IL-24 Gene Polymorphisms Are Associated with Cardiometabolic Parameters and Cardiovascular Risk Factors But Not with Premature Coronary Artery Disease: The Genetics of Atherosclerotic Disease Mexican Study

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Coronary artery disease (CAD) is a multifactorial and polygenic disorder that results from an excessive inflammatory response. We analyzed whether interleukin-24 (*IL-24*) gene polymorphisms are associated with premature CAD in a case-control association study. Four polymorphisms (rs1150253, rs1150256, rs1150258, and rs3762344) of the *IL-24* gene were analyzed by 5' exonuclease TaqMan genotyping assays in a group of 952 patients with premature CAD, 284 individuals with subclinical atherosclerosis (SA), and 912 controls. The studied polymorphisms were not associated with the risk of premature CAD or SA ($P > 0.05$). Under dominant models adjusted for age, sex, body mass index, and medication, the polymorphisms were associated with cardiometabolic parameters and cardiovascular risk factors. Three polymorphisms (rs1150253, rs1150256, and rs3762344) were associated with hypertension and increased levels of systolic blood pressure in controls. In SA, 2 polymorphisms (rs1150256 and rs3762344) were associated with type 2 diabetes mellitus, gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase, whereas rs1150253 was associated with GGT and type 2 diabetes mellitus and rs1150258 with GGT and alkaline phosphatase. In premature CAD, the 4 polymorphisms were associated with total cholesterol > 200 mg/dL, low-density lipoprotein cholesterol (LDL-C), and GGT, whereas rs1150256 was associated also with ApoA. On the other hand, rs1150258 was associated with ApoA, LDL-C > 100 mg/dL, and apoB/apoA ratio, and rs3762344 with ApoA, apoB/apoA ratio, LDL-C > 100 mg/dL, and total cholesterol. On the basis of single-nucleotide polymorphism functional prediction software, rs1150253 and rs1150258 polymorphisms seem to be functional. The 4 studied polymorphisms were in linkage disequilibrium and had a similar haplotype distribution in patients and controls. Our study demonstrates the association of *IL-24* polymorphisms with metabolic and cardiovascular risk factors in individuals with premature CAD, SA, and controls.

Introduction

ATHEROSCLEROSIS, THE MAIN CAUSE of morbidity and mortality in industrialized societies, is a complex disease with both genetic and environmental causes (Leeper and others 2012). A growing body of evidence implies that atherosclerosis can be considered an inflammatory disease (McPherson and Davies 2012; Raman and others 2013). Inflammation is recognized as a major contributor to athero-

genesis through adverse effects on lipoprotein metabolism and arterial wall biology (Hansson 2005). Infiltrates of activated macrophages and T cells are prominent in both human and murine atherosclerotic lesions (Chinetti-Gbaguidi and Staels 2011; Westerterp and others 2013). Foam cell macrophages are generally thought to play a major role in the pathology of the disease (Glass 2001). Activated macrophages secrete cytokines and chemokines that directly amplify the local immune response. Increased

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expression of several chemokines and cytokines in human and animal atherosclerotic lesions has been reported (Wolfs and others 2011; Di Taranto and others 2012; Tuttolomondo and others 2012; Salem and others 2013).

Interleukin-24 (IL-24) is a member of the IL-10 family of cytokines, and it signals through 2 heterodimeric receptors: IL-20R1/IL-20R2 and IL-22R1/IL-20R2. Its gene has been located in chromosome 1, within a 195-kb cytokine cluster containing 5 genes, *IL-10*, *IL-19*, *IL-20*, *IL-22*, and *IL-24* in linear order (Huang and others 2001). IL-24 can induce expression of other cytokines, such as TNF- α , IL-6, and interferon- γ , suggesting that IL-24 may be a member of a complex cascade of cytokines involved in inflammation. IL-24 was recently shown to be able to inhibit angiogenesis by endothelial cells in a receptor-dependent manner (Ramesh and others 2003). Lee and others (2012) reported that IL-24 inhibits β -glycerophosphate-induced calcification of vascular smooth muscle cells by inhibiting apoptosis, suggesting a novel mechanism of action of IL-24 in cardiovascular disease. Recently, Lee and others (2013) showed that exogenous administration of IL-24 attenuated the expression of vascular inflammation and hypertension-related genes induced by H₂O₂ treatment in mouse vascular smooth muscle cells, suggesting that IL-24 could be a therapeutic target for hypertension and cardiovascular diseases. These data suggest that the gene encoding IL-24 could be an important candidate gene to study in atherosclerosis. The aim of the present study was to analyze if *IL-24* gene polymorphisms are associated with premature coronary artery disease (CAD) in a case-control association study (genetics of atherosclerotic disease, GEA).

