombia, research has explored the relationship between genetic variants in the RAS and susceptibility to cardiovascular diseases, albeit these studies are less common.

In Mexico, evidence specifically addressing the *AGTR1* (rs5186) polymorphism and its association with CVD remains limited. While previous studies have examined other polymorphisms within the renin-angiotensin system and their relationship with hypertension, data on the *AGTR1* rs5186 are scarce.

This study not only fills a critical gap in the existing literature but also provides valuable data specific to the Mexican population. These findings are particularly relevant given the unique genetic architecture of the Mexican population, shaped by Indigenous, European, and African ancestry components, which may influence gene expression and disease susceptibility in ways not captured in predominantly European-based studies. Accordingly, identifying genetic risk factors such as the rs5186 polymorphism may facilitate early detection and management of high-risk individuals, thereby reducing the overall burden of CVD. Furthermore, by incorporating inflammatory (serum amyloid A) and metabolic (homocysteine) biomarkers into the analysis, this study goes beyond genotype-phenotype associations to provide insight into potential biological mechanisms linking genetic variation to cardiovascular pathology.

The high prevalence of overweight, unhealthy behaviors, and physical inactivity observed in this cohort, together with the presence of the *AGTR1* (rs5186) polymorphism, highlights the potential interplay between modifiable and genetic factors in shaping CVD risk. In this context, accumulating evidence points to the relevance of systemic inflammation as a contributor to cardiovascular risk, supporting the value of incorporating inflammatory markers into comprehensive risk assessment. Accordingly, our findings suggest that genetic variation, particularly in admixed populations, may provide additional information for risk stratification; however, further studies are needed to determine its clinical utility.

Nonetheless, certain limitations should be acknowledged. No a priori power calculation was performed for the genetic association analysis; however, a post-hoc power analysis based on the observed effect size (OR = 2.51), the frequency of the dominant genotype in the reference group (46.73%), and the study sample sizes

(n = 112 CVD group, n = 214 reference group) at $\alpha = 0.05$ indicated a statistical power of 92.6%, suggesting adequate power to detect the observed association. The sample size, although adequate for detecting the observed association, remains modest in comparison to large-scale genomic studies and may limit the generalizability of the results. The CVD group included patients with heterogeneous cardiovascular phenotypes, including prior myocardial infarction, coronary angioplasty, revascularization surgery, and carotid stenosis, which may limit the biological specificity of the observed genetic association; phenotype-specific analyses were not feasible given the available sample size. The absence of ancestry-informative markers or population stratification analyses represents an additional limitation of this study. The case-control design precludes causal inference, and the lack of evaluation of additional RAS-related polymorphisms may also restrict a more comprehensive understanding of genetic contributions to CVD susceptibility.

Future research should aim to elucidate the complex interplay between genetic variants, inflammatory processes, and lifestyle determinants in order to inform more precise and culturally appropriate interventions that effectively reduce the burden of CVD in the Mexican population. Longitudinal and multi-omic approaches, incorporating broader panels of RAS-related polymorphisms and inflammatory biomarkers, would contribute to a more comprehensive understanding of genetic susceptibility to CVD in admixed Latin American populations.

Conclusion

This study identifies a significant association between the *AGTR1* (rs5186) A1166C polymorphism and increased CVD risk in an admixed Mexican population, independent of traditional risk factors. Additionally, inflammatory (serum amyloid A) and metabolic (homocysteine) biomarkers were significantly associated with CVD, reinforcing their role in cardiovascular risk. These findings highlight a potential interaction between genetic susceptibility and inflammatory pathways in the development of CVD.

The results of this study are particularly relevant to the Mexican population, which is characterized by a unique genetic architecture shaped by Indigenous, European, and African ancestry, and where evidence regarding this polymorphism remains limited. The observed associations underscore the importance of considering both genetic predispositions and lifestyle determinants in comprehensive cardiovascular risk assessment. The high prevalence of overweight, low physical activity, and harmful behaviors in the study population, together with the identified genetic susceptibility, highlights the need for multifaceted public health strategies that integrate genetic screening with targeted behavioral interventions.

