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CLINICAL RESEARCH

The *MHC2TA* gene polymorphisms are not associated with restenosis after coronary stenting in Mexican patients

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Genetic susceptibility;
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Abstract

Objective: The aim of this study was to test for association between *MHC2TA* gene polymorphisms and risk for restenosis after coronary stent placement in a group of Mexican patients.

Methods: The *MHC2TA-168A>G* (rs3087456), *1614C>G* (rs4774), and *2536G>A* (rs2229320) single nucleotide polymorphisms were genotyped using 5′ exonuclease TaqMan genotyping assays in a group of 202 patients, who underwent coronary artery stenting. Basal and procedure coronary angiography were analyzed, looking for angiographic predictors of restenosis and follow-up angiography was performed to screen for binary restenosis.

Results: The results obtained in this study showed that the frequency of the three polymorphisms studied was similar in patients with and without restenosis. Univariate analysis showed that the use of drug-eluting stent (DES) reduces the risk of developing restenosis ($p < 0.001$, OR = 0.26). In contrast, the diameter < 2.5 mm of the stent and bifurcations increased the risk of developing restenosis ($p = 0.049$, OR = 1.74 and $p = 0.041$, OR = 1.8).

Conclusion: The present study suggests that the *MHC2TA* polymorphisms are not involved in the risk of developing restenosis after coronary stent placement.

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PALABRAS CLAVE
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Los polimorfismos del gen *MHC2TA* no se asocian con la reestenosis después del implante de *stent* coronario en pacientes mexicanos

Resumen
Objetivo: El propósito de este estudio fue evaluar la asociación de los polimorfismos del gen MHC2TA y el riesgo de desarrollar reestenosis, después del implante de stent coronario en un grupo de pacientes mexicanos.
Métodos: Los polimorfismos de un solo nucleótido MHC2TA-168A>G (rs3087456), 1614C>G (rs4774) y 2536G>A (rs2229320), se determinaron en un grupo de 202 pacientes tratados con stent coronario. Los polimorfismos fueron evaluados utilizando ensayos de genotipificacion Taq-Man 5′ exonucleasa. El procedimiento basal y la búsqueda de predictores de reestenosis fueron analizados por medio de angiografía coronaria, y seguimiento angiográfico con el fin de detectar reestenosis binaria.
Resultados: Los resultados obtenidos en este estudio mostraron que la distribución génica de los tres polimorfismos estudiados fue muy similar, en pacientes con o sin reestenosis. Sin embargo, el análisis univariado mostró que el uso de los stent medicados reducen el riesgo de desarrollar reestenosis ($p < 0.001$, OR = 0.26). En contraste, con las bifurcaciones y el diámetro < 2.5 mm del stent que se incrementa el riesgo de desarrollar reestenosis ($p = 0.049$, OR = 1.74 y $p = 0.041$, OR = 1.8).
Conclusión: El presente estudio sugiere que los polimorfismos del gen MHC2TA no están asociados con el riesgo de desarrollar reestenosis, después del implante de stent coronario.

Introduction

In the 20th century, coronary artery disease (CAD) was the major cause of morbidity and mortality in the world, and World Health Organization (WHO) statistics demonstrate that this trend will continue well into the future.[1] The invasive treatment strategies are coronary artery bypass grafting, percutaneous transluminal coronary angioplasty (PTCA), and intracoronary stent. However, after PTCA, restenosis occurs in about 30% to 32% of patients and after intracoronary stent placement in 12% to 32% of patients.[2–5]

The restenosis is the arterial wall's healing response to mechanical injury and comprises two main processes-neointimal hyperplasia (i.e., smooth muscle migration/proliferation, extracellular matrix deposition) and vessel remodeling.[6] Immediately after coronary stenting, thrombus formation and acute inflammation occur, followed by neointimal hyperplasia.[7–9]