Subjects and Methods

The primary aim of the GEA study was to investigate genetic factors associated with premature CAD, subclinical atherosclerosis (SA), and other coronary risk factors in the Mexican population. All participants provided written informed consent, and the Ethics Committees of the Instituto

Nacional de Cardiología Ignacio Chávez and Instituto Nacional de Medicina Genómica approved the study.

Subjects

All GEA participants were unrelated and of self-reported Mexican Mestizo ancestry (3 generations). A Mexican Mestizo was 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 XVI century. The study included 952 patients with premature CAD, 284 individuals with SA, and 912 healthy controls from the GEA Mexican Study. Selection of patients and controls of the GEA study has been described previously (Villarreal-Molina and others 2012). Demographic, clinical, anthropometric, and biochemical parameters and cardiovascular risk factors were evaluated in patients and controls.

Genetic analysis

Genomic DNA from whole blood containing EDTA was isolated by standard techniques. The (C>T) rs1150253, (C>T) rs1150256, (T>C) rs1150258, and (G>A) rs3762344 single-nucleotide polymorphisms (SNPs) 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).

Statistical analysis

All calculations were performed using SPSS version 18.0 (SPSS, Chicago, IL) statistical package. Means $\pm$ 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, sex, body mass index (BMI), and total cholesterol (TC)

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF THE POPULATION

	Control (n=912)	SA (n=284)	Premature CAD (n=952)	P ^a
Age (years)	51.88 $\pm$ 8.89	58.62 $\pm$ 8.41	53.43 $\pm$ 7.58	<0.0001
Sex (% male)	38.0	72.2	82.9	<0.0001
Body-mass index (kg/m ²)	28.37 $\pm$ 4.47	28.94 $\pm$ 4.55	28.738 $\pm$ 4.80	0.098
Obesity (%)	31.0	35.2	36.9	<0.0001
Waist circumference (cm)	93.84 $\pm$ 11.70	97.69 $\pm$ 11.18	98.71 $\pm$ 11.09	<0.0001
Central obesity (%)	62.4	56.7	83.8	<0.0001
Total abdominal fat (cm ²)	448.81 $\pm$ 145.95	469.65 $\pm$ 159.50	444.83 $\pm$ 145.20	0.045
Subcutaneous abdominal fat (cm ²)	300.06 $\pm$ 113.59	280.49 $\pm$ 119.38	264.92 $\pm$ 103.79	<0.0001
Visceral abdominal fat (cm ²)	148.81 $\pm$ 63.22	188.86 $\pm$ 68.54	180.29 $\pm$ 72.48	<0.0001
Visceral/subcutaneous adipose tissue ratio	0.55 $\pm$ 0.31	0.75 $\pm$ 0.33	0.74 $\pm$ 0.34	<0.0001
Current smokers (%)	22.4	21.5	12.5	<0.0001
Former smokers (%)	29.6	45.4	64.6	<0.0001
Hypertension (%)	17.8	38.0	66.9	<0.0001
Hypertensive medication (%)	14.8	28.5	66.4	<0.0001
Diastolic blood pressure (mm Hg)	116.08 $\pm$ 16.42	128.39 $\pm$ 20.01	119.50 $\pm$ 18.56	<0.0001
Systolic blood pressure (mm Hg)	71.60 $\pm$ 9.20	77.59 $\pm$ 10.55	72.95 $\pm$ 10.02	<0.0001
Heart rate (bpm)	66.05 $\pm$ 9.23	66.45 $\pm$ 10.28	63.61 $\pm$ 12.29	<0.0001

Data are expressed as means $\pm$ SD. Log-transformed values were used for statistical analysis.