Methods

Study population

A case-control study was conducted in volunteers (n = 356) from the Mexican Institute of Social Security in Nayarit, Mexico. Patients were classified according to the diagnosis of the cardiologist into three groups. CVD group (n = 118), participants with a documented diagnosis of chronic ischemic cardiovascular disease, previous myocardial infarction, coronary revascularization surgery, or coronary angioplasty. All patients in this group presented carotid stenosis $\geq 70\%$ according to ultrasound, and they were evaluated and enrolled in this study during a stable phase of chronic ischemic cardiovascular disease by a cardiologist and all were free of ischemic events during at least 6 months. HTN group (n = 119), patients with a documented diagnosis of hypertension based on either clinic SBP/DBP $\geq 140/90$ mmHg, but without CVD, diabetes, or kidney disease. Control group (n = 119), healthy subjects who attended a routine health check-up and did not have coronary artery disease or chronic illness, diagnosed by medical personnel. The groups were matched by sex and age, and all patients were invited to participate in the study and signed an informed consent. A structured questionnaire was applied to assess clinical history, lifestyle, drug consumption, and risk factors for CVD. The physical activity index was determined by a validated questionnaire reported by Baecke et al.³⁹. 81.36% from CVD subjects and 24.14% from HTN received statins; 7.63% and 7.89% respectively, received fibrates; 3.39% from CVD received cholesterol absorption inhibitors (CAI); while 61.86% from CVD and 22.12% from HTN received β -blockers; 33.05% from CVD and 25.00% from HTN consumed IACE; also 42.37% from CVD and 58.04% from HTN received Angiotensin-II Antagonist (Table 1). This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital Antonio González Guevara, Tepic Nayarit, México (registry number COMBIOET/05/13).

Clinical parameters

After 12 h of overnight fasting, intravenous blood samples were obtained in heparin, EDTA, and dry-tubes. Samples were centrifuged for 25 min at 2500 rpm, 5 °C, and serum and plasma were collected and immediately analyzed or frozen at -80 °C until analysis. Glucose, creatinine, uric acid, and serum calcium were determined in a certified laboratory (ISO 9001 F-010). Total cholesterol, triglycerides, HDL-C, ApoA1 and ApoB were measured by standardized enzymatic procedure in a Cobas 311 analyzer (Roche/Hitachi, Germany) from the National Institute of Cardiology Ignacio Chávez. LDL-C was calculated with the Friedewald formula modified by DeLong et al.⁴⁰. The accuracy and precision of the laboratory are under periodic surveillance by U.S. Center for Disease and Prevention (Atlanta, GA, USA). The coefficients of intra and inter-assay variation were 3%.

Serum amyloid A

SAA concentration was evaluated from serum by commercial ab100635 Serum Amyloid A Human Kit from Abcam, according to the manufacturer's instructions. The kit has a minimum detectable dose of < 0.5 ng/mL,

with intra-assay and inter-assay coefficients of variation of < 10% and < 12%, respectively. All samples were measured in duplicate, and the coefficient of variation across replicates did not exceed 5%.

Homocysteine

Hcys concentration was evaluated from plasma using a commercial Homocysteine ELISA kit STA-670 from Cell Biolabs, inc., according to the manufacturer's instructions. The kit has a detection sensitivity limit of 10 ng/mL and a concentration range of 0 - 40 µg/mL. All samples were measured in duplicate, and the coefficient of variation across replicates did not exceed 5%.

DNA isolation and Angiotensin II Type 1 Receptor *A1166C* genotyping

Of the 356 participants enrolled, 30 samples (8.4%) could not be successfully genotyped due to insufficient DNA yield or quality, and were therefore excluded from the genetic analyses only. Genomic DNA was extracted from blood samples using the "sucrose gradient extraction" method⁴¹ with modifications to isolate DNA from total blood samples from study groups.

500 µL of frozen blood were taken. This volume was then transferred into a falcon tube with 6.75 mL lysis buffer (sucrose 320 mM, MgCl₂ 5 mM, Triton X-100 1%, Tris-HCl 10 mM, pH 7.4). It was mixed in vortex until homogenized (30 seconds) with subsequent centrifugation at 2000 g (rcf) for 15 minutes at 4 °C and the supernatant was decanted. The pellet was resuspended in 200 µL of suspension buffer (NaCl 150 mM, EDTA 60 mM, SDS 1%, Tris-HCl 400 mM, pH 7.4) and 50 µL of sodium perchlorate 5M. The sample was then mixed by gentle rotation for 15 minutes and incubated at 65 °C for 30 minutes. 350 µL chloroform was added at -20 °C. Mixing was done by gentle rotation at room temperature for 10 minutes. Centrifugation was performed at 1400 g (rcf) for 15 minutes at 4 °C. The aqueous phase was obtained, which is transferred to a new microtube. Two volumes of cold absolute ethanol were added at 4 °C and mixed by inversion, until the formation of the pellet corresponding to DNA is observed. This pellet was washed by adding 250 µL of 70% ethanol at 4 °C with subsequent centrifugation at 1000 rpm for 10 minutes at 4 °C. The supernatant was decanted and two more washes were performed. The DNA pellet was left to dry and resuspended in 30 µL of nuclease-free sterile water.