The major histocompatibility complex class II transactivator (*MHC2TA*) is considered a candidate gene in the inflammatory process regulation. The *MHC2TA* is a transcriptional co-activator, it is the key intermediate responsible for INF-γ inducible and is required for expression of MHC class II and other genes related to antigen presentation in antigen-presenting cells.[10–13] On the other hand, the *MHC2TA* is a master regulator of MHC class II transcription and has an important role in the activation of several genes[14–19] that play a role in the processes-neointimal hyperplasia and arterial remodeling after coronary stent placement. The *MHC2TA* gene is located in the 16p13 region.[10] This gene presents three polymorphisms *(-168* A>G, *1614 C>G*, and *2536 G>A*) that have been associated with low and abnormal expression of the *MHC2TA* in lupus erythomatosus, rheumatoid arthritis, multiple sclerosis, myocardial infarction, metabolic syndrome, and atherosclerosis.[20–23]

Considering the prominent role of the *MHC2TA* as regulator of several genes, this study was based on the assumption that the *MHC2TA* gene polymorphisms have a measurable influence in the arterial remodeling after coronary stent placement and contribute to or reduce the occurrence of restenosis. The objective of this study was to establish the role of the *MHC2TA* gene polymorphisms in the risk of developing restenosis after coronary stent placement in a group of Mexicans patients.

Methods

The study included 202 Mexican mestizo patients with symptomatic coronary artery disease who underwent coronary stent implantation at our institutions 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 for angiographic predictors of restenosis, and follow-up angiography was performed to screen for binary restenosis. Using a > 50% stenosis at follow-up (50% reduction in the luminal diameter of the stenosis compared with the coronary angiography findings immediately following angioplasty) as the criterion to define restenosis, we selected 79 patients with restenosis and 123 without restenosis. All subjects were ethnically matched, and we considered as Mexican Mestizo only those who for two generations, including their own, had been born in Mexico. A Mexican Mestizo is defined as someone born in Mexico who is descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards of Caucasian and/or black origin, who came to American during the 16th century. The Institutional Ethics and Research Committee approved the study, and all subjects signed informed consent.

Table 1 Baseline clinical characteristics of the studied individuals.

	With restenosis (n = 79)	Without restenosis (n = 123)	*p*
Age years Mean ± standard deviation	59.9 (±10.7)	58.6 (±10.03)	NS
Male n (%)	67 (84.8)	91 (74)	NS
Hypertension n (%)	44 (55.7)	66 (53.7)	NS
Diabetes n (%)	32 (40.5)	40 (32.5)	NS
Elevated cholesterol n (%)	42 (53.2)	73 (59.3)	NS
Smoker n (%)	46 (58.2)	80 (65)	NS
Statin Therapy (%)	68 (86.1)	109 (88.6)	NS
Stable angina n (%)	8 (10.1)	27 (22)	0.036
STEMI n (%)	31 (39.2)	63 (51.2)	NS
ACS n (%)	61 (77.2)	84 (68.3)	NS

NS: not significant (data are proportions or mean ± standard deviation); *p*: *p* value; STEMI: acute myocardial infarction; ACS: acute coronary syndrome; n: number.

DNA extraction

Genomic DNA from whole blood containing EDTA was extracted by standard techniques.[24]

Determination of the MHC2TA genotype

The *MHC2TA-168 A>G* (rs3087456), *1614 C>G* (rs4774), and *2536 G>A* (rs2229320) single nucleotide polymorphisms were genotyped using 5′ exonuclease TaqMan genotyping assays on an ABI Prism 7900 HT Fast Real time PCR System, according to the manufacturer's instructions (Applied Biosystems®, Foster City, CA, USA).

Statistical analysis

Gene frequencies of *MHC2TA* polymorphisms in the two patient categories were obtained by direct counting. Hardy-Weinberg equilibrium was evaluated by a chi-square test. Continuous variables are expressed as the mean ± standard deviation; discrete variables are expressed as percentages. Differences in genotyping distribution were assessed by chi-square analysis of the relevant 2×2 contingency table or Fisher's exact test, as appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were also calculated. Multiple logistic regressions were used to assess the association between the presence of a particular genotype and the presence of restenosis. We tested for independent association in multiple variable models (multiple logistic regression) of restenosis that included all the variables that were $p < 0.1$ in the univariate analysis using forward stepwise (conditional) analysis. Clinical variables (gender, age, diabetes mellitus, hypertension, hypercholesterolemia, smoking habit, co-morbidities, prior acute myocardial infarction, prior percutaneous coronary angioplasty, prior coronary artery bypass graft, reason for intervention, and use of statins) were analyzed separately from angiographic characteristics (treated vessel, vessel diameter, type of lesion, lesion length, percentage of the lesion, chronic total occlusion, evidence of thrombus or calcification, ostial lesions, bifurcation lesions, bare metal stent (BMS), drug-eluting stent (DES) and diameter and length of stent). The analysis was performed by the SPSS 11 statistical package. 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, USA).

