Genetic Variants and Haplotypes in *OPG* Gene Are Associated with Premature Coronary Artery Disease and Traditional Cardiovascular Risk Factors in Mexican Population: The GEA Study

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Basic and clinical research have demonstrated that osteoprotegerin (OPG) plays an important role in the development and progression of cardiovascular diseases. The aim of this study was to evaluate the association of four polymorphic sites (rs2073618, rs3134069, rs3134070, and rs3102735) of *OPG* gene with premature coronary artery disease (pCAD), and with cardiometabolic parameters. The polymorphisms were genotyped using 5' exonuclease TaqMan genotyping assays with real-time PCR in 1098 individuals with pCAD and 1041 healthy controls. rs2073618 polymorphism was associated with a high risk of developing pCAD according to different inheritance models: additive ($p=0.001$; odds ratio [OR]=1.283), dominant ($p=0.006$; OR=1.383), recessive ($p=0.011$; OR=1.423), and codominant 2 ($p=0.001$; OR=1.646). The four polymorphisms were associated with different cardiovascular risk factors in individuals with pCAD and controls. Our results suggest that *OPG* rs2073618 polymorphism is associated with an increased risk of pCAD. In addition, two haplotypes were associated with pCAD, one increasing the risk (*CACT*) and another one as protective (*GACC*).

Keywords: premature coronary artery disease, osteoprotegerin, vascular calcification, polymorphisms, haplotypes, genetic association

Introduction

VASCULAR CALCIFICATION (VC) is a pathological process that involves the deposition of calcium within the blood vessel wall, particularly in intimal and medial layers. This process is considered the main cause of adverse cardiovascular events, including coronary artery disease (CAD), which represents a major health problem worldwide (Wu *et al.*, 2013; Forde *et al.*, 2018; Hou *et al.*, 2020). Therefore, the awareness of coronary artery calcification (CAC) has received great interest since it participates in several stages of atherosclerotic plaque formation and it is a risk marker for CAD (Sharma *et al.*, 2010; Greenland *et al.*, 2018).

Reports suggest that calcification biomarkers are a helpful tool for early detection and for the application of preventing

measures against CAD (Faggiano *et al.*, 2019; Clemente *et al.*, 2020). Within these biomarkers, the osteoprotegerin (OPG) is considered a key molecule that exerts its function through a signaling pathway complex: OPG/receptor activator of nuclear factor- κ B ligand (RANKL)/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) that regulates the physiopathogenesis of VC (Forde *et al.*, 2018).

Several studies have identified an increased expression of OPG in atherosclerotic plaque lesions; furthermore, high serum levels of OPG have been associated with cardiovascular disease progression (Sandberg *et al.*, 2006; Hakimi *et al.*, 2013; Løgstrup *et al.*, 2013; Tousoulis *et al.*, 2013; Ovchinnikova *et al.*, 2014; Morisawa *et al.*, 2015; Siasos *et al.*, 2018).

The *OPG* gene is located on the 8q24 region and contains five exons. This gene encodes a glycoprotein of 401 amino

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acids conformed as a propeptide; when it loses a 21 amino acid fragment, it remains as a mature protein composed of 380 amino acids, which is secreted as a soluble protein (Simonet *et al.*, 1997; Yasuda *et al.*, 1998).

Considering that the genetic component has been associated with cardiovascular diseases, the identification of *OPG* gene polymorphisms associated with the presence of premature coronary artery disease (pCAD) could be useful to better understand this pathology. In addition, there are few association studies between these polymorphisms and CAD; nevertheless, the results are controversial in different populations.

Therefore, the purpose of this study was to evaluate the association of four *OPG* gene polymorphisms with the presence of pCAD and with cardiovascular risk factors.

Materials and Methods

Study participants

This research enrolled 2139 unrelated individuals: 1041 healthy controls and 1098 individuals with pCAD from the Genetics of Atherosclerotic Disease (GEA) Mexican cohort. The control group included asymptomatic individuals, apparently healthy, without family history of pCAD, recruited from blood donor centers and through brochures at social services centers in primary care.

Diagnoses of pCAD were performed through the clinical history of myocardial infarction, revascularization surgery, angioplasty, or coronary stenosis >50% on angiography. To be considered premature, the CAD had to be diagnosed before 65 years of age in women and before 55 years of age in men. Exclusion criteria in both groups were renal, thyroid, liver, or heart failure or oncological disease.

