Research Article

The \((G>A)\) rs11573191 Polymorphism of \(PLA2G5\) Gene Is Associated with Premature Coronary Artery Disease in the Mexican Mestizo Population: The Genetics of Atherosclerotic Disease Mexican Study

Gilberto Vargas-Alarcón, 1 Carlos Posadas-Romero, 2 Teresa Villarreal-Molina, 3 Edith Alvarez-León, 1 Javier Angeles-Martínez, 1 María Elena Soto, 3 Irma Monroy-Muñoz, 1 Juan Gabriel Juárez, 2 Carlos Jerges Sánchez-Ramírez, 1 Julian Ramirez-Bello, 5 Silvestre Ramírez-Fuentes, 1 José Manuel Fragoso, 1 and José Manuel Rodríguez-Pérez 1

1 Departments of Molecular Biology, Endocrinology, and Immunology, National Institute of Cardiology Ignacio Chávez, 1 4080 Mexico City, DF, Mexico
2 Department of Endocrinology, National Institute of Cardiology Ignacio Chávez, Juan Badiano 1, Sección XVI, Tlalpan, 1 4080 Mexico City, DF, Mexico
3 Cardiovascular Genomics Laboratory, National Institute of Genomic Medicine, 14610 Mexico City, DF, Mexico
4 Department of Immunology, National Institute of Cardiology Ignacio Chávez, Juan Badiano 1, Sección XVI, Tlalpan, 1 4080 Mexico City, DF, Mexico
5 Laboratory of Genomic Medicine, Research Unit, Juárez de México Hospital, 07760 Mexico City, DF, Mexico

Correspondence should be addressed to Gilberto Vargas-Alarcón; gvargas63@yahoo.com

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Coronary artery disease (CAD) is a multifactorial disorder that results from an excessive inflammatory response. Secretory phospholipase A\(_2\)-V (sPLA\(_2\)-V) encoded by \(PLA2G5\) gene promotes diverse proinflammatory processes. The aim of the present study was to analyze if \(PLA2G5\) gene polymorphisms are associated with premature CAD. Three \(PLA2G5\) polymorphisms (rs11573187, rs2148911, and rs11573191) were analyzed in 707 patients with premature CAD and 749 healthy controls. Haplotypes were constructed after linkage disequilibrium analysis. Under dominant, recessive, and additive models, the rs11573191 polymorphism was associated with increased risk of premature CAD (OR = 1.51, \(P_{\text{dom}} = 3.5 \times 10^{-3}\); OR = 2.95, \(P_{\text{rec}} = 0.023\); OR = 1.51, \(P_{\text{add}} = 1.2 \times 10^{-3}\)). According to the informatics software, this polymorphism had a functional effect modifying the affinity of the sequence by the MZF1 transcription factor. \(PLA2G5\) polymorphisms were in linkage disequilibrium and the CGA haplotype was associated with increased risk of premature CAD (OR = 1.49, \(P = 0.0023\)) and with hypertension in these patients (OR = 1.75, \(P = 0.0072\)). Our results demonstrate the association of the \(PLA2G5\) rs11573191 polymorphism with premature CAD. In our study, it was possible to distinguish one haplotype associated with increased risk of premature CAD and hypertension.

1. Introduction

Coronary artery disease (CAD) is a complex multifactorial and polygenic disorder resulting from an excessive inflammatory response to various forms of injurious stimuli to the arterial wall [1–3]. Although the precise mechanisms responsible for the onset of the disease are still unknown, multiple genetic factors may cooperate with environmental factors to confer susceptibility to CAD. The secretory phospholipase A\(_2\) (sPLA\(_2\)) family of enzymes hydrolyzes the sn-2 ester bond of phospholipids and cell membranes, generating nonesterified free fatty acids and lysophospholipids, which may promote diverse proinflammatory processes [4].
Ten sPLA2 enzymes have been described in humans and four of them (sPLA2-IIA, sPLA2-III, sPLA2-V, and sPLA2-X) have been implicated in atherosclerosis [5–11]. Hydrolysis by sPLA2-V reduces the capacity of HDL to promote cellular cholesterol efflux from lipid-loaded macrophages [12]. Some experiments have shown that LDL hydrolyzed by sPLA2-V induces foam cell formation in mouse peritoneal macrophages [6, 7]. On the other hand, immunohistochemical analysis has shown sPLA2-V to be associated with smooth muscle cells and foam cells in the lipid cores of both human and mouse atherosclerotic lesions [13]. The sPLA2-V is encoded by the PLA2G5 gene located in chromosome lp34-36.1 [14]. Polymorphisms in this gene have been reported and some of them have been associated with LDL and oxLDL levels in a group of patients with type II diabetes mellitus [15]. These data suggest that the gene that encodes sPLA2-V could be an important candidate gene to be studied in atherosclerosis. The aim of the present study was to analyze if PLA2G5 gene polymorphisms are associated with premature coronary artery disease (CAD) in a case-control association study (GEA or genetics of atherosclerotic disease).