Results

Baseline characteristic of the patients included in the study are shown in Table 1. No significant differences between patients with and without restenosis with regard to age, diabetes, hypertension, or smoking were observed. However, the presence of stable angina was a protective factor for restenosis ($p = 0.036$). Table 2 shows the angiographic characteristics of the treated coronary lesions (n = 327). We observed that the 95 lesions present in the patients that were treated with bare metal stent (BMS) implantation, the 73.7% develop more restenosis in contrast with the patients that were treated with drug-eluting stent (DES) ($p < 0.001$). Restenosis was independent of the artery treated. In addition, stent with diameters smaller 2.5 mm and bifurcations increased the risk of developing restenosis ($p = 0.05$ and $p = 0.041$, respectively). No differences were observed in the American Heart Association/American College of Cardiology (AHA/ACC) angiographic classification between patients with and without restenosis.

Allele and genotype frequencies

The observed and expected frequencies in the three polymorphic sites of the major histocompatibility complex class II transactivator gene (*MHC2TA*) were in Hardy-Weinberg equilibrium. The allele and genotype frequencies of *MHC2TA* gene at positions *-168 A>G* (rs3087456), *1614 C>G* (rs4774), and *2536 G>A* (rs2229320) were similar between patients with and without restenosis (data not shown). The univariate analysis in patients with and without restenosis is presented in Table 3. Patients with DES implantation showed a decrease risk of developing restenosis ($p < 0.001$, OR = 0.26). In addition, the diameter of the stent (≤ 2.5 mm)

Table 2 Angiographic characteristics of the coronary lesion treated (n = 327).

	With estenosis (n = 95)	Without restenosis (n = 232)	*p*
BMS	70 (73.7)	90 (42.2)	< 0.001
DES	25 (26.3)	134 (57.8)	
Diameter smaller 2.5 mm	32 (34)	53 (22.8)	0.05
Stent length (mm)	21.02 (6.51)	20.33 (6.58)	NS
Ostial lesion	11 (11.7)	29 (12.8)	NS
Bifurcation	29 (30.5)	45 (19.6)	0.04
Artery treated			
LAD	45 (46.9)	109 (47)	NS
Cx	18 (18.9)	48 (20.7)	
RCA	31 (32.6)	65 (28)	
AHA/ACC angiographic classification (n=327)			
A	9 (9.5)	21 (9.1)	NS
B1	37 (38.9)	89 (38.4)	
B2 and C	48 (50.5)	120 (51.7)	
Unknown	1 (1.1)	2 (0.9)	

BMS: bare-metal stent; DES: drug-eluting stent; LAD: left anterior descendent coronary artery; Cx: circumflex coronary artery; RCA: right coronary artery; AHA/ACC: American Heart Association/American College of Cardiology; NS: not significant; *p*: *p* value.

Table 3 Univariate analysis (202 patients with 327 coronary lesions).

	With restenosis	Without restenosis	Odds ratio	CI (95%)	*p*
Patients	79	123			
Male	67 (84.8)	91 (74)	1.9	0.94-4.09	NS
Stable angina	8 (10.1)	27 (22)	0.4	0.17-0.93	0.036
Statin therapy	68 (86.1)	109 (88.6)	0.79	0.34-1.85	NS
Diameter smaller 2.5 mm	2.92 ± 0.49	3.04 ± 6.58	1.79	1.03-2.94	0.04
Bifurcation	29 (30.5)	45 (19.6)	1.8	1.04-3.11	0.041
DES	25 (26.3)	134 (57.8)	0.026	0.14-0.44	<0.001
Chronic total occlusion	16 (16.8)	48 (20.7)	0.77	0.41-1.44	NS

