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miR-196a2 (rs11614913) polymorphism is associated with coronary artery disease, but not with in-stent coronary restenosis

José Manuel Fragoso[1] · Julian Ramírez-Bello[2] · Marco Antonio Martínez-Ríos[3] · Marco Antonio Peña-Duque[3] · Rosalinda Posadas-Sánchez[4] · Hilda Delgadillo-Rodríguez[3] · Mayra Jiménez-Morales[2] · Carlos Posadas-Romero[4] · Gilberto Vargas-Alarcón[1]

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Abstract

Objective The aim of the study was to evaluate the association of *miRNA-146a G/C* (rs2910164), and *miRNA-196a2 C/T* (rs11614913) polymorphisms with the presence of coronary artery disease (CAD) and/or restenosis in patients with coronary stent.

Materials and methods The polymorphisms were determined in 218 patients with CAD who underwent coronary artery stenting (66 with restenosis and 152 without restenosis) and 611 healthy controls using 5′ exonuclease TaqMan assays.

Results The distribution of both polymorphisms was similar in patients with and without restenosis. However, when the whole group of patients (with and without restenosis) was compared to healthy controls, under co-dominant, dominant and additive genetic models, the *T* allele of the *miRNA-196a2 C/T* (rs11614913) polymorphism was associated with increased risk of CAD (OR = 2.18, $P_{co-dom} = 0.006$, OR = 1.86, $P_{dom} = 0.002$, and OR = 1.52, $P_{add} = 0.002$, respectively). All models were adjusted for age, type 2 diabetes mellitus, dyslipidemia, hypertension and smoking habit. The "*GT*" haplotype was associated with increased risk of developing CAD (OR = 1.36, $P = 0.046$).

Conclusions Our data suggests that the *T* allele of the *miRNA-196a2 C/T* (rs11614913) polymorphism is associated with the risk of developing CAD, but no association with restenosis was observed.

Keywords Coronary artery disease · Coronary stenting · MicroRNA · Polymorphism · Restenosis

Introduction

It is well-known that coronary artery disease (CAD) is a complex, multifactorial disease, influenced by genetic and environmental factors that remain the leading cause of death worldwide. Currently, the coronary artery bypass grafting, percutaneous transluminal coronary angioplasty (PTCA), and intracoronary stent are the principal treatments for this disease. However, an important number of patients treated with these procedures develop restenosis (12–32%) [1–4]. The restenosis is the response of the arterial walls to the implantation of the stent and includes two main processes, one is the neointimal hyperplasia that involves the smooth muscle migration/proliferation, extracellular matrix deposition, and vessel remodeling and the other is the vessel remodeling [5]. Some biological processes such as cell proliferation, differentiation, and apoptosis have been associated with the presence and function of microRNAs (miRNAs) [6]. The miRNAs modulate the expression of genes

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José Manuel Fragoso and Julian Ramírez-Bello are equal and the order of authorship is arbitrary.

✉ Gilberto Vargas-Alarcón
gvargas63@yahoo.com

[1] Department of Molecular Biology, Instituto Nacional de Cardiología Ignacio Chávez, Juan Badiano 1, Sección XVI, Del. Tlalpan, 14080 Mexico City, Mexico

[2] Endocrine and Metabolic Diseases Research Unit, Hospital Juárez de Mexico, Mexico City, Mexico

[3] Interventional Cardiology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

[4] Department of Endocrinology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

