Invest Clin 57(3): 246 - 258, 2016

PAI-1 haplogenotype confers genetic susceptibility for obesity and hypertriglyceridemia in Mexican children.

Ulises de la Cruz-Mosso[1], Luz Elena Ramos-Arellano[2], José Francisco Muñoz-Valle[1], Aralia Berenice Salgado-Bernabé[2], Lorenzo Salgado-Goytia[2], Natividad Castro-Alarcón[2], Arturo Ramírez-Peralta[2] and Isela Parra-Rojas[2].

[1]Instituto de Investigación en Ciencias Biomédicas, Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, México.
[2]Unidad Académica de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, México.

Key words: *PAI-1*; polymorphism; obesity; hypertriglyceridemia; children.

Abstract. The presence of childhood obesity predisposes to the development of cardiovascular and metabolic diseases, such as coronary artery disease and type 2 diabetes mellitus, in adulthood. The polymorphisms described in *PAI-1* gene have been linked with obesity and metabolic syndrome in several populations. The aim of this study was to investigate the association of the -844 G/A (rs2227631), -675 4G/5G (rs1799889) and *HindIII* C/G (rs757716) *PAI-1* polymorphisms with obesity and dyslipidemia in a sample of Mexican children. A cross-sectional study was performed in 222 children with an age range between 6-11 years; 104 children were classified as obese and 118 children with normal-weight. The *PAI-1* polymorphisms were analyzed by PCR-RFLP. Linkage disequilibrium (LD) and haplogenotype analysis among the three polymorphisms were determined. The results showed significant associations with obesity of the -844 G/A genotype and the A allele (OR= 2.75, p<0.001 and OR= 1.76, p=0.01, respectively). The -844 G/A polymorphism was found in LD with -675 4G/5G *PAI-1* polymorphism (D'= 0.77). We found that G-4G-C/A-5G-G is a risk haplogenotype for obesity [OR=2.6; 95% confidence interval (CI) 1.17-4.22; p= 0.01] and with marginal association with hypertriglyceridemia(OR= 2.6; 95% CI 1.04-6.35; p= 0.05). The G-4G-C/A-5G-G *PAI-1* haplogenotype may be a genetic marker of susceptibility for obesity and hypertriglyceridemia in Mexican children.

Corresponding author: Isela Parra-Rojas, Laboratorio de Investigación en Obesidad y Diabetes, Unidad Académica de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero39090, México. E-mail: iprojas@yahoo.com.

Un haplogenotipo de *PAI-1* confiere susceptibilidad genética para la obesidad y la hipertrigliceridemia en niños mexicanos.

Invest Clin 2016; 57(3): 246 - 258

Palabras clave: *PAI-1*; polimorfismo; obesidad; hipertrigliceridemia; niños.

Resumen. La presencia de la obesidad en la infancia predispone al desarrollo de enfermedades cardiovasculares y metabólicas, como la enfermedad arterial coronaria y la diabetes mellitus tipo 2 en la edad adulta. Algunos polimorfismos en el gen *PAI-1* se han relacionado con la obesidad y el síndrome metabólico en varias poblaciones. El objetivo del estudio fue investigar la asociación de los polimorfismos -844 G/A (rs2227631), -675 4G/5G (rs1799889) y *HindIII* C/G (rs757716) en el gen *PAI-1* con la obesidad y las dislipidemias en una muestra de niños mexicanos. Se realizó un estudio transversal en 222 niños con un rango de edad de 6-11 años, de los cuales 104 niños fueron clasificados con obesidad y 118 con peso normal. Los polimorfismos en el gen *PAI-1* fueron analizados por PCR-RFLP. También se determinó el desequilibrio de ligamiento y el análisis de haplogenotipos de los tres polimorfismos. Los resultados mostraron la asociación significativa de la obesidad con el genotipo -844 G/A y el alelo A (OR= 2,75, p<0,001 y OR= 1,76, p=0,01, respectivamente). El polimorfismo -844 G/A se encontró en desequilibrio de ligamiento con el -675 4G/5G (D'= 0.77). También se encontró que el haplogenotipo G-4G-C/A-5G-G es un marcador de riesgo para la obesidad [OR=2,6; 95% intervalo de confianza (CI) 1,17-4,22; p= 0,01], además de que este haplogenotipo presentó una asociación marginal con la hipertrigliceridemia (OR= 2,6; 95% CI 1,04-6,35; p= 0,05). El haplogenotipo G-4G-C/A-5G-G en el gen *PAI-1* puede ser un marcador genético de susceptibilidad para obesidad e hipertrigliceridemia en niños mexicanos.