CI: confidence interval; DES: drug-eluting stent; NS: not significant (Only the most important variables are shown. Non-significant variables are not included but were analyzed); *p*: *p* value.

and bifurcations increased the risk of developing restenosis ($p = 0.049$, OR = 1.74 and $p = 0.041$, OR = 1.8, respectively). However, none of the risk factors analyzed in the univariate analysis showed an interaction with the polymorphisms studied. After multivariate adjustment, the predictors of restenosis were the type of stent (BMS) and the diameter of the stent ($\leq$ 2.5 mm) (Table 4). On the other hand, the polymorphisms *1614 C>G* (rs4774) and *2536 G>A* (rs2229320) showed a strong linkage disequilibrium with a D' = 0.87. However, the distribution of the different haplotypes was similar in patients with and without restenosis.

Discussion

The pathogenesis of restenosis after PTCA and after stent implantation has an inflammatory component.[3–5] Morphological analysis after coronary stenting demonstrates an early thrombus formation and acute inflammation, followed by neointimal hyperplasia after stenting.[6–9] The severity of arterial injury during stent placement correlates with inflammatory processes that play an important role in the developing restenosis and remodeling neointimal.[6] Recent studies provide evidence on the emerging role of

Table 4 Multivariate analysis.

Variable	Alpha coefficient	*p*	CI (95%)
Stent diameter <2.5 mm	0.158	0.018	0.03-0.316
Stent type (DES)	0.332	<0.001	0.182-0.434

CI: confidence interval; DES: drug-eluting stent; NS: not significant (Only the most important variables are shown. Non-significant variables are not included but were analyzed).

MHC2TA gene as master regulator of MHC class II transcription and several genes, such as collagen,[14] matrix metalloproteinase-9,[15] IL-4,[16] cathepsin E, IL-10 and TGF-β,[17–19] play important roles in the arterial remodeling after coronary stent placement. Moreover, several studies associate this gene with several inflammatory diseases, such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, myocardial infarction, metabolic syndrome, and atherosclerosis.[20–23] In the present work, we studied 202 Mexican Mestizo patients with symptomatic coronary artery disease who underwent coronary stent implantation. The patients were divided in seventy-nine with restenosis and one-hundred twenty-three without restenosis. The distribution of the studied polymorphisms was similar in patients with and without restenosis. The *MHC2TA* gene polymorphism association studies in different diseases and different populations are controversial with positive and negative results. Koizumi et al. reported no association of the three *MHC2TA* gene polymorphisms with systemic lupus erythematosus in Japanese patients,[21] agreeing with our results. Yazdani-Biuki et al. studied the *MHC2TA-168 A/G* gene polymorphism in Australian patients with rheumatoid arthritis and no association was observed.[25] In contrast, Swamberg et al. reported that the *-168 G* was significantly associated with increased risk of myocardial infarction and rheumatoid arthritis (OR = 1.39 and OR = 1.29, respectively) in a Caucasian population.[22] However, they did not observe significant differences in the *MHC2TA 1614 C>G* and *MHC2TA 2536 G>A* polymorphisms distribution. On the other hand, Lindholm et al. studied two populations (Finland and Sweden) and reported the association of the *-168 AG* and *GG* genotypes with cardiovascular mortality after myocardial infarction, but only in the Finnish population.[23]

The results of the univariate analysis showed that bare-metal stent (BMS), bifurcations and the diameter of the stent ($\leq$ 2.5 mm) increased the risk of developing restenosis. These data confirm the previous results obtained by our group in a small group of patients.[26–28] In addition, this analysis showed no association between the *MHC2TA* gene polymorphisms with both angiographic and clinical characteristic of the restenosis after coronary stent placement in a group of Mexican patients. Clinical and angiographic measures of restenosis were evaluated over six months after coronary stent placement.

Conclusion

The present study suggests that the *MHC2TA* gene polymorphisms are not a risk factor for restenosis after coronary stenting. Our data are preliminary and additional studies in a larger number of samples and in other populations could help to define the true role of this marker as risk factor for developing restenosis after coronary stenting.

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Conflict of interest

There are no conflicts of interest to disclose.

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