and in consequence participate in the regulation of several cellular events which are involved in vascular dysfunction, ischemic angiogenesis, and vascular restenosis [7, 8]. Thus, they are considered specific biomarkers for cardiovascular diseases such as myocardial infarction, atherosclerosis, coronary artery disease, heart failure, atrial fibrillation, and fibrosis [9–12]. The role of the miRNAs in restenosis has been reported in several studies [13–15]. The miRNAs are non-coding, small RNAs (~22-nucleotide) that regulate gene expression at a post-transcriptional level by translational repression or degradation of messenger RNAs (mRNAs), thus affecting a variety of cell processes [16, 17]. Recent studies have shown that the single nucleotide polymorphisms (SNPs) in *miRNA* genes may affect the function of their respective miRNAs. Three common variants: *miRNA-146a G/C, miRNA-196a2 C/T*, and *miRNA-499 A/G* (rs2910164, rs11614913, and rs3746444, respectively) have been associated with the development of several inflammatory diseases such as pulmonary hypertension, gastric cancer, acute coronary syndrome, CAD, ischemic stroke, colorectal cancer, type 2 diabetes mellitus (T2DM), non-Hodgkin lymphoma, and lung cancer [18–25]. Some studies, including three meta-analysis, have reported association between the *miRNA-146a C/G* (rs2910164) SNP and CAD [21, 26–30]. Regarding the *miR-196a2 C/T* (rs11614913) polymorphism, some studies have evaluated its role in CAD [20, 26, 27, 31, 32], however, none of them showed an association with this disease. Given that the studies have been carried out mainly in Asians, it is necessary to perform the analysis of these variants in other populations with different genetic background. Therefore, the aim of our study was to evaluate the association of the *miRNA-146a C/G* (rs2910164), and *miRNA-196a2 T/C* (rs11614913) polymorphisms with the presence of CAD or restenosis in patients who were treated with a coronary stent.

Materials and methods

Patients and controls

The study included 218 Mexican Mestizo patients with symptomatic CAD who underwent coronary stent implantation at our institution during the period between October 2008 and October 2015 and went to follow-up coronary angiography because of symptoms of ischemia documented in a myocardial perfusion-imaging test. Basal and procedure coronary angiographies were analyzed to establish the angiographic predictors of restenosis, and in the same way, follow-up angiography after 6 months was performed to screen for binary restenosis. Restenosis was defined as a > 50% stenosis at follow-up (50% reduction in the luminal diameter of the stenosis compared with the coronary angiography findings immediately following angioplasty). With this criterion, 66 patients presented restenosis and 152 did not presented restenosis. As a comparison group, we included 611 healthy individuals with neither symptoms nor previous diagnosis of cardiovascular or systemic disease recruited of blood donors at our institute. All subjects included in the study were Mexican Mestizos, defined as individuals who, for three generations including their own, have been born in Mexico. The Institutional Ethics and Research Committees approved the study, and all subjects signed informed consent.

Genetic analysis

DNA extraction was performed from blood peripheral agree to the proposed method by Lahiri and Nurnberger [33]. The genotyping of the *miRNA-146a C/G* (rs2910164), and *miRNA-196a2 T/C* (rs11614913) polymorphisms were performed using 5′ exonuclease TaqMan assays according to manufacturer's instructions (Applied Biosystems, Foster City, USA). As positive controls we included samples previously sequenced of the different genotypes.

Functional prediction analysis

To establish the functional effect of the studied polymorphisms, we used the programs FastSNP (https://omictools.com/fastsnp-tool), and SNP Function Prediction (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) [34, 35]. The programs analyze the location of the SNP and its possible functional effects such as amino acid changes in protein structure, transcription factor binding sites in promoter or intronic enhancer regions, and alternative splicing regulation by disrupting exonic splicing enhancers or silencers.

Statistical analysis

The comparison of continuous variables between control and CAD groups was made with the Mann–Whitney *U* test. Categorical variables were analyzed using the Chi-square or Fisher's exact tests. Logistic regression analysis, using the co-dominant, dominant, recessive, over-dominant, and additive models was used to evaluate the association of the polymorphisms with restenosis and CAD. The inheritance models were adjusted by age, hypertension, dyslipidemia, T2DM, and smoking habit. The analysis of data was performed with SPSS version 18.0 (SPSS, Chicago, IL, USA) statistical package. Genotype frequencies did not deviate from Hardy–Weinberg equilibrium in any case (HWE, $P > 0.05$). Linkage disequilibrium and haplotype analysis were performed with Haploview version 4.1 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA). The statistical power to

detect association with CAD was 0.80, and was estimated with QUANTO software (http://biostats.usc.edu/Quanto.html).