2. Material and Methods

The primary aim of the GEA study is to investigate genetic factors associated with premature CAD and other coronary risk factors in the Mexican population. The study complies with the Declaration of Helsinki. All participants provided written informed consent, and the study was approved by the Ethics Committees of the Instituto Nacional de Cardiología “Ignacio Chávez” and the Instituto Nacional de Medicina Genómica.

2.1. Subjects. All GEA participants are unrelated and of self-reported Mexican-Mestizo ancestry (three generations). A Mexican Mestizo is defined as someone born in Mexico, who is a descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards, of Caucasian and/or African origin, who came to America during the sixteenth century. The study included 707 patients with premature CAD and 749 healthy controls from the genetics of atherosclerotic disease (GEA) Mexican study. The selection of patients and controls of the GEA study has been described previously [16]. Demographic, clinical, anthropometric, and biochemical parameters, as well as cardiovascular risk factors, were evaluated in patients and controls.

2.2. Genetic Analysis. Genomic DNA from whole blood containing EDTA was isolated by standard techniques. The rs11573185, rs2148911, and rs11573191 single nucleotide polymorphisms (SNPs) of the PLA2G5 were genotyped using 5’ exonuclease TaqMan genotyping assays on an ABI Prism 7900HT Fast Real-Time PCR system, according to manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA).

2.3. Statistical Analysis. All calculations were performed using SPSS version 18.0 (SPSS, Chicago, IL) statistical package. Means ± SD and frequencies of baseline characteristics were calculated. Chi-square tests were used to compare frequencies and ANOVA and Student’s t-test were used to compare means. ANCOVA was used to determine associations between the polymorphisms and metabolic variables, adjusting for age, gender, and BMI, as appropriate. Logistic regression analysis was used to test for associations of polymorphisms with premature CAD under inheritance models. The most appropriate inheritance model was selected based on Akaaike information criteria and was adjusted for age, gender, and BMI. Genotype frequencies did not show deviation from Hardy-Weinberg equilibrium (HWE, \( P > 0.05 \)). Pairwise linkage disequilibrium (LD, \( D' \)) estimations between polymorphisms and haplotype reconstruction were performed with Haplovew version 4:1 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA).

2.4. Functional Prediction Analysis. We predicted the potential effect of the PLA2G5 SNPs using the TFSearch program (http://www.cbrj.jp/research/db/TFSEARCH.html).

3. Results

General characteristics of the population studied are shown in Tables 1 and 2.

3.1. Association of Polymorphisms with Premature CAD. Observed and expected frequencies in the polymorphic sites were in HWE. Similar distribution of the rs11573185 and rs2148911 polymorphisms was observed in both groups. Under dominant, recessive, and additive models adjusting for age, gender, and BMI, the rs11573191 polymorphism was associated with increased risk of premature CAD as compared to controls (OR = 1.51, 95% CI: 1.14–1.99, \( P_{\text{dom}} = 3.5 \times 10^{-3} \); OR = 2.95, 95% CI: 1.12–3.76, \( P_{\text{rec}} = 0.023 \); OR = 1.51, 95% CI: 1.17–1.94, \( P_{\text{add}} = 1.2 \times 10^{-3} \)) (Table 3). The statistical power estimated with QUANTO software (http://hydra.usc.edu/GxE/) to detect an association between premature CAD and controls was 0.88 for rs11573191.

3.2. Association of the Polymorphisms with Metabolic Parameters and Cardiovascular Risk Factors. The effect of the three polymorphisms on various metabolic parameters and cardiovascular risk factors was analyzed in premature CAD patients and controls. No associations were observed in this analysis (data is not shown).