Recibido: 23-08-2015 . Aceptado: 14-04-2016

INTRODUCTION

Obesity is a complex multifactorial chronic disease, which is particularly influenced by a wide-range of genetic and environmental factors (1). The presence of obesity in childhood predisposes to the development of cardiovascular and metabolic diseases such as atherosclerosis and type 2 diabetes mellitus in adulthood (2, 3). An increased production of pro-inflammatory molecules and a decrease in the fibrinolytic capacity has been described in the course of this pathology, which generally is attributed to increased levels of plasminogen activator inhibitor-1 (*PAI-1*) (4-7).

PAI-1 is the main inhibitor in the plasminogen activation system, which comprises an inactive proenzyme (plasminogen) that can be converted into its active form the plasmin, by the action of physiological plasminogen activators; plasmin is the main enzyme that degrades fibrin into soluble products (8). Increased *PAI-1* levels in circulation are associated with the development of myocardial infarction and the formation/progression of chronic inflammatory diseases such as atherosclerosis and cardiovas-

cular disease (4, 8, 9). Notably, increased *PAI-1* levels also have been linked with risk factors for type 2 diabetes mellitus such as insulin resistance, glucose intolerance, hypertension, metabolic syndrome (MetS) and dyslipidemias (low HDL-C serum levels and hypertriglyceridemia) (7, 10, 11). Therefore, we consider that *PAI-1* could be an important marker in the course of these inflammatory processes.

The human *PAI-1* gene is located in the chromosome 7q22, and 180 polymorphic sites have been described within the *PAI-1* gene (6, 12). Only three polymorphisms appear to have functional importance (10-12). Of these, two polymorphisms identified in the promoter region are the single nucleotide polymorphism (SNP) -844 G/A (rs2227631) and the insertion/deletion -675 4G/5G (rs1799889). Both polymorphic loci have been associated with increased gene expression and elevated *PAI-1* levels in circulation, as well as risk factors such as insulin resistance, high triglycerides, low HDL-C and with several diseases including obesity, deep vein thrombosis, coronary artery disease and MetS (13-20). The third functional SNP described is the *HindIII* C/G polymorphism (rs757716), which is located in the 3´ un translated region (UTR) of the *PAI-1* gene, has also been associated with elevated circulating *PAI-1* levels as well as increased cholesterol and insulin serum levels according to the genotype in myocardial infarction patients (21).

Association studies have shown that these three functional *PAI-1* polymorphisms could influence on *PAI-1* levels in circulation, and these variants are related with lipid concentrations in several populations with Mexican-mestizo background, most likely by linkage disequilibrium (LD) (13, 14, 19-23).

Based on this knowledge, the functional *PAI-1* polymorphisms are genetic markers that could contribute to the pathological features associated with obesity. Therefore, the aim of this study was to investigate the association of genotypes, haplotypes and haplogenotypes of the -844 G/A (rs2227631), -675 4G/5G (rs1799889) and *HindIII* C/G *PAI-1* (rs757716) polymorphisms with obesity and the lipid profile in a sample of Mexican children.

PATIENTS AND METHODS

Participants

A cross-sectional study was performed in 222 children with an age range of 6-11 years, 104 children were classified with obesity and 118 children with normal-weight. All children enrolled in the study were of Mexican-mestizo population born in the State of Guerrero, Mexico, with a family history of ancestors, at least back to the third generation born in our State.

Informed written consent was obtained from all parents or guardians before enrollment in the study. Approval for the study was obtained from the Research Ethics Committee of the Universidad Autónoma de Guerrero according to the ethical guidelines of the Declaration of Helsinki.

Clinical and anthropometric measurements

Body weight was determined using a Tanita body composition monitor (Tanita BC-553, Arlington, USA) and height was measured to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany). From these measurements, body mass index was calculated (BMI= weight/height^2, kg/m^2). The circumferences were measured by duplicate using a diameter tape accurate to within ± 0.1 cm (Seca 201, Hamburg, Germany). Waist circumference was measured at the level of the umbilicus and the superior iliac crest. The measurement was made at the end of a normal expiration while the subjects stood upright, with feet together and arms hanging freely at the sides. Hip circumference was measured at the maximum point below the waist, without compressing the skin. The thickness of

three skinfolds was measured to the nearest 0.1 mm, in duplicate, using skinfold caliper (Dynatronics Co, Salt Lake City, USA): triceps, biceps and subscapular. The duplicate of all measures was averaged.

Biochemical measurements and definitions

A blood sample was obtained from each child by antecubital venipuncture after an overnight fast. Total serum cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C) and glucose levels were obtained using semi-automated equipment (COBAS MIRA).

