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Association of V249I and T280M variants of fractalkine receptor CX3CR1 with carotid intima-media thickness in a Mexican population with type 2 diabetes

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Abstract

Objective: *To evaluate the association of the V249I and T280M variants of CX3CR1 fractalkine gene with carotid intima-media thickness in Mexican subjects with and without type 2 diabetes.* **Methods:** *We analyzed the V249I and T280M variants of the CX3CR1 receptor by TaqMan assays in 111 subjects with type 2 diabetes and 109 healthy controls. Hemoglobin A1c, glucose, and lipid profile were determined.* **Results:** *A significant increase in carotid intima-media thickness was observed in type 2 diabetes patients (0.979 ± 0.361 mm) compared to healthy controls (0.588 ± 0.175 mm). In subjects carrying the MM variant of the T280M polymorphism, hemoglobin A1c was higher (p = 0.008). Classic risk factors for atherosclerosis showed no differences between carriers of the T280M and V249I variants. Controls with the II249 genotype associated with carotid intima-media thickness (0.747 ± 0.192 mm; p = 0.041), and this difference remained significant even after adjusting factors such as age, gender, and body mass index (OR: 7.7; 95% CI: 1.269-47.31; p = 0.027).* **Conclusions:** *V249I genotype of the fractalkine receptor showed a protector role in patients with type 2 diabetes. The T280M genotype is associated with increased carotid intima-media thickness in Mexican individuals with or without type 2 diabetes.* (Gac Med Mex. 2017;153:45-52)
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Introduction

Fractalkine receptor variants V249I and T280M relate with inflammatory and immune processes in coronary artery disease and obesity. Type 2 diabetes (T2D) and hypertension increase the risk of myocardial infarction, cerebrovascular disease, and atherosclerosis[1,2]. In addition, T2D is associated with low-grade inflammation and the recruitment of leukocytes in adipose tissue, which induces insulin resistance[3]. Moreover, increase in carotid intima-media thickness (cIMT) has been found in T2D patients, and is used as a predictor of cardiovascular disease[4-6]. On the other hand,

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fractalkine receptor CX3CR1 and its ligand (CX3CL1) are key components in leukocyte chemotaxis, and play an important role in the filtration of monocytes, T lymphocytes, and smooth muscle cells on atherosclerotic lesions, inducing platelet activation[7-10]. Fractalkine structure (CX3C) is unique among chemokines by having both soluble and membrane-bound forms, the latter being the one that intervenes in cellular adhesion[8]. The great majority of cells of the immune system, mainly macrophages, T lymphocytes and NK cells, express CX3CR1 receptors in the cell membrane, binding to the G-protein-coupled receptor, and promoting survival and activation[9]. Stimulation of endothelial cells with tumor necrosis factor (TNF)-α and interferon (INF)-γ, results in the induction and stabilization of CX3CL1 mRNA, which is mediated by p38 MAPKMAPKAPK-2 activation[11]. Through the same mechanism, resistin increases CX3CL1/CX3CR1 expression in smooth muscle cells through p38 MAPK and STAT3 phosphorylation, and also involves NF-kB, AP-1, TLR4 and G protein[12]. On the other hand, interleukin-10 and IFN-γ activate their expression, but via the PI3K pathway in monocytes[13].

High concentrations of fractalkine have been correlated with elevated concentrations of interleukin-6, Apo-B, low-density lipoprotein (LDL)-cholesterol and insulin[14]. In addition, fractalkine has been associated with obesity, insulin resistance, atherosclerosis, infarction and T2D[15-17]. A high concentration of fractalkine has been found in adipocytes of obese patients with diabetes[15]. In contrast, other studies have reported non-significant differences in levels of fractalkine in patients with T2D[18]. Interestingly, hyperglycemic experimental mice showed an increased expression of CX3CR1[19], whereas in ischemic deficient mice *CX-3CR1*(-/-) alleles confer protection against inflammation[20]. There is controversy regarding the influence of CX3CL1/CX3R1 on T2D, but its role in inflammation is widely recognized.