This study was approved by Investigation and Bioethics Committee of the “Instituto Nacional de Cardiología Ignacio Chávez” in Mexico City and belongs to the Genetics of Atherosclerosis Disease project, with registration number (INCICH: 18–1071). This research complies with the Helsinki Declaration. All participants signed a written informed consent.

All individuals answered a standardized questionnaire to obtain information related with their nutritional habits, alcohol consumption, smoking habits, familial medical history, and pharmacological treatments. Moreover, anthropometric, clinical, and demographic characteristics, cardiovascular risk factors, as well as metabolic parameters were assessed in all participants as previously described (Medina-Urrutia *et al.*, 2015; Posadas-Sánchez *et al.*, 2017a, 2017b).

Computed axial tomography scans of abdomen and chest were performed in the control group using a 64-channel multidetector computed tomography system (Somatom Cardiac Sensation, Germany). Control individuals with CAC, with Agatston score (Mautner *et al.* 1994) greater than zero were excluded from the analysis, because they were considered individuals with subclinical atherosclerosis (SA).

Genetic analysis

Genomic DNA was extracted from peripheral blood samples using QIAamp DNA Blood Mini Kit (250) catalog number 51106 (QIAGEN, Germany). Four polymorphic sites

were included in the study (rs2073618-ID: C__1971047_40, rs3134069-ID: C__27464534_20, rs3134070-ID: C__27466052_20, and rs3102735-ID: C__1971046_10) and were determined using 5' exonuclease TaqMan genotyping assays on an ABI Prism 7900HT Real-Time PCR equipment, according to the manufacturer's instructions (Thermo Fisher Scientific, CA). In addition, we randomly retested 10% of the samples, considering the three genotypes, and found that 100% were concordant with the results. This was done as an internal quality control.

Statistical analysis

The four polymorphic sites tested were in Hardy-Weinberg equilibrium. We used the Statistical Package for the Social Sciences (SPSS v20.0) for all analyses. Data were analyzed considering their distribution and expressed as mean (standard deviation), median (interquartile range), or frequencies. Analyses of categorical and continuous variables in the study group were evaluated with Student's *t*-test or Mann-Whitney *U* test, and chi-square test as required. The associations of polymorphisms with pCAD under inheritance models (additive, dominant, recessive, heterozygote, codominant 1, and codominant 2) were assessed using logistic regression analyses.

All inheritance models were adjusted for age, gender, body mass index (BMI), subcutaneous abdominal fat, smoking habits, type 2 diabetes mellitus (T2DM), apolipoprotein (a) concentrations, low-density lipoprotein (LDL) cholesterol, serum calcium, and serum phosphorus, as well as alkaline phosphatase, alanine transaminase, and aspartate transaminase activities. To evaluate the associations between polymorphisms and cardiovascular risk factors in healthy controls and individuals with pCAD, the genetic models were adjusted for age, gender, and BMI. A *p*-value <0.05 was considered statistically significant. Finally, the pairwise linkage disequilibrium (LD, *D'*) evaluation was made with Haploview software v4.1 (Broad Institute of Massachusetts [MIT], Cambridge, MA).

Results

Clinical, anthropometric, and metabolic parameters, and frequency of cardiovascular risk factors

The clinical and metabolic parameters obtained from the studied population are represented in (Table 1). Individuals with pCAD had higher values of BMI, glucose, waist circumference, triglycerides, and aspartate transaminase when compared to controls. Figure 1 depicts frequency of obesity, subcutaneous abdominal fat, hypoalphalipoproteinemia, aspartate transaminase >p75, and alkaline phosphatase >p75; these factors were higher in individuals with pCAD in comparison to healthy controls. The raw data are shown in the Supplementary Data.