The classification of obesity was made using the 2000 Center for Disease Control and Prevention growth charts defined as normal-weight 5th-85th percentiles and obesity ≥95thpercentile (24).

Genotyping of *PAI-1* polymorphisms

Total genomic DNA (gDNA) was isolated from peripheral blood leukocyte by the salting out method (25). The -844 G/A, -675 4G/5G and *HindIII*C/G *PAI-1* polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Amplification of these three *PAI-1* polymorphisms was done using the primers and PCR protocols reported in our previous studies (19, 20). To confirm the results, genotyping of the three *PAI-1* polymorphisms was done in duplicate in all cases and confirmed by automatized sequencing of a randomly selected subset of *PAI-1* genotypes (Applied Biosystems, USA).

Statistical analysis

Statistical analysis was performed using the statistical software STATA v 9.2. For the descriptive analysis, nominal variables were expressed as frequencies, continuous variables with parametric distribution were expressed as mean and standard deviation, and variables with nonparametric distribution were expressed as medians and percentiles 5th-95th. We determined genotype and allele frequencies for the *PAI-1* gene polymorphisms by direct counting and performed chi-square test to compare proportions between groups and to evaluate the Hardy-Weinberg equilibrium. To compare parametric quantitative variables we used the Student´s t test and for nonparametric quantitative variables we used the U Mann-Whitney test. To analyze the potential risk for obesity associated with the *PAI-1* gene polymorphisms, Odds Ratio (OR) with 95% Confidence Intervals (CI) were calculated. Haplotype frequencies were estimated using the HEMHAPFRE software based on expectation-maximization algorithm according to Excoffier and Slatkin (26). LD between *PAI-1* polymorphisms was expressed as Lewontin's D' corrected LD coefficient (D') (27). The differences between the biochemical and anthropometric parameters were evaluated by haplogenotypes (G-4G-C/G-4G-C and G-4G-C/A-5G-G *PAI-1*) using the Student's t test and by the U Mann Whitney test. To evaluate the effect of the haplogenotypes we used logistic and linear regression models adjusted by gender and age. For all analysis, the reference haplotype and haplogenotype were the most frequent. Differences were considered statistically significant at $p<0.05$.

RESULTS

Baseline characteristics

A total of 222 children with an age range of 6-11 years were divided in two groups: 104 obese children and 118 with normal-weight as control group. The descriptive characteristics of the sample are summarized in Table I. As expected, body circumferences, skin folds thickness and biochemical measurements such as glucose, total cholesterol and triglycerides levels were significantly ($p<0.001$) greater in obese participants, whereas HDL-C levels were lower compared to normal-weight children.

Genotypic and allelic frequencies of PAI-1 polymorphisms

Table II shows the genotypic and allelic frequencies of the three *PAI-1* polymorphisms, which are in Hardy-Weinberg Equilibrium (-844 G/A,p= 0.13;-675 4G/5G,p= 0.24 and *HindIII*C/G,p= 0.80). Significant differences in the genotypic and allelic frequencies were found between study groups for the -844 G/A *PAI-1* (p= 0.001 and p= 0.003, respectively), the G/A heterozygote genotype and the A allele of -844 G/A *PAI-1* were associated with genetic susceptibility for obesity (OR= 2.75 and OR= 1.76, respectively); for the -675 4G/5G *PAI-1* a similar pattern was observed with a significant association of the 4G/5G heterozygous genotype for obesity (OR=1.83) and a marginal association of the 4G allele with obesity (OR=1.49). In the case of *HindIII* C/G *PAI-1* no differences or significant associations were found.

TABLE I
CLINICAL AND BIOCHEMICAL CHARACTERISTICS BY GROUP

Variables	Obesity *n*=104	Normal-weight *n*=118	*p* value
Age (years)*	9 (6-11)	9 (6-11)	0.30
Gender % (n)**			0.19
Male	56 (58)	48 (57)	
Female	44 (46)	52 (61)	
BMI (kg/m^2)*	23.1(18.8-29.1)	16.3 (13.9-19.1)	<0.001
Body circumferences			
Waist (cm)*	79 (64.5-94)	62 (52-73)	<0.001
Arm (cm)*	24.5 (20.5-30.5)	19 (15-22)	<0.001
Skinfolds			
Biceps (mm)***	18.1±4.2	13.3±4.3	<0.001
Triceps (mm)*	18(11.5-22)	12(8-18)	<0.001
Subscapular (mm)*	18(11.5-24.5)	10(5-18)	<0.001
Biochemical measurements			
Glucose (mg/dL)*	98 (83-112)	95 (72-112)	<0.01
Total cholesterol (mg/dL)***	187.9 ± 29.85	171.36 ± 30.25	<0.001
Triglycerides (mg/dL)*	138 (57-204)	68 (25-150)	<0.001
HDL-C (mg/dL)*	40.5 (23-83)	64 (34-101)	<0.001
LDL-C (mg/dL)*	102.2 (63.5-180.8)	95.3 (53.4-158.9)	0.03
Insulin (μU/mL)*	9.4 (1.3-27.5)	4.7 (0.6-13.6)	<0.001
HOMA-IR*	2.3 (0.2-6.8)	1.2 (0.1-3.1)	<0.001

*Data provided in median and percentile 5th-95th. U Mann-Whitney test.**Data provided in percentages and n. Chi square test X^2. ***Data provided in mean ± SD. Student´s t test.