Results

Characteristics of the study population

The clinical and angiographic data of the CAD patients are presented in Table 1. There were no significant differences between patients with or without restenosis with regard to age, gender, T2DM, hypertension, dyslipidemia, smoking habit, unstable angine, statin therapy, and diameter and stent length. Nonetheless, the patients who underwent coronary bare-metal stent (BSM) implantation developed more restenosis (71%) than those patients who underwent drug-eluting stent (DES) implantation (29%) ($P < 0.001$). In addition, patients with stable angina developed less restenosis ($P = 0.01$). There were significant differences between patients with CAD and healthy controls with regard to T2DM, hypertension, and dyslipidemia, smoking habit and age (Table 2).

Allele and genotype frequencies

Allele and genotype distribution of the *miRNA-146a G/C* (rs2910164), and *miRNA-196a2 C/T* (rs11614913) polymorphisms was similar in patients with and without restenosis (data no shown). Nonetheless, the analysis made comparing the whole group of patients (with or without restenosis) and healthy controls, showed an increased frequency of the "*T*" allele of the *miRNA-196a2 C/T* (rs11614913) polymorphism in CAD patients (43%) when compared to healthy controls (37%). In this case, we estimate the risk according to five models (co-dominant, dominant, recessive, over-dominant and additive), adjusted by cardiovascular risk factors (Table 3). Under co-dominant, dominant, and additive models, the *miRNA-196a2 C/T* (rs11614913) polymorphism was associated with increased risk of CAD (OR = 2.18, 95% CI 1.23–3.86, $P_{\text{co–dom}} = 0.006$, OR = 1.86, 95% CI 1.24–2.79, $P_{\text{dom}} = 0.002$, and OR = 1.52, 95% CI 1.16–2.01,

Table 1 Clinical and angiographic characteristics of the CAD patients with and without restenosis

	With Restenosis [*n* (%)][a]	Without Restenosis [*n* (%)][a]	*P*
Men	51 (77)	119 (78)	NS
Hypertension	42 (64)	83 (55)	NS
Type II diabetes mellitus	31 (47)	57 (38)	NS
Hypercholesterolemia	36 (55)	92 (61)	NS
Smoking habit	41 (62)	93 (61)	NS
Unstable angina	25 (38)	41 (27)	NS
Stable angina	6 (9)	34 (22)	0.01
Statin therapy	53 (80)	130 (86)	NS
DES	19 (29)	95 (63)	<001
BSM	47 (71)	57 (38)	<001
Diameter smaller 2.5 mm	20 (30)	30 (20)	NS
Stent length (mm)	28 (42)	67 (44)	NS
	Median (percentile 25–75)	Median (percentile 25–75)	
Age (years)	60.2 (54–67)	59.0 (53–652)	NS

BMS bare metal stent, *DES* drug-eluting stent, *NS* not significant

[a][*n* (%)] number and proportion of subjects with the clinical and angiographic characteristic in both groups

Table 2 Baseline clinical characteristics of the studied individuals (patients with CAD and healthy controls)

Clinical characteristics	CAD patients [*n* (%)][a] (*n* = 218)	Healthy controls [*n* (%)][a] (*n* = 611)	*P* value
Men	170 (79)	463 (76)	
Hypertension	125 (57)	139 (23)	<0.001
Type II diabetes mellitus	88 (40)	83 (14)	<0.001
Dyslipidemia	128 (59)	216 (35)	<0.001
Smoking habit	134 (61)	137 (22)	<0.001
	Median (percentile 25–75)	Median (percentile 25–75)	
Age (years)	59.4 (53–66)	54 (49–59)	0.01

[a][*n* (%)] number and proportion of subjects with the clinical characteristic in both groups