It has been reported that the presence of single nucleotide polymorphisms (SNP) in the gene encoding the fractalkine receptor (*CX3CR1*) affects its expression and function. Two of the most studied variants of the *CX3CR1* gene, V249I and T280M, are found in the transmembrane domains six and seven of the CX3CR1 protein[21]. The variants cause a valine to isoleucine substitution at position 249 (V249I) and a threonine to methionine substitution at position 280 (T280M), and have been implicated in various low-grade inflammation conditions, including obesity and coronary artery disease[22-24].

The purpose of this study was to explore the association between V249I and T280M variants of the *CX-3CR1* gene with cIMT in a study with Mexican patients.

Methods

The study was approved by the Ethics and Investigation Review Board of the *Hospital de Especialidades, Centro Medico Nacional Siglo XXI, Instituto Mexicano de Seguro Social*, and conforms to the Declaration of Helsinki currently in effect. A total of 111 Mexican outpatients (48.6% females) with T2D according to the American Diabetes Association criteria[25] were recruited from consecutive cases at the *Hospital de Especialidades del Centro Médico Nacional Siglo XXI, del Instituto Mexicano del Seguro Social* (IMSS), Mexico City. Healthy controls, 109 (58.7% females), attending the same outpatient from hospital workers in the IMSS system served as control group. The age of subjects was 20-74 years. All patients with type 1 diabetes and with secondary causes of diabetes or alcoholism, smoking, renal failure, and primary dyslipidemia were excluded. After giving informed consent, all study subjects underwent a thorough clinical examination. After an overnight fast of 12 hours, venous blood was collected from the antecubital vein. Serum glucose, hemoglobin A1c (HbA1c), total cholesterol, high-density lipoprotein (HDL)-cholesterol, LDL-cholesterol, and triglyceride levels were determined by CX analyzer (Beckman Systems, Fullerton CA). Details of clinical features and laboratory investigations were recorded, including age, sex, weight, waist circumference, body mass index (BMI), time with diagnosis of diabetes, and systolic and diastolic blood pressure. Entry criteria for the control group included: fasting glucose < 100 mg/dl, blood pressure < 130/85 mmHg, total cholesterol < 200 mg/dl, and triglycerides < 150 mg/dl.

Carotid ultrasonogram

Doppler and real-time ultrasound studies were performed at the imaging department of the *Hospital de Especialidades, Centro Médico Nacional Siglo XXI*, IMSS. Ultrasound was used for automatic measurements of cIMT and performed in a blinded manner by a single skilled radiologist. High-resolution ultrasound equipment (Philips HDI 5000) was used with specific software for carotid exams and a wide band lineal transducer (7-12-MHz). All examinations were

performed according to predetermined, standardized protocols for left and right carotid arteries. The measurements were made in a 1 cm segment in the distal of the common carotid artery, 1 cm of the carotid artery bifurcation (carotid bulb), and internal carotid artery were the same in each survey (1 cm after the carotid bulb). Images of the carotid segments were obtained at two angles: anterior oblique (30° from the median line) and lateral (100° from the median line). The posterior wall of the artery was focused and the approximation function was used to amplify the image of the artery. To measure cIMT, an image of 1 cm of the external carotid bulb, a lateral longitudinal view of 10 mm of the posterior wall of the carotid artery was used. The distance between the first echogenic line and the second hypoechogenic line of the upper layer of the tunica adventitia was measured during diastole in both carotid arteries. The study included information about the mean of IMT of the right and left carotid arteries, and presence or absence of carotid plaque of each segment by longitudinal circumferential scan in each subject[5]. All explorations were photographed and stored. Images were analyzed by the same radiologist with the Philips's QLAB 4.2.1 Advanced Ultrasound Quantification Program (Philip's QLAB 4.2.1, USA).

Genotyping

Genomic DNA was isolated from peripheral blood white cells using the AutoGenFlex Star equipment (AutoGen, USA). The DNA samples were genotyped for the polymorphisms of T280M (rs3732378) and V249I (rs3732379) using the same method and equipment as co-authors had used previously[26].