Association of *OPG* gene polymorphisms with pCAD

The genotype distributions of rs3134069, rs3134070, and rs3102735 were similar in controls and individuals with pCAD (data not shown). On the other hand, the rs2073618 *C* allele was associated with a high risk of developing pCAD under additive ($p=0.001$; odds ratio [OR]=1.283), dominant ($p=0.006$; OR=1.383), recessive ($p=0.011$;

TABLE 1. CLINICAL AND METABOLIC CHARACTERISTICS OF THE STUDY GROUPS

Characteristics	Control (n=1041)	pCAD (n=1098)	p
Gender (% male)	42.3	81.2	<0.0001
Age (years)	51±9	54±8	<0.0001
BMI (kg/m ²)	27.8 [25.4–30.9]	28.3 [25.9–31.1]	0.002
Glucose (mg/dL)	90 [84–97]	95 [87–116]	<0.0001
Waist circumference (cm)	94±11	98±10	<0.0001
Subcutaneous abdominal fat (cm ²)	286 [218–365]	247 [193–314]	<0.0001
Alkaline phosphatase (IU/L)	81 [68–96]	76 [63–95]	<0.0001
Apolipoprotein A (mg/dL)	134 [114–156]	120 [101–138]	<0.0001
Serum phosphorus (mg/dL)	3.5±0.5	3.6±0.9	0.137
Triglycerides (mg/dL)	145 [107–202]	162 [119–220]	<0.0001
Low-density lipoprotein cholesterol (mg/dL)	115 [95–133]	91 [68–116]	<0.0001
Aspartate transaminase (IU/L)	25 [20–30]	26 [22–31]	0.002
Alanine transaminase (IU/L)	24 [18–34]	25 [19–36]	0.045
Serum calcium (mg/dL)	9.7±0.6	9.7±0.7	0.343

The data are shown as mean±standard deviation, median [interquartile range], or percentage. Student's *t*-test, Mann–Whitney U test or chi-square test.

BMI, body mass index; CAD, coronary artery disease; pCAD, premature coronary artery disease.

OR=1.423), and codominant 2 ($p=0.001$; OR=1.646) models (Fig. 2). The analysis stratified by gender showed that rs2073618 polymorphism was associated with a high risk of pCAD in women (p recessive=0.035; OR=1.668) as well as in men (p additive=0.006; OR=1.297; p dominant=0.003; OR=1.530; p codominant 1=0.009; OR=1.494;

and p codominant 2=0.012; OR=1.613) (Fig. 3). All the analyses were adjusted for age, gender, BMI, subcutaneous abdominal fat, smoking habits, T2DM, concentrations of apolipoprotein (a), LDL-cholesterol, calcium and phosphorus serum, and alkaline phosphatase, alanine transaminase, and aspartate transaminase activities.

FIG. 1. Frequency of cardiovascular risk factors in the study population. * $p \leq 0.001$, ** $p = 0.005$.

FIG. 2. Association of *OPG* gene polymorphisms with pCAD. The models were adjusted for age, gender, BMI, subcutaneous abdominal fat, smoking habits, T2DM, apolipoprotein (a) concentrations, LDL-cholesterol, serum calcium, serum phosphorus, and alkaline phosphatase, alanine transaminase, and aspartate transaminase activities. BMI, body mass index; CI, confidence interval; LDL, low-density lipoprotein; MAF, minor allele frequency; OPG, osteoprotegerin; OR, odds ratio; pCAD, premature coronary artery disease; T2DM, type 2 diabetes mellitus.

Association of *OPG* polymorphisms with metabolic abnormalities

Associations between *OPG* polymorphisms and metabolic parameters were explored independently in healthy controls and in individuals with pCAD. Table 2 shows associations observed in the control group: rs2073618 was associated with a low risk of fatty liver (p dominant=0.031; OR=0.728 and p codominant 1=0.047; OR=0.732). The

rs3134069 was associated with a high risk of T2DM (p recessive=0.015; OR=5.031 and p codominant 2=0.024; OR=4.526), low risk of insulin resistance of adipose tissue (p additive=0.045; OR=0.749), low risk of having high levels of alkaline phosphatase (p additive=0.047; OR=0.749 and p dominant=0.043; OR=0.729), and low risk of having high levels of gamma glutamyl transpeptidase (p additive=0.043; OR=0.744). rs3134070 was associated with a high risk of T2DM (p recessive=0.018; OR=4.709

FIG. 3. Association of *OPG* gene polymorphisms with pCAD analyzed by gender. The models were adjusted for age, gender, BMI, subcutaneous abdominal fat, smoking habits, T2DM, apolipoprotein (a) concentrations, LDL-cholesterol, serum calcium, serum phosphorus, and alkaline phosphatase, alanine transaminase, and aspartate transaminase activities.