^aP values were computed using ANOVA for continuous variables and Pearson's chi-square test for categorical values.

CAD, coronary artery disease; SA, subclinical atherosclerosis.

TABLE 2. COMPARISON OF BIOCHEMICAL PARAMETERS IN INDIVIDUALS WITH PREMATURE CAD, SA, AND HEALTHY CONTROLS

	Control (n=912)	SA (n=284)	Premature CAD (n=952)	P ^a
TC (mg/dL)	192.20 ± 36.65	197.59 ± 36.76	168.19 ± 47.50	<0.0001
TC >200 mg/dL (%)	37.0	46.1	21.6	<0.0001
HDL-C (mg/dL)	48.50 ± 14.14	44.29 ± 11.67	40.12 ± 10.45	<0.0001
Hypo- α -lipoproteinemia (%)	48.1	50.4	56.4	0.001
LDL-C (mg/dL)	116.74 ± 31.89	123.36 ± 30.69	97.02 ± 39.07	<0.0001
LDL-C > 100 mg/dL (%)	29.7	41.5	17.0	<0.0001
Triglycerides (mg/dL)	168.45 ± 109.07	182.78 ± 105.69	193.09 ± 123.09	<0.0001
Hypertriglyceridemia (%)	46.5	54.9	58.6	<0.0001
apoA (mg/dL)	139.07 ± 37.37	138.40 ± 36.48	84.31 ± 30.87	<0.0001
apoB (mg/dL)	93.40 ± 27.14	98.14 ± 27.61	120.67 ± 26.18	<0.0001
apoB/apoA	0.71 ± 0.25	0.74 ± 0.24	0.72 ± 0.28	0.186
Metabolic syndrome (%)	39.9	58.1	29.4	<0.0001
Type 2 diabetes mellitus (%)	8.6	6.3	35.9	<0.0001
Glucose mg/dL	97.92 ± 30.65	101.44 ± 33.72	112.68 ± 44.65	<0.0001
HOMA-IR	5.04 ± 7.77	4.89 ± 3.08	6.63 ± 5.77	<0.0001
Aspartate transaminase (IU/L)	27.54 v 11.67	28.18 ± 13.81	27.97 ± 11.10	0.627
Alanine transaminase (IU/L)	28.30 ± 18.31	30.08 ± 21.50	29.50 ± 18.02	0.240
Alkaline phosphatase (IU/L)	82.67 ± 24.99	84.59 ± 29.98	80.63 ± 25.43	0.049
Gamma-glutamyl transpeptidase (IU/L)	36.74 ± 39.25	38.91 ± 34.83	44.47 ± 41.38	<0.0001
Creatinine	0.82 ± 0.19	0.91 ± 0.19	0.97 ± 0.21	<0.0001
Uric acid	5.41 ± 1.46	5.99 ± 1.51	6.44 ± 1.55	<0.0001

Data are expressed as means ± SD. Log-transformed values were used for statistical analysis.

^aP values were estimated using ANOVA for continuous variables and Pearson's chi-square test for categorical values.

HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment–insulin resistance; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

TABLE 3. ASSOCIATION OF THE rs1150253 (C>T), rs1150256 (C>T), rs1150258 (T>C), AND rs3762344 (G>A) POLYMORPHISMS WITH PREMATURE CAD AND SA

rs1150253	CC	CT	TT	MAF	OR	95% CI	P
Control (n=912)	0.29	0.491	0.219	0.465			
SA (n=284)	0.261	0.542	0.197	0.428	0.99	0.80–1.23 ^a	0.93
Premature CAD (n=952)	0.291	0.512	0.198	0.453	0.95 1.04	0.82–1.10 ^a 0.85–1.27 ^b	0.49 0.72
rs1150256	CC	CT	TT	MAF	OR	95% CI	P
Control (n=912)	0.280	0.493	0.223	0.470			
SA (n=284)	0.272	0.534	0.194	0.461	0.93	0.75–1.15 ^a	0.50
Premature CAD (n=952)	0.286	0.509	0.205	0.460	0.96 0.97	0.82–1.11 ^a 0.79–1.18 ^b	0.55 0.76
rs1150258	TT	TC	CC	MAF	OR	95% CI	P
Control (n=912)	0.286	0.497	0.218	0.466			
SA (n=284)	0.272	0.534	0.194	0.461	0.96	0.78–1.20 ^a	0.73
Premature CAD (n=952)	0.290	0.506	0.204	0.457	0.96 0.99	0.83–1.11 ^a 0.81–1.21 ^b	0.59 0.91
rs3762344	GG	GA	AA	MAF	OR	95% CI	P
Control (n=912)	0.286	0.488	0.226	0.470			
SA (n=284)	0.272	0.526	0.201	0.465	0.95	0.77–1.18 ^a	0.64
Premature CAD (n=952)	0.296	0.486	0.218	0.461	0.96 0.99	0.83–1.11 ^a 0.82–1.21 ^b	0.57 0.94

Associations were tested using logistic regression adjusted for age, sex, BMI, and TC levels. Only the dominant model is showed.

^aCompared to controls.

^bCompared to individuals with SA.

BMI, body-mass index; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio.

levels, 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 Akaike information criteria and was adjusted for age, sex, and BMI. Genotype frequencies did not show deviation from Hardy–Weinberg equilibrium ($P > 0.05$). Pairwise linkage disequilibrium (LD, D') estimations between polymorphisms and haplotype reconstruction were performed with Haploview version 4:1 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA).

Functional prediction analysis

We predicted the potential effect of the *IL-24* SNPs using bioinformatics tools, including FastSNP (Yuan and others 2006), SNP Function Prediction (<http://snpinfo.niehs.nih.gov/snpfunc.htm>), Human-transcriptome Database for Alternative Splicing (www.h-invitational.jp/h-dbas/), Splice Port: An Interactive Splice Site Analysis Tool (www.spliceport.cs.umd.edu/SplicingAnalyser2.html), ESE finder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese_finder.cgi), HSF (www.umd.be/HSF/), and SNPs3D (www.snps3d.org/).

Results

General characteristics of the population are shown in Tables 1 and 2. Because 284 (23.7%) of the apparently healthy individuals recruited as controls showed a positive coronary artery calcification (CAC) score, 3 independent groups were considered for the analysis: controls (CAC score = 0), SA (CAC score > 0), and premature CAD.

Association of polymorphisms with premature CAD and SA

Observed and expected frequencies in the polymorphic sites were in Hardy–Weinberg equilibrium. The distribution of the studied polymorphisms was similar in patients with premature CAD, individuals with SA, and healthy controls in all the models analyzed (Table 3). In this case, the models were adjusted for age, sex, BMI, and TC.

Association of the polymorphisms with metabolic cardiovascular risk factors and metabolic parameters

The effect of the *IL-24* polymorphisms on various metabolic cardiovascular risk factors and metabolic parameters was explored separately in controls (CAC score = 0), SA (CAC score > 0), and premature CAD. Under dominant models, adjusted for age, sex, BMI, and medication, the polymorphisms were associated with several cardiometabolic parameters and cardiovascular risk factors. Three polymorphisms were associated with hypertension and increased levels of systolic blood pressure in healthy controls ($P = 0.026$ and $P = 0.001$ for rs1150253, $P = 0.001$ and $P < 0.001$ for rs1150256, $P = 0.027$ and $P = 0.001$ for rs3762344) (Table 4). In SA individuals, 2 polymorphisms (rs1150256 and rs3762344) were associated with type 2 diabetes mellitus (T2DM; $P = 0.033$ and $P = 0.026$), gamma-glutamyl transpeptidase (GGT; $P = 0.018$ and $P = 0.009$), and alkaline phosphatase (ALP; $P = 0.012$ and $P = 0.028$),