Statistical analysis

Data are expressed as medians with their 25 and 75 percentiles, according to distribution. Kruskal-Wallis tests were applied to analyze groups and determine the *p* value according to data distribution. Multiple logistic regression analyses were used to examine the effects of various traditional and non-traditional risk factors for T2D over the healthy controls. All values of $p < 0.05$ were considered significant. All statistical analysis was done with the program SPSS, version 16.0 for Windows (SPSS Inc. Chicago Il). Hardy-Weinberg equilibrium was determined using the χ^2 test with 1 degree of freedom; $p < 0.05$ was considered in disequilibrium.

Results

We evaluated the association of cIMT with polymorphisms of T280M and V249I of the fractalkine receptor *CX3CR* gene in 220 subjects, which included 111 (50.5%) patients with T2D and 109 (49.5%) healthy controls. The Hardy-Weinberg equilibrium was evaluated in the control group ($p = 0.568$ and $p = 0.054$ for T280M and V249I, respectively). Patients with T2D exhibited 76.6% obesity, 70.3% hypertriglyceridemia, 12.6% high blood pressure, and 44.1% hypoalphalipoproteinemia. Only 28.8% (32/111) of the patients with T2D achieved the threshold for percentage of HbA1c < 7%.

Age, systolic and diastolic blood pressure, glucose levels, total cholesterol, HDL- cholesterol, LDL-cholesterol, total cholesterol/HDL-cholesterol ratio, and triglycerides did not differ between the study subjects with the TT, TM, and MM 280 genotype of the *CX3CR* gene. Similar results were obtained for the V249I polymorphisms; however, HbA1c was only significant in subjects with the T280M polymorphisms (Table 1).

As we did not have a reference value to indicate abnormal cIMT in our study population, in controls we determined this value from the 75 percentile (≥ Pc75), the cut-off value being found to be 0.600 mm. The expected results were obtained; cIMT was greater in patients with T2D than healthy controls (0.979 ± 0.361 vs. 0.588 ± 0.175 mm). Presence of atheromatous plaque was found in four (3.7%) controls and 24 (21.6%) patients; all but one was classified as below 50% stenosis. However, there was no correlation between the presence of plaque and any specific genotype. In summary, TT280 genotype showed cIMT of 0.964 ± 0.341 in patients vs. 0.605 ± 0.172 in controls; TM280 showed 0.958 ± 0.355 vs. 0.532 ± 0.162, respectively, and MM280 presented 1.104 ± 0.450 vs. 0.742 ± 0.364, respectively. On the other hand, with the VV249 the values were 1.017 ± 0.388 vs. 0.577 ± 0.154 in patients vs. controls, respectively and V249I presented 0.973 ± 0.391 vs. 0.577 ± 0.207, respectively. The most significant finding was in II249, which showed 0.901 ± 0.179 in patients vs. 0.747 ± 0.192 in controls.

As shown in table 2, significant differences werefound in the odds ratios (OR) of the genotypes: while MM280 and II249 genotypes were risk factors for increased cIMT in our study population, only II249 genotype remained significant, and V249I genotype demonstrated lower risk of cIMT when the study population was stratified.

Table 1. Anthropometric and clinical characteristics of the study population, according to T280M and V249I variants of the *CX3CR gene*