TABLE 2. ASSOCIATION OF OSTEOPROTEGERIN GENE POLYMORPHISMS WITH CARDIOVASCULAR RISK FACTORS IN THE CONTROL GROUP

<i>Polymorphisms</i>	<i>Genotype frequency</i>			<i>MAF</i>	<i>Model</i>	<i>OR [95% CI]</i>	<i>p</i>
rs2073618	<i>GG</i>	<i>GC</i>	<i>CC</i>	<i>C</i>			
Fatty liver							
No (<i>n</i> =712)	0.333	0.490	0.177	0.422	Dominant	0.728 [0.546–0.971]	0.031
Yes (<i>n</i> =329)	0.405	0.442	0.153	0.374	Codominant 1	0.732 [0.539–0.995]	0.047
rs3134069	<i>AA</i>	<i>AC</i>	<i>CC</i>	<i>C</i>			
T2DM							
No (<i>n</i> =941)	0.751	0.239	0.010	0.129	Recessive	5.031 [1.363–18.57]	0.015
Yes (<i>n</i> =100)	0.810	0.150	0.040	0.115	Codominant 2	4.526 [1.222–16.76]	0.024
Insulin resistance of adipose tissue							
No (<i>n</i> =545)	0.734	0.248	0.019	0.142	Additive	0.749 [0.566–0.993]	0.045
Yes (<i>n</i> =496)	0.784	0.210	0.006	0.111			
Alkaline phosphatase >p75							
No (<i>n</i> =652)	0.737	0.250	0.014	0.139	Additive	0.749 [0.563–0.996]	0.047
Yes (<i>n</i> =389)	0.794	0.196	0.010	0.108	Dominant	0.729 [0.573–0.991]	0.043
Gama glutamyl transpeptidase >p75							
No (<i>n</i> =609)	0.739	0.245	0.017	0.139	Additive	0.744 [0.599–0.991]	0.043
Yes (<i>n</i> =432)	0.786	0.207	0.007	0.110			
rs3134070	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>T</i>			
T2DM							
No (<i>n</i> =941)	0.749	0.240	0.011	0.131	Recessive	4.709 [1.305–16.99]	0.018
Yes (<i>n</i> =100)	0.810	0.150	0.040	0.115	Codominant 2	4.234 [1.170–15.32]	0.028
Gama glutamyl transpeptidase >p75							
No (<i>n</i> =609)	0.734	0.248	0.018	0.142	Additive	0.713 [0.536–0.948]	0.020
Yes (<i>n</i> =432)	0.788	0.205	0.007	0.109	Dominant	0.716 [0.527–0.973]	0.030
Low apolipoprotein (a)							
No (<i>n</i> =694)	0.738	0.245	0.017	0.140	Additive	0.700 [0.521–0.941]	0.018
Yes (<i>n</i> =347)	0.797	0.198	0.006	0.105	Dominant	0.704 [0.513–0.967]	0.030
rs3102735	<i>TT</i>	<i>TC</i>	<i>CC</i>	<i>C</i>			
Diabetes mellitus							
No (<i>n</i> =941)	0.666	0.309	0.024	0.179	Recessive	4.407 [1.800–10.79]	0.001
Yes (<i>n</i> =100)	0.690	0.230	0.080	0.195	Codominant 2	4.030 [1.632–9.955]	0.003
Alanine aminotransferase >p75							
No (<i>n</i> =568)	0.663	0.293	0.044	0.191	Recessive	0.357 [0.141–0.904]	0.030
Yes (<i>n</i> =423)	0.679	0.308	0.013	0.157	Codominant 2	0.354 [0.139–0.901]	0.029
Alkaline phosphatase >p75							
No (<i>n</i> =652)	0.644	0.320	0.035	0.196	Additive	0.750 [0.586–0.959]	0.022
Yes (<i>n</i> =389)	0.714	0.265	0.021	0.153	Dominant	0.731 [0.544–0.964]	0.026
					Codominant 1	0.747 [0.562–0.994]	0.046

The models were adjusted for age, gender, and BMI.

CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; T2DM, type 2 diabetes mellitus.

and *p* codominant 2=0.028; OR=4.234), low risk of having high levels of gamma glutamyl transpeptidase (*p* additive=0.020; OR=0.713 and *p* dominant=0.030; OR=0.716), and low risk of having low apolipoprotein (a) levels (*p* additive=0.018; OR=0.700 and *p* dominant=0.030; OR=0.704). The rs3102735 was associated with a high risk of T2DM (*p* recessive=0.001; OR=4.407 and *p* codominant 2=0.003; OR=4.030), low risk of having high levels of alanine aminotransferase (*p* recessive=0.030; OR=0.357 and *p* codominant 2=0.029; OR=0.354), and low risk of having high levels of alkaline phosphatase (*p* additive=0.022; OR=0.750; *p* dominant=0.026; OR=0.731; and *p* codominant 1=0.046; OR=0.747).