3.3. Haplotype Analysis and Functional Effect. The three PLA2G5 polymorphisms were in strong linkage disequilibrium (\( D' > 0.95 \)) and four haplotypes were observed: AGG, CGG, CAG, and CGA. The CGA haplotype was associated with increased risk of premature CAD (OR = 1.49, 95% CI: 1.15–1.93, and \( P = 0.0023 \)) (Table 4). The effect of the haplotypes on diverse metabolic parameters and cardiovascular risk factors was analyzed in premature CAD patients and healthy controls. Only the CGA haplotype was associated with increased risk of hypertension in the group of patients.
### Table 1: Demographic characteristics of the studied population.

|                        | Controls (n = 749) | Premature CAD (n = 707) | P    |
|------------------------|-------------------|-------------------------|------|
| Age (years)            | 53.99 ± 9.82      | 53.3 ± 7.4              | 0.982|
| Gender (% male)        | 49.7              | 81.4                    | <0.0001|
| Body mass index (kg/m²)| 28.47 ± 4.44      | 28.77 ± 4.29            | 0.184|
| Obesity (%)            | 31.5              | 35.3                    | 0.067|
| Waist circumference (cm)| 94.38 ± 11.62    | 98.06 ± 11.32           | <0.0001|
| Central obesity (%)    | 79.7              | 82                      | 0.144|
| Total abdominal fat (cm²)| 438.44 ± 163.17  | 424.59 ± 166.62         | 0.110|
| Subcutaneous abdominal fat (cm²)| 181.30 ± 120.63 | 248.95 ± 110.14        | <0.0001|
| Visceral abdominal fat (cm²)| 157.09 ± 70.70 | 175.71 ± 81.70         | <0.0001|
| Visceral/subcutaneous adipose tissue ratio| 1.99 ± 1.00 | 1.54 ± 0.81 | <0.0001|
| Current smokers (%)    | 21.4              | 12.3                    | <0.0001|
| Former smokers (%)     | 36.8              | 64.4                    | <0.0001|
| Hypertension (%)       | 24.9              | 64.9                    | <0.0001|
| Hypertensive medication (%) | 11.2        | 89.9                    | <0.0001|
| Diastolic blood pressure (mmHg) | 74.53 ± 10.09 | 74.16 ± 10.25    | 0.497|
| Systolic blood pressure (mmHg) | 121.56 ± 18.83 | 121.48 ± 19.45       | 0.942|
| Heart rate (bpm)       | 65.54 ± 9.20      | 64.99 ± 11.00           | 0.301|

Data are expressed as means ± SD; log-transformed values were used for statistical analysis. P values were estimated using ANOVA for continuous variables and Pearson's Chi-square test for categorical values. CAD: coronary artery disease.

### Table 2: Comparison of biochemical parameters in individuals with premature coronary artery disease and controls.

|                        | Controls (n = 749) | Premature CAD (n = 707) | P    |
|------------------------|-------------------|-------------------------|------|
| Total cholesterol (mg/dL) | 19338 ± 36.46  | 169.31 ± 48.18          | <0.0001|
| TC > 200 mg/dL (%)       | 40.0              | 20.7                    | <0.0001|
| HDL-C (mg/dL)           | 46.61 ± 13.69     | 39.90 ± 10.82           | <0.0001|
| Hipo-a-lipoproteinemia (%) | 51.2         | 57.5                    | 0.009|
| LDL-C (mg/dL)           | 118.69 ± 32.37    | 97.22 ± 40.25           | <0.0001|
| Triglycerides (mg/dL)   | 170.47 ± 113.01   | 193.72 ± 127.26         | <0.0001|
| Hypertriglyceridemia (%)| 46.9              | 59.8                    | <0.0001|
| ApoAI (mg/dL)           | 139.50 ± 43.68    | 119.32 ± 26.74          | <0.0001|
| ApoB (mg/dL)            | 91.32 ± 28.85     | 82.71 ± 31.01           | <0.0001|
| Statin and/or fibrate treatment (%) | 4.2         | 15.4                    | <0.0001|
| Type 2 diabetes mellitus (%) | 7.6        | 35.3                    | <0.0001|
| Glucose (mg/dL)         | 100.75 ± 35.75    | 112.33 ± 44.31          | 0.001|
| HOMA-IR                 | 5.29 ± 8.38       | 6.73 ± 6.00             | <0.0001|
| Insulin (µU/mL)         | 20.32 ± 13.82     | 24.23 ± 17.26           | <0.0001|
| Metabolic syndrome (%)  | 46.3              | 46.1                    | 0.488|
| Uric acid (mg/dL)       | 5.47 ± 1.78       | 6.07 ± 2.06             | <0.0001|
| Creatinine (mg/dL)      | 0.84 ± 0.25       | 0.92 ± 0.31             | <0.0001|
| Alanine transaminase (IU/L) | 27.75 ± 12.45 | 28.07 ± 11.49          | 0.619|
| Aspartate transaminase (IU/L) | 26.84 ± 11.34 | 27.97 ± 17.77          | 0.222|
| Alkaline phosphatase (IU/L) | 83.07 ± 28.36 | 78.99 ± 25.97          | 0.004|
| Gamma-glutamyl transpeptidase (IU/L) | 35.91 ± 32.83 | 44.36 ± 44.63 | <0.0001|