Parameters	T280M (n = 220)				V249I (n = 220)			
	TT (n = 137)	TM (n = 67)	MM (n = 16)	p value	VV (n = 122)	VI (n = 71)	II (n = 27)	p value
Age (years)	46 (40.5-52.0)	46 (39-53)	48.5 (43-55)	0.341	46 (41-52)	47 (40-53)	50 (40-54)	0.543
Gender F (%)	71 (51.8)	40 (59.7)	7 (43.8)	–	63 (51.6)	43 (60.6)	12 (44.4)	–
SBP (mmHg)	110 (100-120)	110 (100-120)	110 (110-130)	0.279	110 (100-120)	110 (100-120)	110 (110-120)	0.128
DBP (mmHg)	70 (70-80)	70 (70-80)	70 (70-80)	0.749	70 (70-80)	70 (70-80)	70 (70-80)	0.769
HbA1c (%)	6.1 (5.5-7.0)	6.3 (5.7-8.2)	7.5 (6.1-10.4)	**0.008***	6.1 (5.5-6.9)	6.4 (5.8-8.8)	6.1 (5.8-8.5)	0.066
Glucose, mg/dl	98 (85.5-139.0)	105 (89-188)	148 (97.0-159.7)	0.061	98 (86-139)	105 (86-205)	133 (92-182)	0.153
Total Cholesterol, mg/dl	196 (173.5-221.0)	188 (160-225)	210 (173.7-237.2)	0.490	196 (174.7-216.2)	184 (161-235)	201 (179-234)	0.440
HDL-C, mg/dl	47 (38-56)	43 (36-53)	44.5 (33.0-47.7)	0.109	47 (38.7-55.2)	44 (35-53)	43 (37-51)	0.080
LDL-C, mg/dl	116 (91.5-140.0)	110 (84-139)	124.9 (80.0-156.2)	0.625	115.3 (91.0-138.5)	109 (87-144)	125 (94-157)	0.165
TC/HDL-C	4.2 (3.2-5.3)	4.6 (3.6-6.1)	4.6 (4.0-6.1)	0.244	4.2 (3.2-5.0)	4.6 (3.2-6.0)	4.8 (4.0-6.0)	0.081
Triglycerides, mg/dl	150 (111.5-221.0)	147 (118-226)	208 (159.0-263.5)	0.100	148.5 (115.0-214.5)	148 (113-242)	198 (152-244)	0.193
With T2D n (%)	59 (43.1)	38 (56.7)	14 (87.5)	–	49 (40.2)	42 (59.2)	20 (74.1)	–
Time of disease (years)	9 (6-15)	8 (2-10)	8 (2.7-15.0)	0.239	9 (6-15)	8 (2.7-13.5)	6.5 (2.2-14.7)	0.379

Data are median (interquartile range, Q25-Q75). Kruskal-Wallis test; p value < 0.05 was considered significant. *p < 0.05.
DBP: diastolic blood pressure; F: female; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC/HDL-C: total cholesterol/HDL-C ratio; T2D: type 2 diabetes.

We also performed a multiple logistic regression analysis to find the relationship of the anthropometric and biochemical risk factors common to cardiovascular disease with V249I and T280M polymorphisms, considering the cIMT in our study population. Significant differences were found in genotypes for anthropometric and biochemical risk factors. Interestingly, we found the MM280 genotype with significant differences and an OR as risk factor for cIMT only in the total population when this model was adjusted for age (OR: 1.0; 95% CI: 1.053-1.137; p < 0.001) and gender (OR: 2.7; 95% CI: 1.480-5.113; p < 0.001). As shown in table 3, with respect to the V249I variant, significant differences were found only for the II249 genotype associated with an increased cIMT in the three models tested when regression was made in the total population adjusted for anthropometric and biochemical risk factors, but significant differences in mean arterial

Table 2. Association of genotypes with cIMT (> 75 Pc) for the T280M and V249I variants of the *CX3CR* gene

T280M					V249I				
Genotypes	**cIMT**	**OR**	**95% CI**	***p* value**	**Genotypes**	**cIMT**	**OR**	**95% CI**	***p* value**
TT	79/137 (57.7%)	1.0	–	–	VV	65/122 (53.3%)	1.0	–	–
TM	40/67 (59.7%)	1.0	0.668-1.790	0.735	VI	45/71 (63.4%)	1.5	0.9723-2.607	0.064
MM	15/16 (93.8%)	11.0	1.969-234.1	**0.003***	II	24/27 (88.9%)	7.0	2.331-29.31	**0.001***
Patients with T2D (T280M)					Patients with T2D (V249I)				
TT	54/59 (91.5%)	1.0	–	–	VV	46/49 (93.9%)	1.0	–	–
TM	33/38 (86.8%)	0.6	0.253-1.765	0.299	VI	36/42 (85.7%)	0.3	0.173-1.022	**0.027***
MM	14/14‡ (100.0%)	1.2	0.229-27.68	0.801	II	19/20 (95.0%)	1.2	0.227-26.01	0.834
Healthy controls (T280M)					Healthy controls (V249I)				
TT	25/78 (32.1%)	1.0	–	–	VV	19/73 (26.0%)	1.0	–	–
TM	7/29 (24.1%)	0.6	0.267-1.536	0.361	VI	9/29 (31.0%)	1.2	0.554-2.772	0.538
MM	1/2 (50.0%)	2.1	0.054-82.68	0.586	II	5/7 (71.4%)	7.1	1.401-52.880	0.060†