TABLE II
FREQUENCY DISTRIBUTION OF *PAI-1* POLYMORPHISMS BY GROUPS

Polymorphism	Obesity % (*n* =104)	Normal-weight % (*n* =118)	*p value**	OR (CI 95%)	*p value**
-844 G/A *PAI-1*					
Genotype			0.001		
G/G†	46 (48)	68 (80)		1	-
G/A	49 (51)	27 (31)		2.75 (1.49-5.06)	<0.001
A/A	5 (5)	5 (7)		1.19 (0.28-4.63)	0.77
Allele			0.003		
G†	71 (147)	81 (191)		1	-
A	29 (61)	19 (45)		1.76 (1.10-2.80)	0.01
-675 4G/5G *PAI-1*					
Genotype			0.08		
5G/5G†	41 (43)	56 (66)		1	-
4G/5G	47 (49)	35 (41)		1.83 (1.0-3.35)	0.03
4G/4G	12 (12)	9 (11)		1.67 (0.61-4.60)	0.36
Allele			0.06		
5G†	65 (135)	73 (173)		1	-
4G	35 (73)	24 (63)		1.49 (0.97-2.27)	0.05
HindIII C/G *PAI-1*					
Genotype			0.58		
C/C†	50 (52)	57 (67)		1	-
C/G	43 (45)	38 (45)		1.28 (0.72-2.32)	0.36
G/G	7 (7)	5 (6)		1.5 (0.40-5.75)	0.49
Allele			0.18		
C†	72 (149)	76 (179)		1	-
G	28 (59)	24 (57)		1.24 (0.80-1.94)	0.31
PAI-1 Haplotype					
G-4G-C†	58.7 (122)	66.2 (156)		1	-
A-5G-G	18.3 (38)	11.9 (28)		1.76 (0.76-4.12)	0.05
G-5G-G	6.7 (14)	6.8 (16)		1.13 (0.33-3.81)	0.81
A-5G-C	5.8 (12)	4.2 (10)		1.56 (0.37-6.76)	0.47
G-5G-C	3.8 (8)	4.2 (10)		1.04(0.19-5.06)	0.95
A-4G-C	3.8 (8)	1.7 (4)		2.6 (0.35-29.4)	0.26
G-4G-G	1.9 (4)	4.2 (10)		0.52 (0.04-3.32)	0.43
A-4G-G	1 (2)	0.8 (2)		1.3 (0.01-103.3)	0.85

*Chi square test X^2; OR: Odds Ratio; CI: Confidence Interval; †reference category.

Based on these findings regarding the associations between *PAI-1* polymorphisms with obesity in our population, it was decided to determine the LD between the three *PAI-1* polymorphisms. In this regard, the -844 G/A *PAI-1* was found in LD with -675 4G/5G *PAI-1* (D'= 0.77).

Haplotypic and haplogenotypic frequencies of *PAI-1* polymorphisms

The haplotypic frequencies are shown in Table II. The most frequent haplotypes were the G-4G-C followed by the A-5G-G, no significant differences or associations were found in the comparison between study groups. To examine the combined effect of three *PAI-1* polymorphisms a haplogenotype analysis considering the most frequent haplotypic combinations G-4G-C andA-5G-G was performed (Table III). According to this analysis it was found that the G-4G-C/A-5G-G heterozygous combination a risk haplogenotype for obesity (OR=2.6; CI1.17-4.22; p= 0.01).