Data are expressed as means ± SD; log-transformed values were used for statistical analysis. P values were estimated using ANOVA for continuous variables and Pearson's Chi-square test for categorical values. CAD: coronary artery disease.
Table 3: Association of rs11573185, rs2148911, and rs11573191 PLAC2G5 gene polymorphisms with premature CAD.

| rs11573185 | Genotype frequency (%) | MAF | Model | OR (95% CI) | P       |
|------------|------------------------|-----|-------|-------------|---------|
|            | A/A  | A/C  | C/C  |            |         |
| Control (n = 749) | 0.410 | 0.437 | 0.153 | 0.371       |         |
| Premature CAD (n = 707) | 0.395 | 0.462 | 0.142 | 0.373       |         |

| rs2148911 | Genotype frequency (%) | MAF | Model | OR (95% CI) | P       |
|-----------|------------------------|-----|-------|-------------|---------|
|            | G/G  | G/A  | A/A  |            |         |
| Control (n = 749) | 0.780 | 0.206 | 0.015 | 0.117       |         |
| Premature CAD (n = 707) | 0.782 | 0.203 | 0.014 | 0.115       |         |

| rs11573191 | Genotype frequency (%) | MAF | Model | OR (95% CI) | P       |
|------------|------------------------|-----|-------|-------------|---------|
|            | G/G  | G/A  | A/A  |            |         |
| Control (n = 749) | 0.825 | 0.166 | 0.009 | 0.092       |         |
| Premature CAD (n = 707) | 0.777 | 0.202 | 0.021 | 0.122       |         |

Associations were tested using logistic regression adjusting for age, gender, and BMI. CAD: coronary artery disease; MAF: minor allele frequency.

Table 4: Haplotype frequencies in premature CAD patients and healthy controls.

| rs11573185 | rs2148911 | rs11573191 | Frequencies | Control | Premature CAD | OR (95% CI) | P       |
|------------|-----------|------------|-------------|---------|---------------|-------------|---------|
| A          | G         | G          | Total       | 0.625   | 0.625         | 1.49 (1.15–1.93) | 0.0023  |
| C          | G         | G          | Total       | 0.151   | 0.165         | 0.86 (0.69–1.08) | 0.19    |
| C          | A         | G          | Total       | 0.115   | 0.115         | 1.2 (0.88–1.44)  | 0.36    |
| C          | G         | A          | Total       | 0.105   | 0.091         | 1.49 (1.15–1.93) | 0.0023  |

The ORs were adjusted for age, gender, medication, and BMI. The AGG haplotype was used as reference.

4. Discussion

The role of sPLA2-IIA in atherogenesis has been well studied; however, the involvement of sPLA2-V is less understood. sPLA2-V is highly expressed in the heart and is present in other tissues as well, including eye, placenta, lung, and brain [17–21]. A number of human cells, including macrophages, neutrophils, bronchial and renal tubular epithelia, subendocardial cells (cardiomyocytes), and interstitial fibroblasts of gastric submucosa, have been shown to express sPLA2-V [20, 22–26]. Recently, Ohta et al. [27] identified a unique function of sPLA2-V in activation of macrophages and in their capacity to recruit T cells to amplify the effector phase of pulmonary inflammation. However, the possible effect of the sPLA2-V in the developing of atherosclerosis is contradictory.