*p < 0.05 is significant. †Borderline significance. ‡One was added to the denominator in order to calculate CI. cIMT > 75 Pc was ≥ 0.600 mm, as found in Mexican population.
CI: confidence interval; OR: odds ratio.

pressure and hypertriglyceridemia were not found. When the study population was stratified, the significant differences to the II249 genotype remained only in healthy controls in all models, but without significant differences in age and mean arterial pressure in model 2 or age, mean arterial pressure, and hypertriglyceridemia in model 3 (Table 3), whereas the patients with T2D did not show significant differences to the V249I variant, which suggests that II249 genotype has an impact on cIMT in healthy populations (0.747 ± 0.192 mm; p = 0.04).

Discussion

The association of cIMT with atherogenic dyslipidemia, obesity, hypertension, and diabetes has been well demonstrated[4-6]. In this study, significant differences were not found between traditional clinical cardiovascular risk factors in either T280M and V249I variants (Table 1). These variants of *CX3CR1* are consistently associated with increased cIMT in people with or without diabetes. Whether the frequency of the polymorphisms is ethnically linked remains unclear[27-29].

Additionally, our results confirm the previously described association between cIMT and age, gender as well as with BMI in other populations[30-33]. Other risk factors that have been associated with cIMT are hypertension, HDL-cholesterol, triglycerides, and glucose. However, after multiple logistic regression analysis, only age, gender, and BMI remained significant with cIMT for the II249 genotype. It is important to note that an increase in cIMT is an aspect of arterial wall aging, related to cellular and molecular changes that underlie cIMT in the development and progression of atherosclerosis. In addition, cIMT is age-dependent and increases at a rate of 0.005-0.010 mm/year of age[34].

Increased cIMT and carotid artery distension have been found in other conditions related to diabetes, such as obesity[35] and cardiovascular diseases[36]. The

Table 3. Multiple logistic regression analyses, according to cIMT (> 75 Pc) with the T280M and V249I polymorphisms of the *CX3CR gene*

Models	Genotypes	Total group OR (95% CI) *p*value	Patients with T2D OR (95% CI) *p* value	Healthy controls OR (95% CI) *p* value
T280M polymorphism				
1	TT	1	1	1
	TM	1.2 (0.662-2.435) 0.473	0.6 (0.157-2.455) 0.497	0.8 (0.297-2.326) 0.725
	MM	8.6 (1.078-68.654) **0.042***	–	1.5 (0.081-29.654) 0.769
2	TT	1	1	1
	TM	1.2 (0.647-2.561) 0.472	0.6 (0.156-2.501) 0.506	1.3 (0.072-24.08) 0.851
	MM	7.8 (0.976-63.731) 0.053†	–	1.0 (0.989-1.112) 0.112
3	TT	1	1	1
	TM	1.1 (0.544-2.514) 0.688	0.6 (0.164-2.867) 0.606	0.7 (0.244-2.353) 0.632
	MM	6.6 (0.737-60.853) 0.091	–	1.4 (0.074-26.935) 0.820
V249I polymorphism				
1	VV	1	1	1
	VI	1.7 (0.906-3.425) 0.095	0.3 (0.074-1.603) 0.174	1.6 (0.612-4.694) 0.310
	II	6.7 (1.854-24.813) **0.004***	1.1 (0.102-12.247) 0.929	7.7 (1.269-47.31) **0.027***
2	VV	1	1	1
	VI	1.7 (0.858-3.479) 0.126	0.3 (0.071-1.594) 0.170	1.5 (0.503-4.52) 0.463
	II	5.8 (1.558-22.102) **0.009***	1.1 (0.1-12.368) 0.930	7.7 (1.133-53.334) **0.037***
3	VV	1	1	1
	VI	1.3 (0.606-3.150) 0.442	0.3 (0.063-1.605) 0.165	1.6 (0.508-5.147) 0.416
	II	6.2(1.394-27.710) **0.017 ***	0.9 (0.078-11.584) 0.968	7.6 (1.086-53.912) **0.041***