TABLE 4. ASSOCIATION OF THE *IL-24* POLYMORPHISMS WITH METABOLIC PARAMETERS AND CARDIOVASCULAR RISK FACTORS IN CONTROLS

SNP	Parameter	Model	P
rs1150253	HT (%)	Dominant	0.026
	Systolic blood pressure (mm Hg)	Dominant	0.001
rs1150256	HT (%)	Dominant	0.001
	Systolic blood pressure (mm Hg)	Dominant	<0.001
rs1150258	HT (%)	Dominant	0.892
	Systolic blood pressure (mm Hg)	Dominant	0.399
rs3762344	HT (%)	Dominant	0.027
	Systolic blood pressure (mm Hg)	Dominant	0.001
	Diastolic blood pressure (mm Hg)	Dominant	0.007

All associations were tested using logistic regression adjusted for age, sex, BMI, and medication when appropriate.

HT, hypertension; SNP, single-nucleotide polymorphism.

whereas rs1150253 was associated with T2DM ($P = 0.045$) and GGT ($P = 0.013$), and rs1150258 was associated with GGT ($P = 0.013$) and ALP ($P = 0.019$) (Table 5). In premature CAD patients, rs1150253 was associated with TC > 200 mg/dL ($P = 0.014$), low-density lipoprotein cholesterol (LDL-C; $P = 0.035$) and GGT ($P = 0.028$); rs1150256 was associated with TC > 200 mg/dL ($P = 0.019$), LDL-C ($P = 0.039$), GGT ($P = 0.039$), and ApoA ($P = 0.045$); rs1150258 was associated with TC > 200 mg/dL ($P = 0.030$), LDL-C ($P = 0.033$), LDL-C > 100 mg/dL ($P = 0.022$), ApoA ($P = 0.035$), apoB/apoA ratio ($P = 0.028$), and GGT

TABLE 5. ASSOCIATION OF THE *IL-24* POLYMORPHISMS WITH METABOLIC PARAMETERS AND CARDIOVASCULAR RISK FACTORS IN INDIVIDUALS WITH SUBCLINICAL ATHEROSCLEROSIS

SNP	Parameter	Model	P
rs1150253	Type 2 diabetes (%)	Dominant	0.045
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.013
rs1150256	Type 2 diabetes (%)	Dominant	0.033
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.018
	Alkaline phosphatase (UI/L)	Dominant	0.012
rs1150258	Type 2 diabetes (%)	Dominant	0.202
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.013
	Alkaline phosphatase (UI/L)	Dominant	0.019
rs3762344	Type 2 diabetes (%)	Dominant	0.026
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.009
	Alkaline phosphatase (UI/L)	Dominant	0.028

All associations were tested using logistic regression adjusted for age, sex, BMI, and medication when appropriate.

TABLE 6. ASSOCIATION OF THE *IL-24* POLYMORPHISMS WITH METABOLIC PARAMETERS AND CARDIOVASCULAR RISK FACTORS IN PREMATURE CAD PATIENTS

SNP	Parameter	Model	P
rs1150253	TC >200 mg/dL (%)	Dominant	0.014
	LDL-C (mg/dL)	Dominant	0.035
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.028
rs1150256	TC >200 mg/dL (%)	Dominant	0.019
	LDL-C (mg/dL)	Dominant	0.039
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.039
rs1150258	apoA (mg/dL)	Dominant	0.045
	TC >200 mg/dL (%)	Dominant	0.030
	LDL-C (mg/dL)	Dominant	0.033
	LDL-C >100 mg/dL (%)	Dominant	0.022
	apoA (mg/dL)	Dominant	0.035
	apoB/apoA	Dominant	0.028
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.037
rs3762344	TC (mg/dL)	Dominant	0.022
	TC >200 mg/dL (%)	Dominant	0.004
	LDL-C (mg/dL)	Dominant	0.015
	LDL-C >100 mg/dL (%)	Dominant	0.008
	apoA (mg/dL)	Dominant	0.010
	apoB/apoA	Dominant	0.020
	Gamma-glutamyl transpeptidase (IU/L)	Dominant	0.028

All associations were tested using logistic regression adjusted for age, sex, BMI, and medication when appropriate.