Association of the *PAI-1* haplogenotypes with obesity and triglyceride levels

Following the haplogenotype analysis proposed for this study, demographic, clinical and biochemical variables were compared with the most frequent haplogenotypes (frequency >10%). The G-4G-C/A-5G-G haplogenotype was associated with an increased percentage of obesity in children in comparison with the G-4G-C/G-4G-C haplogenotype carriers (61 vs. 37%; p= 0.01), as well as with high triglyceride levels (103 vs. 81 mg/dL; p= 0.01) (Table IV). In order to estimate the risk for high triglyceride levels attributed to the G-4G-C/A-5G-G PAI-1 haplogenotype in all children, a logistic and linear regression models adjusted by age and gender was used. The G-4G-C/A-5G-G haplo-

TABLE III
FREQUENCIES OF THE FOUR MORE COMMONS HAPLOGENOTYPES OF *PAI-1* POLYMORPHISMS BY GROUPS

PAI-1 Haplogenotype	Obesity % (*n*= 104)	Normal-weight % (*n*= 118)	OR (CI 95%)	*p* value*
G-4G-C/G-4G-C†	30 (31)	44 (52)	-	-
G-4G-C/A-5G-G	27 (28)	15.2 (18)	2.6 (1.17-4.22)	0.01
G-4G-C/G-5G-G	7.8 (8)	9 (11)	-	NS
G-4G-C/A-5G-C	7.8 (8)	4 (5)	-	NS
Others	27.4 (29)	27.8 (32)	-	NS

†Reference category; NS= Not Significant; OR= Odds Ratio; CI= Confidence Interval; * Chi square test X^2.

TABLE IV
GENERAL CHARACTERISTICS STRATIFIED BY *PAI-1* HAPLOGENOTYPES IN ALL CHILDREN

Variables	*PAI-1* Haplogenotypes		
	G-4G-C/G-4G-C *n*=83	G-4G-C/A-5G-G *n*=46	*p* value
Age (years)*	9 (6-11)	9 (6-11)	0.30
Gender % (n)**			0.10
Male	58 (48)	43 (20)	
Female	42 (35)	57 (26)	
BMI (kg/m^2)*	17.4 (13.9-25.9)	20.1 (14.4-28.2)	0.13
Obesity % (n) **			0.01
Yes	37 (31)	61 (28)	
No	63 (52)	39 (18)	
Body circumferences			
Waist (cm)*	66 (53-86)	68 (56-90)	0.20
Arm (cm)*	20 (17-28)	21.5 (16-29)	0.10
Skinfolds			
Biceps (mm)***	15 ± 4.7	16.1 ± 4.7	0.20
Triceps (mm)*	14 (9-21.5)	15.3 (8.5-20.5)	0.30
Subscapular (mm)*	12.5 (6-21.5)	14.3 (7-22)	0.07
Biochemical measurements			
Glucose (mg/dL)*	95 (74-107)	93 (70-108)	0.50
Total cholesterol (mg/dL)***	173.1 ± 32.4	182.9 ± 29.6	0.09
Triglycerides (mg/dL)*	81 (31-200)	103 (39-200)	0.01
HDL-C (mg/dL)*	58 (27-101)	45 (28-99)	0.20
LDL-C (mg/dL)*	97 (58-172)	98.2 (61-199)	0.86
Insulin (µU/mL)*	6 (0.8-25)	9.9 (0.8-22.6)	0.16
HOMA-IR*	1.4 (0.2-6)	2.3 (0.2-5.8)	0.20

*Data provided in median and percentile 5th-95th. U Mann-Whitney test.**Data provided in percentages and n. Chi square test X^2. ***Data provided in mean ± SD. Student´s t test.

TABLE V
ASSOCIATION OF *PAI-1* HAPLOGENOTYPE WITH TRIGLYCERIDE LEVELS

PAI-1 haplogenotype	Triglyceride levels		OR	(95% CI)	*p* value*
	Normal	High			
	<150 mg/dL	≥ 150 mg/dL			
	% (*n*=105)	% (*n*=24)			
G-4G-C/G-4G-C†	68.6 (72)	45.8 (11)	1	-	-
G-4G-C/A-5G-G	31.4 (33)	54.2 (13)	2.6	1.04-6.35	0.05

†reference category; *Chi square test X^2; OR= Odds Ratio, CI= Confidence Interval.

genotype presented a marginal association with hypertriglyceridemia (OR= 2.6; CI 1.04-6.35; p= 0.05) (Table V). Besides, the G-4G-C/A-5G-G *PAI-1* haplogenotype contributed to a significant increase in triglycerides serum levels (β= 21.12; CI 2.7-39.5; R2= 0.04;p= 0.02) (Data not shown in tables).

DISCUSSION

The present study shows the genetic contribution of *PAI-1* polymorphisms for obesity and hypertriglyceridemia in Mexican-mestizo children. In previous studies in a Mexican-mestizo population we found that the −844 G/A *PAI-1* polymorphism was related with the risk of developing MetS, obesity and atherogenic dyslipidemia, and the *HindIII* C/G *PAI-1* polymorphism, with increased total cholesterol levels (19), whereas the -675 4G/5G *PAI-1* polymorphism was associated with an increase of body adiposity measures (20). Based on these findings we decided to increase the sample of children studied and to analyze together these three polymorphisms and their association with obesity in a sample of Mexican children.