*p < 0.05 is significant. †Borderline significance.
Model 1 was adjusted for age and gender, while model 2 included age, gender, arterial pressure mean and body mass index, and model 3 was adjusted for age, gender, arterial pressure mean, body mass index, diabetes (only to total group), low-density lipoprotein cholesterol, total cholesterol/high-density lipoprotein cholesterol ratio and hypertriglyceridemia. carotid intima-media thickness > 75 Pc was ≥ 0.600 mm, as found in Mexican population.
CI: confidence interval; OR: odds ratio.

cIMT measurements that we obtained corresponding to the genotypes of the variants in the healthy controls are similar to those reported in previous studies[22,37].

In our population analyzed, we found the MM280 genotype associated with increased cIMT (Table 2), but this association was lost when adjusting for confounding factors in logistic regression. In addition, MM280 genotype has a high frequency in T2D (100%) compared with controls (50%) (Table 2). The MM280 genotype has previously been associated with low risk and reduction of cIMT in patients with carotid atherosclerosis[37-39]; this genotype has also been associated with obesity[24].

Women with obesity carrying the MM280 genotype show significant increase in mean waist circumference, while men carrying the T280M genotype show a significant increase in mean waist circumference[24] and present greater insulin resistance, with high levels of leptin and reduction in adiponectin[40].

Various reports have analyzed the role of I249/M280 in relation to the risk of coronary events and disease[41,42]. In those studies, the M280 allele was the only one associated with lower risk. McDermott, et al.[43] found that leukocytes are significantly affected by the presence of I249 and M280. It has also been suggested that they have a protective effect in regard to acute

coronary syndrome and internal carotid artery occlusive disease, as well as atherosclerosis and its inflammatory response, in the case of the M280 allele[22,28,44]. On the other hand, in European populations the II249 genotype has been found as a risk factor for cerebral infarction[39], for cardiovascular arterial disease with acute coronary syndrome[23], and is also associated with obesity[24]. We found that healthy controls that carry the II249 genotype have a risk factor for cIMT (Tables 2 and 3). However, one Italian study reported that the presence of the II249 polymorphism does not play a major role on the progression of carotid atherosclerosis[22]. Interestingly, we found the V249I genotype with lower risk factor for cIMT in patients with T2D (Table 2), but one study has shown no correlation between V249I genotype and the presence of carotid thickening[39]; the difference in these findings could be the result of the ethnic mixture in the Mexican population.

The important results obtained from the regression analysis indicate that the II249 genotype is associated with an increased cIMT (OR 7.6; 95% CI: 1.086-53.912; p = 0.041) in Mexican healthy controls. Nevertheless, there were few study subjects with this genotype, so this finding should be interpreted with caution. In patients with diabetes the II249 genotype did not have any association, which could be due to the evolution of the disease and the influence of anthropometric and clinical traditional cardiovascular risk factors, which may have already increased cIMT.

In fact, in patients with T2D, the effect of fractalkine receptor variants on cIMT was very limited. This may be due to the fact that diabetes itself is the main determinant of differences in cIMT in this population.

In conclusion, we show that V249I genotype of the fractalkine receptor is associated with lower cIMT in patients with T2D. However, the association between II249 and cIMT is observed only in healthy populations.

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Disclosure of interest

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