DNA purity and concentration were determined using a Nanodrop system. Real-time PCR assays of Angiotensin II Type 1 Receptor *A1166C* (*AGTR1 A1166C*) (rs5186) exchange of adenine y cytosine (VIC/FAM) were performed using primers specific fluorescent TaqMan probes from Applied Biosystem (Foster City, CA, USA). The PCR reaction mixture contained 200 nM of the TaqMan probe, 900 nM of each primer, 1X TaqMan Universal PCR Master Mix, and 50 ng of DNA. PCR were performed using a StepOne Real-Time PCR system according to the standard protocol as follows: 50 °C for 2 minutes, 95 °C for 10 minutes, then 40 cycles at 95 °C for 15 seconds and 60 °C for 1 minute. A negative control was included in each determination. In addition, an internal positive control with a previously verified genotype was included in all reactions to confirm allelic discrimination accuracy. Allelic discrimination was performed using the StepOne ver. 2.0 software.

Statistical analysis

The normal distribution of continuous variables was assessed using the Skewness and Kurtosis test. Non-normally distributed data are presented as median with interquartile range (IQR), and frequencies as percentages. Frequency comparisons were evaluated using Chi-squared tests, while non-parametric variables were analyzed using the Kruskal-Wallis test with post hoc Dunn's test. Hardy-Weinberg equilibrium was determined using the chi-square test to assess the reliability of genotyping and the expected distribution of genotype frequencies, with a significance threshold of $p < 0.05$ to indicate deviation from equilibrium.

Firth's penalized maximum likelihood logistic regression models were performed to evaluate the association of variables with the risk of HTN and CVD, as this method provides less biased estimates in the context of modest sample sizes and quasi-complete separation⁴². We used dichotomized variables as follows: for HTN group, we compared the participants with a documented diagnosis of hypertension with the control group (healthy subjects); the CVD group was compared with a reference group composed of the control group plus the HTN group. This decision was based on the clinical definition of the CVD group, which included participants with established chronic ischemic cardiovascular disease regardless of HTN status. The clinically appropriate comparator was therefore the absence of established CVD, a condition shared by both the HTN and control groups. Potential confounding variables and predictors of inflammation indices were identified based on the existing literature. The results are expressed as OR and 95% confidence intervals (95% CI), and the models were adjusted for sex, BMI, current alcohol consumption, SAA, Hcys, total cholesterol, and cardiovascular medication use. The CVD model additionally included physical activity index and serum calcium concentration. Variables were selected a priori based on clinical relevance, biological plausibility, and evidence from prior literature, following a hierarchical framework that considered distal sociodemographic factors, intermediate behavioral variables, and proximal clinical and inflammatory markers (Supplementary Tables S1 and S2). Studies that previously evaluated the association between this polymorphism and the development of CVD have reported the adjustment for sociodemographic variables such as sex and age⁴³, BMI, harmful habits; as well as lifestyle variables, and clinical parameters including cholesterol levels, which may modify the modulation of the *A1166C* genotype, influencing its expression at the phenotypic level⁴⁴⁻⁴⁶. Furthermore, calcium, Hcys, and SAA levels are potential modulators of *AGTR1* activity, thus underscoring the importance of controlling for these potential confounding variables in order to enhance the estimates regarding the influence of the polymorphism on the development of CVD^{19,20,47}. Lastly, cardiovascular medications (β -blockers, calcium channel blockers, and IACE) were also considered as confounding variables, considering that the use of these medications has anti-inflammatory effects that could influence protein expression and regulation. Consequently, this may alter the relationship between the polymorphism and CVD and potentially dilute the phenotypic effects⁴⁸. No a priori power calculation was performed for the genetic association analysis. A post-hoc power analysis was conducted

using the CaTS Power Calculator⁴⁹ under a dominant inheritance model, based on the observed effect size, the frequency of the dominant genotype in the reference group, and the study sample sizes. Values of $p < 0.05$ were considered statistically significant. All analyses were conducted using Stata version 14 (Stata Statistical Software, Stata Corporation, College Station, Texas, USA).

Data availability

The primary datasets generated and/or analyzed during the current study are not publicly archived due to the lack of access to an appropriate public repository at this time. However, the data are available from the corresponding authors upon reasonable request.

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Author contributions

J.F.H.M., N.P.R., and I.M.M.D. contributed to the conceptualization of the study. J.F.H.M., N.P.R., K.G.M., and I.M.M.D. performed the formal analysis. J.F.H.M., N.P.R., A.E.R.G., Y.Y.B.H., and J.P.G. participated in sample collection. J.F.H.M., N.P.R., A.E.R.G., A.D.R., B.S.B.V., Y.Y.B.H., C.A.G.A., K.G.M., G.A.V., and I.M.M.D. contributed to drafting the manuscript and revising it critically for important intellectual content. J.F.H.M. and I.M.M.D. contributed to project administration. I.M.M.D. provided resources, supervision, and funding acquisition. All authors reviewed and approved the final manuscript.

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Declarations

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital Antonio González Guevara, Tepic Nayarit, México (registry number COMBIOET/05/13).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-58713-6>.

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