The present results show that heterozygous genotypes of the -844 G/A and -675 4G/5G *PAI-1* polymorphisms were associated with genetic susceptibility for obesity. In the case of *HindIII* C/G *PAI-1* no differences or significant associations were found with a similar pattern to those reported in our previous studies. In addition, both *PAI-1* promoter polymorphisms were found in LD, which indicates that *PAI-1* alleles are segregated in block from one generation to another and may confer a similar risk.

However, in the haplotype analysis, no significant differences or associations were found between study groups. Interestingly it was found that the G-4G-C/A-5G-G *PAI-1* heterozygous combination is a risk haplogenotype for obesity and moderately for hypertriglyceridemia.

Other studies have determined the functional effect of the *PAI-1* polymorphisms on protein levels. Several studies have reported the base change of G to A at position -844 of the promoter *PAI-1* gene generates a binding site consensus sequence for the Ets nuclear protein, which could be involved in regulating gene expression and may influence the increase in *PAI-1* levels(28, 29). Nevertheless, the associations reported for -844 G/A and *HindIII* C/G PAI-1 polymorphisms with *PAI-1* levels in other po-

pulations are not consistent. In other studies in adults, where the-675 4G/5G polymorphism was determined, the 4G/5G heterozygous genotype was related with increased *PAI-1* levels (30, 31).

Few association studies have evaluated the influence of the *PAI-1* polymorphisms in phenotypes related with obesity in children. Some authors have reported a relationship between the 4G/4G homozygous genotype with insulin resistance and increased adipose tissue in Caucasian populations, where the 4G allele is considered the risk allele, since it has been associated with high *PAI-1* levels due to the lack of a binding site for a transcriptional repressor gene (30). In European Caucasian children with obesity, Estelles et al., observed no association of the -675 4G/5G polymorphism with *PAI-1* levels (32). A similar pattern was observed in Turkish children with obesity, where the -675 4G/5G polymorphism was not associated with lipid and glucose parameters (33, 34).

In this study we found that the heterozygous genotypes and their combinations in haplogenotype may confer increased susceptibility for obesity in comparison with homozygous genotypes. This pattern observed in our population could be due to racial influence; our population has a genetic background with Amerindian (21-25%), Caucasian (60-64%) and African (15%) ancestry, which gave origin to the Mexican-mestizo population (35). Similarly to that reported in previous studies conducted in Mexican-mestizo population and ethnic groups of Mexico, we found that the 5G allele of the -675 4G/5G *PAI-1* polymorphism is predominant in our population (> 50%) compared with the 4G allele (22, 23).This suggests that the high frequency of the 5G allele may be due to the contribution of Amerindian genes in the genetic background of the Mexican population.

One of the limitations in our study was that we did not measure the *PAI-1* levels in circulation or *PAI-1* activity. Studies of *PAI-1* knockout mice have shown an effect of *PAI-1* on weight gain and increased adipose cellularity associated with high-fat dieting (36). Moreover, studies in which the *PAI-1* gene was disrupted in ob/ob mice showed a reduction in adiposity of these mice. This suggests that the *PAI-1* gene can control fat mass, although the mechanism of action is not yet known, it may be that the *PAI-1* gene could control fat mass at least in part, by inducing adipocyte proliferation through the expression of pro-inflammatory cytokine genes such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), leptin and the hormone insulin (37). It has been reported that a very-low-density lipoprotein (VLDL)-responsive element in the *PAI-1* promoter could be responsible for the effect of plasma lipids on *PAI-1* expression (6) and the increase in *PAI-1* levels may be a causal link between obesity and cardiovascular disease. Therefore, further studies are necessary in order to evaluate the functional contribution of the *PAI-1* polymorphisms in the study population.

In conclusion, our results suggest that the G-4G-C/A-5G-G *PAI-1* haplogenotype may be a genetic marker of susceptibility for obesity and hypertriglyceridemia in Mexican children.

ACKNOWLEDGMENT

This work was supported by Grants from PROMEP-SEP (UAGRO-EXB-057) and FOMIX-CONACYT-Gobierno del Estado de Guerrero 2010-01 (No. 147778).

REFERENCES

1. **Arslan N, Erdur B, Aydin A.** Hormones and cytokines in childhood obesity. Indian Pediatr 2010;47(10):829-839.
2. **Han JC, Lawlor DA, Kimm SY.** Childhood obesity. Lancet 2010;375(9727):1737-1748.
3. **Morandi A, Maffeis C.** Predictors of me-

tabolic risk in childhood obesity. Horm Res Pædiatrics 2014;82(1):3-11.