Enzyme deficiency in sPLA2-V-null mice leads to marked attenuation of airway inflammation [28, 29] and reduced atherosclerosis [9, 30]. It has been reported that sPLA2-V can hydrolyze phospholipids in LDL, leading to the production of proatherogenic modified LDL in vitro [7]. PLAC2G5 overexpression in bone marrow cells worsens atherosclerosis, whereas its deficiency decreases modestly the atherosclerosis [9]. In the same way, the PLAC2G5 deficiency does not affect the atherosclerotic lesion development in mice [30] and pan-sPLA2 inhibitor varespladib did not reduce the risk of cardiovascular events after acute coronary syndrome [31]. The genes that encode sPLA2-IIA and sPLA2-V molecules are linked in a negative orientation on the same chromosome [14]. Polymorphisms in both genes have been associated with variations in the lipid levels [15, 32]. In the present work, three PLAC2G5 gene polymorphisms (rs11573185, rs2148911, and rs11573191) were analyzed in order to establish their role as susceptibility markers for premature CAD, metabolic parameters, and cardiovascular risk factors. The functional prediction software used here predicted that the rs11573191 polymorphism is functional with an effect on the affinity of the sequence for the MZF1 transcriptional factor. The A allele of this polymorphism presents major affinity for the transcription factor than the G allele, having important consequences on sPLA2-V production. This result obtained with premature CAD (OR = 1.75, 95% CI: 1.17–2.60, and P = 0.0072) (data is not shown). This analysis was adjusted for age, gender, medication, and BMI.

Based on SNP functional prediction software, the rs11573191 polymorphism seems to be functional. This polymorphism modifies the binding affinity of the transcription factor MZF1, having greater affinity by the A allele. The differences in affinity could have important consequences in the expression of sPLA2-V protein.
using informatics software is in agreement with our genetic results because, in the association analysis, the rs11573191 A allele was associated with increased risk of developing premature CAD. However, our study did not include expression analysis and we have no evidence that the PLA2G5 expression is different in premature CAD patients with the risk allele. The distribution of the other two PLA2G5 polymorphisms was similar in CAD patients and healthy controls. Recently, Holmes et al. [33] using data from the Advanced Study of Aortic Pathology identified that the PLA2G5 rs525380 polymorphism was strongly associated with PLA2G5 mRNA expression levels. However, the association of this polymorphism with sPLA activity and coronary heart disease was not corroborated. This polymorphism was not included in our analysis. Wootton et al. [15] studied seven PLA2G5 polymorphisms in patients with type II diabetes mellitus to investigate the association of these polymorphisms with coronary heart disease risk factors. Of the seven SNPs, three of them (rs11573185, rs11573203, and rs11573248) showed significant association with cholesterol and LDL levels. In our study, none of the studied polymorphisms was associated with lipid levels in premature CAD or healthy controls. In the study by Wootton et al. [15], the haplotype analysis showed associations of some haplotypes with significantly higher cholesterol and LDL. In our work, the three studied polymorphisms were in linkage disequilibrium, and one of the haplotypes (CGA) was associated with risk of developing premature CAD and with hypertension in the premature CAD patients. This haplotype included the A allele associated independently with the disease. In a previous work, Mancini et al. [34], using a genome-wide association analysis in a spontaneously hypertensive rat model, identified four candidate genes for hypertension, one of them was the PLA2G5 gene. This agrees with our study, in which an association of the PLA2G5 haplotype with hypertension was detected.

Study limitations need to be addressed. This study only included the analysis of three polymorphisms of the PLA2G5 gene. Considering that this is the first work to report an association of the PLA2G5 polymorphisms with premature CAD and hypertension, replication in another group of patients is necessary. The predicted functional consequences of the rs11573191 polymorphism, using informatics tools, need experimental testing.

5. Conclusion

In summary, our study demonstrates the association of the PLA2G5 rs11573191 polymorphism with premature CAD and with hypertension in this group of patients. According to the informatics software, this polymorphism had a functional effect in modifying the affinity of the sequence by the MZF1 transcription factor. The associations reported in the present work should be explored in other populations to establish the true role of these polymorphisms in cardiovascular diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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