On the other hand, (Table 3) depicts associations between polymorphisms and cardiovascular risk factors in individuals with pCAD: rs2073618 were associated with high levels

of interleukin-1 β (IL-1 β) (*p* additive=0.008; OR=1.371; *p* recessive=0.005; OR=1.706; and *p* codominant 2=0.007; OR=1.870). The rs3134069 was associated with a high risk of insulin resistance of adipose tissue (*p* dominant=0.031; OR=1.412; *p* heterozygote=0.014; OR=1.500; and *p* codominant 1=0.016; OR=1.489) and high levels of IL-1 β (*p* recessive=0.030; OR=2.895 and *p* codominant 2=0.028; OR=2.945). rs3134070 was associated with a risk of insulin resistance of adipose tissue (*p* heterozygote=0.021; OR=1.465 and *p* codominant 1=0.026; OR=1.446).

Finally, rs3102735 was associated with a high risk of insulin resistance of adipose tissue (*p* dominant=0.010; OR=1.469; *p* heterozygote=0.002; OR=1.604; and *p* codominant 1=0.003; OR=1.583) and high levels of IL-1 β (*p* recessive=0.042; OR=2.261 and *p* codominant 2=0.045; OR=2.248); this polymorphism was also associated with a

TABLE 3. ASSOCIATION OF OSTEOPROTEGERIN GENE POLYMORPHISMS WITH CARDIOVASCULAR RISK FACTORS IN PREMATURE CORONARY ARTERY DISEASE

Polymorphism	Genotype frequency				MAF	Model	OR [95% CI]	p
rs2073618	GG	GC	CC	C				
IL-1 β >p75						Additive	1.371 [1.086–1.731]	0.008
No (n=903)	0.319	0.476	0.204	0.340		Recessive	1.706 [1.174–2.481]	0.005
Yes (n=195)	0.253	0.441	0.306	0.526		Codominant 2	1.870 [1.186–2.949]	0.007
rs3134069	AA	AC	CC	C				
Insulin resistance of adipose tissue						Dominant	1.412 [1.033–1.932]	0.031
No (n=484)	0.784	0.194	0.022	0.119		Heterozygote	1.500 [1.087–2.071]	0.014
Yes (n=614)	0.725	0.261	0.013	0.144		Codominant 1	1.489 [1.078–2.058]	0.016
IL-1 β >p75								
No (n=903)	0.759	0.226	0.015	0.128		Recessive	2.895 [1.107–7.569]	0.030
Yes (n=195)	0.724	0.235	0.041	0.159		Codominant 2	2.945 [1.121–7.737]	0.028
rs3134070	CC	CT	TT	T				
Insulin resistance of adipose tissue								
No (n=484)	0.784	0.192	0.024	0.120		Heterozygote	1.465 [1.060–2.025]	0.021
Yes (n=614)	0.729	0.259	0.011	0.141		Codominant 1	1.446 [1.045–2.000]	0.026
rs3102735	TT	TC	CC	C				
Insulin resistance of adipose tissue						Dominant	1.469 [1.095–1.969]	0.010
No (n=484)	0.734	0.233	0.034	0.150		Heterozygote	1.604 [1.184–2.173]	0.002
Yes (n=614)	0.661	0.316	0.023	0.181		Codominant 1	1.583 [1.167–2.147]	0.003
Non-HDL cholesterol >160 mg/dL						Additive	0.656 [0.482–0.894]	0.008
No (n=882)	0.678	0.285	0.037	0.179		Dominant	0.630 [0.444–0.893]	0.009
Yes (n=216)	0.769	0.213	0.019	0.125		Heterozygote	0.677 [0.472–0.971]	0.034
						Codominant 1	0.657 [0.458–0.943]	0.023
IL-1 β >p75								
No (n=903)	0.699	0.275	0.027	0.164		Recessive	2.261 [1.031–4.956]	0.042
Yes (n=195)	0.682	0.259	0.059	0.188		Codominant 2	2.248 [1.018–4.963]	0.045

The models were adjusted for age, gender, and BMI.
HDL, high-density lipoprotein; IL-1 β , interleukin-1 β .