4. **Hamsten A, de Faire U, Walldius G,-Dahlen G, Szamosi A, Landou C, Blomback M, Wiman B.** Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet 1987;2(8549):3-9.
5. **Juhan-Vague I, Thompson SG, Jespersen J.** Involvement of the hemostatic system in the insulin resistance syndrome. A study of 1500 patients with angina pectoris. The ECAT Angina Pectoris Study Group. Arterioscler Thromb 1993;13(12):1865-1873**.**
6. **Binder BR, Christ G, Gruber F, Grubic N, Hufnagl P, Krebs M, Mihaly J, Prager GW.** Plasminogen activator inhibitor 1: physiological and pathophysiological roles. News Physiol Sci 2002;17(2):56-61.
7. **Bouchard L, Vohl MC, Lebel S, Hould FS, Marceau P, Bergeron J, Perusse L, Mauriege P.** Contribution of genetic and metabolic syndrome to omental adipose tissue PAI-1 gene mRNA and plasma levels in obesity. Obes Surg 2010;20(4):492-499.
8. **Aso Y.** Plasminogen activator inhibitor (PAI)-1 in vascular inflammation and thrombosis. Front Biosci 2007;12(8):2957-2966.
9. **Ha H, Oh EY, Lee HB.** The role of plasminogen activator inhibitor 1 in renal and cardiovascular diseases. Nat Rev Nephrol 2009;5(4):203-211.
10. **Morange PE, Saut N, Alessi MC, Yudkin JS, Margaglione M, Di Minno G, Hamsten A, Humphries SE, Tregouet DA, Juhan-Vague I.** Association of plasminogen activator inhibitor (PAI)-1 (SERPINE1) SNPs with myocardial infarction, plasma PAI-1, and metabolic parameters: the HIFMECH study. Arterioscler Thromb Vasc Biol 2007;27(10):2250-2257.
11. **Naran NH, Chetty N, Crowther NJ.** The influence of metabolic syndrome components on plasma PAI-1 concentrations is modified by the PAI-1 4G/5G genotype and ethnicity. Atherosclerosis 2008;196(1):155-163.
12. **Ma Z, Paek D, Oh CK.** Plasminogen activator inhibitor-1 and asthma: role in the pathogenesis and molecular regulation. Clin Exp Allergy 2009;39(8):1136-1144.
13. **Arya R, Blangero J, Williams KL, Almasy L, Dyer TD, Leach RJ, O'Connell P, Stern MP, Duggirala R.** Factors of insulin resistance syndrome-related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic mexican-americans. Diabetes 2002;51(3):841-847.
14. **Duggirala R, Blangero J, Almasy L,Dyer TD,Williams KL, Leach RJ, O'Connell P, Stern MP.** A major susceptibility locus influencing plasma triglyceride concentrations is located on chromosome 15q in Mexican Americans. Am J Hum Genet 2000;66(4):1237-1245.
15. **Adamski MG, Turaj W, Slowik A, Wloch-Kopec D, Wolkow P Szczudlik A.** A-G-4G haplotype of PAI-1 gene polymorphisms -844 G/A, HindIII G/C, and -675 4G/5G is associated with increased risk of ischemic stroke caused by small vessel disease. Acta Neurol Scand 2009;120(2):94-100.
16. **Lopes C, Dina C, Durand E, Froguel P.** PAI-1 polymorphisms modulate phenotypes associated with the metabolic syndrome in obese and diabetic Caucasian population. Diabetologia 2003;46(9):1284-1290.
17. **Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, Hamsten A.** Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci USA 1995;92(6):1851-1855.