($P=0.037$); rs3762344 was associated with TC ($P=0.022$), TC >200 mg/dL ($P=0.004$), LDL-C ($P=0.015$), LDL-C >100 mg/dL ($P=0.008$), ApoA ($P=0.010$), apoB/apoA ratio ($P=0.020$), and GGT ($P=0.028$) (Table 6).

To establish if the detected associations in the independent groups were present more broadly, the association of the polymorphisms with metabolic parameters and cardiovascular risk factors was analyzed in the whole group of individuals studied. In this analysis, under a dominant model, rs1150253 and rs3762344 polymorphisms were associated with central obesity ($P=0.004$ and $P=0.005$, respectively) (data not shown).

Haplotype analysis and SNP function prediction

The 4 *IL-24* polymorphisms were in strong linkage disequilibrium ($D'>0.9$ and $r^2>0.85$). None of the haplotypes was associated with premature CAD, SA, or metabolic parameters and cardiovascular risk factors (data not shown).

On the basis of SNP functional prediction software (<http://snpinform.nih.gov/snpfunc.htm>), the rs1150253 and rs1150258 polymorphisms seem to be functional. For rs1150253, the presence of the T allele produced a DNA binding site for the transcription factors GATA1, GATA2, and GATA3 with possible consequences in the expression of *IL-24*. On the other hand, the rs1150258 polymorphism located in exon 5 produced an amino acid change (histidine → tyrosine). The FastSNP indicated that this change

affects the structure of the protein and consequently could affect the function of the molecule.

Discussion

IL-24 is an important cytokine in the inflammatory process because it can induce expression of other cytokines, such as TNF- α , *IL-6*, and interferon- γ (Wang and Liang 2005). *IL-24* belongs to the *IL-10* family, which includes *IL-19*, *IL-20*, and *IL-22*. Some studies suggest a role of *IL-24* in cardiovascular diseases (Ramesh and others 2003; Lee and others 2012; Lee and others 2013). However, no association studies of *IL-24* gene polymorphisms and cardiovascular disease has been reported yet. In the present work, 4 *IL-24* gene polymorphisms (rs1150253, rs1150256, rs1150258, and rs3762344) were analyzed to establish their role as susceptibility markers for premature CAD. No association with premature CAD or SA was observed in our study; however, the 4 polymorphisms were associated with some cardiovascular risk factors and metabolic parameters in premature CAD, SA, and healthy controls. In the healthy controls, the polymorphisms were associated with hypertension, mainly with high levels of systolic blood pressure. We have no physiological explanation for this differential association; however, it has been reported that systolic pressure is a better predictor of cardiovascular risk than diastolic pressure (Williams and others 2008; Zanchetti and others 2009). Association of cytokine polymorphisms with hypertension has been reported (Li 2012; Li and others 2012; Park and others 2013); however, our study is the first to report an association with *IL-24* polymorphisms. In SA, *IL-24* polymorphisms were associated with GGT, ALP, and T2DM. A recent GWAS identified loci influencing concentrations of liver enzymes in plasma, including loci related with inflammation and immunity (STAT4, MAPK10, CD276, and HPR); however, no association with *IL-10* family genes was detected in that study (Chambers and others 2011). Lee and others (2003 and 2004) reported that GGT is an independent predictor of T2DM in 2 studies. These studies are in line with our data on the association of *IL-24* polymorphisms with diabetes in individuals with SA. On the other hand, some studies reported that GGT is an independent predictor for future cardiovascular mortality and all-cause mortality and that it is associated with metabolic syndrome (Du and others 2013). In our study, GGT levels were associated also with *IL-24* polymorphisms in patients with premature CAD. In this group of patients, *IL-24* polymorphisms were associated also with variations in lipid levels principally TC and LDL-C. Some studies have reported an association of cytokine polymorphisms with lipid levels (Valladares-Salgado and others 2010; Fabris and others 2012; Manica-Cattani and others 2012). A recent GWAS meta-analysis identified 95 loci associated with circulating lipid levels (Teslovich and others 2010). Some polymorphisms in genes located in the same chromosome as *IL-24* (chromosome 1) were associated with variations in triglycerides, LDL and high-density lipoprotein levels. These variants were evaluated to establish their role in the risk of developing myocardial infarction (Song and others 2013). Only rs4149313 located in *ABCA1* was associated with the risk of developing myocardial infarction in this study. In our study, the *IL-24* polymorphisms associated with lipid levels in premature CAD were not associated with clinical or sub-clinical disease.