FIG. 4. Association of *OPG* haplotypes with PCAD. The order of polymorphisms in the haplotypes analysis follows the position that they occupy in the chromosome (rs2073618, rs3134069, rs3134070, and rs3102735). NS, not significant.

low risk of having non-high-density lipoprotein (HDL) cholesterol >160 mg/dL (p additive=0.008; OR=0.656; p dominant=0.009; OR=0.630; p heterozygote=0.034; OR=0.677; and p codominant=0.023; OR=0.657). All inheritance models were adjusted for age, gender, and BMI.

Haplotype analysis of OPG polymorphisms

The four polymorphic sites we studied showed linkage disequilibrium. Four haplotypes were constructed; two of them were associated with pCAD. One haplotype was associated with an increased risk of pCAD (*CACT*; p =0.006; OR=1.203), while another haplotype was associated with a decreased risk of pCAD (*GACC*; p =0.021; OR=0.710). When the haplotype analysis was made separately in women and men, we observed that one haplotype was associated with a high risk of pCAD in men (*CACT*; p =0.020; OR=1.236) (Fig. 4).

Discussion

Evidence has shown that VC is regulated by complex processes and it is associated with adverse cardiovascular events (Leopold, 2015). Currently, OPG is considered a marker of calcification and it is involved in vascular and bone remodeling (Kosmopoulos *et al.*, 2019). Thus, we studied four polymorphisms (rs2073618, rs3134069, rs3134070, and rs3102735) of *OPG* gene, in 2139 subjects (1041 healthy controls and 1098 individuals with pCAD) to investigate the association between *OPG* polymorphisms and the susceptibility to develop pCAD in Mexican Mestizo population. In addition, we analyzed the association of these polymorphisms with cardiovascular risk factors. A different distribution of rs2073618 polymorphism was observed in individuals with pCAD when compared to healthy controls.

The high frequency of the *C* allele of this genetic variant suggests that it could be a susceptibility marker for pCAD in Mexican population. Increased serum levels of OPG have been positively associated with cardiovascular diseases and VC (Nitta *et al.*, 2004; Ziegler *et al.*, 2005; Ciccone *et al.*, 2013; Tousoulis *et al.*, 2013). In addition, the presence of calcified areas is associated with CAD (Makarović *et al.*, 2015); however, the role of polymorphism rs2073618 as a genetic marker is controversial in different diseases, with negative and positive data.

For instance, an association was reported in diabetic retinopathy (Mankoč Ramuš *et al.*, 2013), bone mineral density, osteoporosis fractures (Sheng *et al.*, 2017), diabetic foot (Mrozikiewicz-Rakowska *et al.*, 2018), and osteonecrosis of the femoral head (Chen *et al.*, 2019), whereas no association was observed in rheumatoid arthritis (Yang *et al.*, 2019), osteoporosis (Wu *et al.*, 2019), and periodontitis (Petean *et al.*, 2019). It is important to notice that this polymorphism is located in exon 1 of the gene and produces a substitution of lysine for asparagine, which has an effect on the signal peptide necessary for the OPG to be secreted from the cell (Zhao *et al.*, 2005). Probably, this could explain the association of this polymorphism with the risk of pCAD.

Luo and Xie reported an association of rs2073618 polymorphism with an increased risk of acute coronary syndrome in Han population (Luo and Xie, 2012). In the same way, a recent meta-analysis reported an association of this polymorphism with an increased risk of CAD (Jia *et al.*, 2017). These studies agree with our results. On the contrary, Lin *et al.* (2019) reported that rs2073618 polymorphism was not associated with CAD in Han Chinese population.

Similarly, other *OPG* polymorphisms (rs3102735, and rs3134069) were not associated with CAD in Korean individuals (Rhee *et al.*, 2006). Various situations could explain the different associations observed between *OPG* polymorphisms and CAD: (1) the studies by Yang *et al.*, Wu *et al.*, and Petean *et al.* did not include ischemic patients; (2) the study by Lin *et al.*, only included 536 individuals with CAD and 86 with peripheral artery disease; and (3) finally, these studies were performed in populations with different genetic backgrounds than Mexican Mestizo (Lisker *et al.*, 1996; Juárez-Cedillo *et al.*, 2008).