18. **McCormack LJ, Nagi DK, Stickland MH, Mansfield MW, Mohamed-Ali V, Yudkin JS, Knowler WC, Grant PJ.** Promoter (4G/5G) plasminogen activator inhibitor-1 genotype in Pima Indians: relationship to plasminogen activator inhibitor-1 levels and features of the insulin resistance syndrome. Diabetologia 1996;39(12):1512-1518.
19. **De la Cruz-Mosso U, Muñoz-Valle JF, Salgado-Goytia L, García-Carreón A, Illades-Aguiar B, Castañeda-Saucedo E, Parra-Rojas I.** Relationship of metabolic syndrome and its components with -844 G/A and HindIII C/G PAI-1 gene polymorphisms in Mexican children. BMC Pediatr 2012;12:41.
20. **De la Cruz-Mosso U, Muñoz-Valle JF, Salgado-Bernabé AB, Castro-Alarcón N, Salgado-Goytia L, Sánchez-Corona J, Flores-Martínez SE, Parra-Rojas I.** Body adiposity but not insulin resistance is associated with -675 4G/5G polymorphism in the PAI-1 gene in a sample of Mexican children. J Pediatr (Rio J) 2013;89(5):492-498.
21. **Dawson S, Hamsten A, Wiman B, Henney A, Humphries S.** Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb 1991;11(1):183-190.
22. **Ruiz-Quezada S, Vázquez-Del Mercado M, Parra-Rojas I, Rangel-Villalobos H, Best-Aguilera C, Sánchez-Orozco LV, Muñoz-Valle JF.** Genotype and allele frequency of PAI-1 promoter polymorphism in healthy subjects from the west of Mexico. Association with biochemical and hematological parameters. Ann Genet 2004; 47(2):155-162.
23. **Nuño-Arana I, Páez-Riberos LA, Sandoval-Ramírez L, Muñoz-Valle JF, Pinto-Escalante D, Ceballos-Quintal JM, Rangel-Villalobos H.** High prevalence of 5G allele in Amerindian tribes and Mestizos from Mexico at 4G/5G *PAI-1* gene promoter polymorphism. Thromb Haemost 2005;93 (5):1005-1007.
24. **Centers for Disease Control and Prevention, National Center for Health Statistics.** Clinical Growth Charts [http://www.cdc.gov/growthcharts/clinical_charts.htm].
25. **Miller SA, Dykes DD, Polesky HF.** A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16 (3):1215.
26. **Excoffier L, Slatkin M.** Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995;12 (5): 921-927.
27. **Lewontin RC.** The interaction of selection and linkage. I. General considerations; heterotic models. Genetics 1964;49 (1): 49-67.
28. **Grubic N, Stegnar M, Peternel P, Kaider A, Binder BR.** A novel G/A and the 4G/5G polymorphism within the promoter of the plasminogen activator inhibitor-1 gene in patients with deep vein thrombosis. Thromb Res 1996;84 (6): 431-443.
29. **Henry M, Chomiki N, Scarabin PY, Alessi MC, Peiretti F, Arveiler D, Ferrières J, Evans A, Amouyel P, Poirier O, Cambien F, Juhan-Vague I.** Five frequent polymorphisms of the PAI-1 gene: lack of association between genotypes, PAI activity, and triglyceride levels in a healthy population. Arterioscler Thromb Vasc Biol 1997;17 (5): 851-858.
30. **Hoffstedt J, Andersson I-L, Persson L, Isaksson B, Arner P.** The common -675 4G/5G polymorphism in the plasminogen activator inhibitor -1 gene is strongly associated with obesity. Diabetologia 2002;45 (4): 584-587.

31. **Sartori MT, Vettor R, De Pergola G, De Mitrio V, Saggiorato G, Della Mea P, Patrassi GM, Lombardi AM, Fabris R, Girolami A.** Role of the 4G/5G polymorphism of PAI-1 gene promoter on PAI-1 levels in obese patients: influence of fat distribution and insulin-resistance. Thromb Haemost 2001;86 (5): 1161-1169.
32. **Estellés A, Dalmau J, Falcó C, Berbel O, Castelló R, España F, Aznar J.** Plasma PAI-1 levels in obese children-effect of weight loss and influence of PAI-1 promoter 4G/5G genotype. Thromb Haemost 2001;86 (2): 647-652.
33. **Kinik ST, Ataç FB, Verdi H, Cetintaş S, Sahin FI, Ozbek N.** The effect of plasminogen activator inhibitor-1 gene 4G/5G polymorphism on glucose and lipid metabolisms in Turkish obese children. Clin Endocrinol (Oxf) 2005;62 (5): 607-610.
34. **Kinik ST, Ozbek N, Yuce M, Yazici AC, Verdi H, Ataç FB.** PAI-1 gene 4G/5G polymorphism, cytokine levels and their relations with metabolic parameters in obese children. Thromb Haemost 2008;99 (2): 352-356.
35. **Rangel-Villalobos H, Muñoz-Valle JF, González-Martín A, Gorostiza A, Magaña MT, Páez-Riberos LA.** Genetic admixture, relatedness, and structure patterns among Mexican populations revealed by the Y-chromosome. Am J Phys Anthropol 2008;135 (4): 448-461.
36. **Morange PE, Lijnen HR, Alessi MC, Kopp F, Collen D, Juhan-Vague I.** Influence of PAI-1 on adipose tissue growth and metabolic parameters in a murine model of diet-induced obesity. Arterioscler Thromb Vasc Biol 2000;20 (4): 1150-1154.
37. **Schäfer K, Fujisawa K, Konstantinides S, Loskutoff DJ.** Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. FASEB J 2001;15 (10): 1840-1842.