Table 3 Distribution of *miRNA-146a G/C* (rs2910164), and *miRNA-196a2 C/T* (rs11614913) polymorphisms in CAD patients and healthy controls

	n (genotype frequency)			MAF	Model	OR (95% CI)	*P*
miRNA-146a							
rs2910164							
Controls	*GG*	*GC*	*CC*				
(*n* = 595)	277 (0.465)	267 (0.449)	51 (0.086)	0.31	Co-dominant	0.94 (0.46–1.91)	0.20
					Dominant	0.73 (0.50–1.07)	0.11
					Recessive	1.11 (0.56–2.20)	0.77
CAD	116 (0.532)	85 (0.390)	17 (0.078)	0.27	Over-dominant	0.70 (0.48–1.04)	0.07
(*n* = 218)					Additive	0.84 (0.62–1.14)	0.25
miRNA-196a2							
rs11614913							
Controls	*CC*	*CT*	*TT*				
(*n* = 588)	235 (0.400)	266 (0.452)	87 (0.148)	0.37	Co-dominant	2.18 (1.23–3.86)	**0.006**
					Dominant	1.86 (1.24–2.79)	**0.002**
					Recessive	1.57 (0.94–2.62)	0.08
CAD	67 (0.307)	113 (0.518)	38 (0.174)	0.43	Over-dominant	1.40 (0.96–2.04)	0.08
(*n* = 218)					Additive	1.52 (1.16–2.01)	**0.002**

The *P* values were calculated from logistic regression analysis and the ORs were adjusted for age, T2DM, hypertension, dyslipidemia, and smoking habit. Bold numbers indicate significant associations

CAD coronary artery disease, *MAF* minor allele frequency, *OR* odds ratio, *CI* confidence interval

P_{add} = 0.002, respectively). All models were adjusted by age, T2DM, dyslipidemia, hypertension and smoking habit. On the other hand, the haplotype analysis revealed that the *GT* haplotype conformed by *miRNA-146a G/C* (rs2910164), and *miRNA-196a2 C/T* (rs11614913) polymorphisms was associated with increased risk for development CAD when compared to healthy controls (OR = 1.36, 95% CI 0.92–1.51, *P* = 0.046) (Table 4).

Discussion

The present study conducted in Mexican population explored the association of the *miRNA-146a G/C* (rs2910164), and *miRNA-196a2 C/T* (rs11614913) polymorphisms with the risk of developing restenosis or CAD. Interestingly, the *TT* and *TC* + *TT* genotypes of *miRNA-196a-2 C/T* (rs11614913) were found to be associated with increased risk of CAD. Zhou et al., studied three polymorphisms (*miRNA-146a G/C*, *miRNA-196a2 C/T*, and *miRNA-499 A/G*) and reported the association of the *T* allele of the *miRNA-196a2* (rs11614913) and the *G* allele of the *miRNA-499 A/G* (rs3746444) polymorphisms with increased risk for developing dilated cardiomyopathy (OR = 1.73, OR = 1.79, respectively) in Chinese population [36]. In addition, Buraczynska et al., reported that the presence of the *T* allele of the *miRNA-196a2 C/T* (rs11614913) polymorphism was associated with increased risk of developing cardiovascular disease in Caucasian patients with T2DM (OR = 1.75) [23]. Xu et al., determined that the *CC* genotype of *miRNA-196a2 T/C* (rs11614913) polymorphism is associated with the risk of developing congenital heart disease (OR = 1.51) in Chinese population [37], however, in our study, we identified an association of the *TT* and *TC* + *TT* genotypes with the risk of developing CAD. This discrepancy may be due to the following: The congenital heart disease includes abnormalities in the heart structure and occur before birth [38], while, CAD is a disease in which the arteries that supply blood to the heart

Table 4 Frequencies (%) of haplotypes of the *miRNA-146a C/G* (rs2910164), and *miRNA-196a2 T/C* (rs11614913) polymorphisms in CAD patients and healthy controls

rs2910164	rs11614913	CAD	Controls	OR	95% CI	*P* value
Haplotype		Hf	Hf			
G	*C*	0.409	0.439	0.88	0.64–1.20	0.43
G	*T*	0.317	0.251	1.36	0.92–1.51	**0.046**
C	*C*	0.157	0.187	0.90	0.83–1.43	0.60
C	*T*	0.115	0.123	0.99	0.67–1.48	0.98