On the other hand, in the haplotype analysis, we detected two haplotypes, one of risk (*CACT*) to develop CAD and another one of protection (*GACC*) against CAD. When the analysis was made separately in women and men, the *CACT* haplotype remained significantly associated with CAD in men only. These results agree with those reported by Soufi *et al.* (2004) who observed a risk haplotype (that included the rs2073618 polymorphism) for CAD in Caucasian men.

In individuals with pCAD, rs2073618, rs3134069, and rs3102735 were associated with increased levels of IL-1 β , cytokine that has been considered a biomarker related with atherosclerosis (Polonskaya *et al.*, 2017; Wang and Wan, 2019), while rs3134069, rs3134070, and rs3102735 were associated with an increased risk of insulin resistance of adipose tissue, a metabolic parameter associated with CAD (Fraysn and Coppack, 1992; Ferrara *et al.*, 2019). In addition, rs3102735 was associated with a low risk of having increased levels of non-HDL-cholesterol; this finding is apparently contradictory, but it is important to consider that our group of pCAD patients were under pharmacological treatment, which could be affecting the lipid profile in these individuals (Brea *et al.*, 2019; Masson *et al.*, 2019).

In the control group, rs2073618 polymorphism was associated with a low risk of fatty liver. Recent reports have shown that fatty liver is associated with a higher prevalence of atherosclerotic cardiovascular diseases (Sinn *et al.*, 2017; Sao and Aronow, 2018; Wójcik-Cichy *et al.*, 2018; Chang *et al.*, 2019). On the other hand, rs3134069 and rs3102735 were significantly associated with a low risk of having high serum levels of alkaline phosphatase. Several studies have demonstrated that high alkaline phosphatase levels are associated with an increased risk of cardiovascular disease and CAC (Oh *et al.*, 2017; Panh *et al.*, 2017). rs3134070 polymorphism has been associated with low serum levels of apolipoprotein (a), a molecule considered a risk factor for atherosclerosis (Ehrlich and Ehrlich, 1990; Ooi *et al.*, 2018; Tmoyan *et al.*, 2018; Sabbah *et al.*, 2019).

Furthermore, rs3134069, rs3134070, and rs3102735 have been associated with decreased levels of gamma glutamyl transpeptidase and alanine aminotransferase, and high levels of liver enzymes have been considered a risk factor for developing cardiovascular disease and even as a cause of cardiovascular mortality (Kelishadi *et al.*, 2018; Ndrepepa *et al.*, 2018; Chung *et al.*, 2019; Ma *et al.*, 2019; Rahmani *et al.*, 2019; Jeon *et al.*, 2020). On the other hand, we observed that rs3134069, rs3134070, and rs3102735 were associated with an increased risk of T2DM; this outcome was opposite to what we expected. However, the individuals in our control group were not under pharmacological treatment, which could explain this result.

In addition, we performed a bioinformatics analysis using MatInspector software (Cartharius *et al.*, 2005). This

analysis demonstrated that rs2073618 modifies a DNA binding site for FOXH1 and XBP1 transcriptional factors. In particular, FOXH1 is involved in signaling pathways of the transforming growth factor beta (TGF- β) and it is related to developmental biology in vertebrates. Some studies have suggested that TGF- β is associated with the progression of atherosclerosis and several implications in heart function (Uluçay *et al.*, 2015). On the other hand, XBP1 participates in the proliferation of endothelial cells induced by the vascular endothelial growth factor, as well as in the formation of blood vessels during embryonic development. This factor also participates in angiogenesis in adult tissues in ischemic conditions (Zeng *et al.*, 2009, 2013).

It is important to mention that the transversal design of our study could be a limitation. However, this study also has interesting strengths: (1) we enrolled a large cohort of individuals with pCAD and control subjects and (2) individuals with SA (CAC score > zero) evaluated with computed tomography were excluded from the control group.

Conclusion

In summary, our findings indicate that rs2073618 polymorphism of *OPG* gene is associated with a high risk of developing pCAD. In addition, we detected two haplotypes associated with coronary disease in the whole population, and one risk haplotype associated just in men. Finally, all polymorphic sites showed association with different cardiovascular risk factors in both, individuals with pCAD and healthy controls.

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No competing financial interests exist.

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Supplementary Material

Supplementary Data

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