In the human immune system, certain stimuli promote secretion of IL-24 by peripheral blood mononuclear cells, preferably monocytes and T and B cells (Caudell and others 2002; Wolk and others 2002). In addition, IL-24 induces secretion of proinflammatory cytokines (INF- γ , IL-6, and TNF- γ) by human peripheral blood mononuclear cells, along with lower levels of IL-1, IL-12, and GM-CSF, favoring a TH1-type immune response (Caudell and others 2002). The functional prediction software used here predicted that rs1150253 and rs1150258 *IL-24* polymorphisms are functional. For rs1150253, the presence of the T allele produced a DNA binding site for the transcription factors GATA1, GATA2, and GATA3. On the other hand, the rs1150258 polymorphism located on exon 5 produced an amino acid change (histidine $\rightarrow$ tyrosine). The FastSNP indicates that this change affects the structure of the protein. These 2 polymorphisms could have functional effects by increasing the production of IL-24 with the consequent increase of proinflammatory cytokines. The increased inflammation could have an effect on the metabolic parameters and cardiovascular risk factors. The results obtained using the informatics software agree with the genetic results because, in our study, the rs1150253 and rs1150258 polymorphisms were associated with metabolic parameters and cardiovascular risk factors in the 3 studied groups.

Study limitations need to be addressed. This study included only the analysis of 4 polymorphisms of *IL-24*. Since this is the first work to report an association of *IL-24* polymorphisms with metabolic and cardiovascular risk factors, replication in another group of patients is necessary. The associations detected in the group of individuals with SA should be taken with caution given the size of the analyzed sample.

In our study, *IL-24* polymorphisms were in strong linkage disequilibrium; however, none of the haplotypes was associated with premature CAD, SA, or metabolic parameters and cardiovascular risk factors. Crawford and others (2004) reported that the haplotype architecture of candidate genes across the human genome is complex and demonstrated that a large amount of sequence variation has not been described yet. Considering this information, we believe that without the full knowledge of the complete genetic variation within the *IL-24* gene or of the structure of linkage disequilibrium in the studied region, the lack of association of *IL-24* haplotypes observed in our study should be interpreted with caution.

In summary, our study demonstrates the association of *IL-24* polymorphisms with several metabolic and cardiovascular risk factors in individuals with premature CAD, SA, and healthy controls. According to the informatics software, the rs1150253 and rs1150258 polymorphisms had a functional effect, producing DNA binding sites for some transcriptional factors. These 2 polymorphisms could be used as risk factors for hypertension, liver injury enzymes, diabetes, and increased levels of lipids in the Mexican population. The Mexican population has a characteristic genetic background and important differences in regard to other populations (Lisker and others 1986, 1988, 1990; Juárez-Cedillo and others 2008). Because of these genetic characteristics of the Mexican population, we considered that the association of *IL-24* polymorphisms with metabolic parameters and cardiovascular risk factors, detected in our study, should be explored in other populations.

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Author Disclosure Statement

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