Bold numbers indicate significant associations

Hf haplotype frequency, *CAD* coronary artery disease, *CI* confidence intervals

muscle harden and narrow due to the progressive deposition of lipids and fibrous matrix in the arterial wall [39]. Thus, although both diseases are cardiovascular, the first affects heart structures before birth, mean while, CAD is a progressive chronic disease that occurs in the course of the life. On the other hand, several studies have failed to identify association of this polymorphism with CAD [20, 26, 27, 31, 32]. Although Sung et al. identified an association between the *miRNA-196a2 C/T* (rs11614913) SNP and protection against CAD in a population from the South Korea, after the OR was adjusted, the association was lost [31]. In addition, a meta-analysis carried out by Zhou et al. did not identify association between this variant and CAD [27]. However, our results showed association of this polymorphism with increased risk of CAD. Thus, our study is the first to identify association of this variant with susceptibility to developing CAD (but not to restenosis). Considering the previously mentioned reports, the association of the *miRNA-196a2 C/T* (rs11614913) polymorphism with several cardiovascular diseases in different populations is controversial. This could be due to the allelic distribution of this polymorphism varies depending of the ethnic origin of the population studied. Data obtained from the National Center for Biotechnology Information, showed that Mexican Mestizos, Colombians, and individuals from Los Angeles with Mexican ancestry, present a high frequency of the *miRNA-196a2 C/T* (rs11614913) *C* allele (62%, 65%, and 57%, respectively), and low frequency of the *T* allele (38%, 35%, 43%, respectively). Similar distribution of the *T* allele was observed in Spaniards and Italians (37% and 35%, respectively). Contrary, Asian populations present a high frequency of the *T* allele (58%) (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). The detection of specific alleles in SNPs of miRNAs and is application as biomarkers in CAD could help to resolve the ethnic discrepancies observed in the different association studies performed.

To do a functional approach, we performed an informatics analysis of the *miRNA-196a2 C/T* (rs11614913) polymorphism. In spite that this analysis did not shown a functional effect of the miRNA-196a2 *T/C* (rs11614913) polymorphism, it has been suggested that this miRNA may target TRAF6, TLR5 and NFKB1 among others [40], which are involved in inflammation and in consequence in cardiovascular diseases [41]. In addition, Xu et al., [37] showed that the miRNA-196a2 *T/C* (rs11614913) polymorphism affect the expression of the gene HOXB8. In this work, the authors hypothesized that miRNA-196a2 *T/C* (rs11614913) SNP might influence miRNA-196a2-HOXB8-Shh signaling and thus be associated with congenital heart disease susceptibility. Another study has shown that *miRNA-196a2 T/C* (rs11614913) is associated with regulation of annexin A1 (ANXA1), molecule associated with decreased TNF-alpha levels [42]. Thus, would be informative to measure some inflammatory markers, as well as, inflammatory enzymes (inducible COX-2 or phospholipase A2) that can be affected by Annexin in our samples.

Some limitation of the study should be pointed out: first, the number of individuals with CAD is reduced principally when they are divided into patients with and without restenosis. Considering this limitation, studies in other populations and in a higher number of individuals could help to define the effect of the *miRNAs* polymorphisms as susceptibility markers for restenosis and/or CAD. As can be seen, in our work, a high percentage of the studied population is male, however, there is no difference in male distribution in CAD patients and healthy controls. Moreover, only two polymorphisms were analyzed. In spite of these limitations, our study contributes with a new argument in which the *miRNA-196a2 C/T* (rs11614913) polymorphism may have a role as a risk factor for developing CAD.

In summary, this study demonstrates that *miRNA-196a2 C/T* (rs11614913) polymorphism is associated with increased risk of developing CAD, but not with restenosis after coronary stenting in Mexican population.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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